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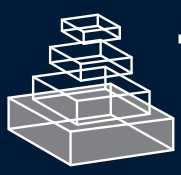

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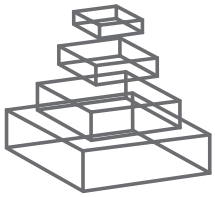

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BASAL GANGLIA CIRCUITS

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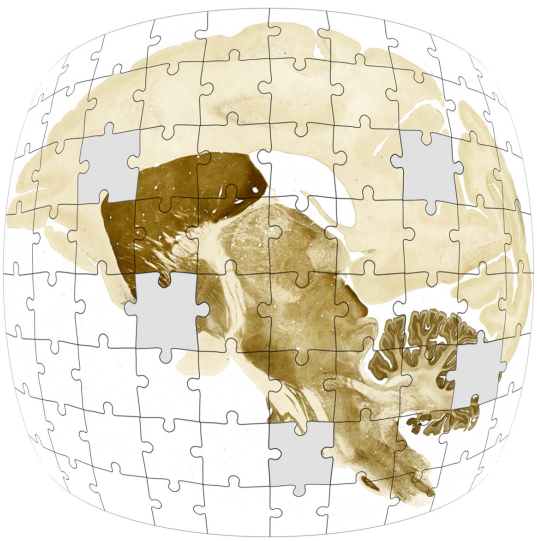

The current basal ganglia model has been introduced 25 years ago and has settled the basis for most of our current understanding of Parkinson's disease. Despite the tremendous success of the model, a number of experimental evidences have been made available over the past 25 years and the "classical" basal ganglia model is somewhat obsolete. I believe that it would be possible to recruit a number of international leading experts that have generated new data on basal ganglia circuits and therefore a Research Topic of this kind would lead to the introduction of a fully updated basal ganglia model, incorporating all the new basal ganglia circuits that have been characterized over the past 25 years.

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EDITORIAL
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Basal ganglia circuits: what's now and next?

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The current model of the basal ganglia was introduced two decades ago and has become the basis for most of our current understanding of basal ganglia function and dysfunction. Extensive research efforts have been carried out in recent years leading to unprecedented levels of understanding of the main operational principles underlying the pathophysiology of the basal ganglia. Although somewhat obsolete, the "classical" basal ganglia model developed in the mid 1980s by the "founding fathers" (Penney and Young, 1986; Crossman, 1987; Albin et al., 1989; DeLong, 1990) still maintains a remarkable appeal. This model was shaped mainly by the preponderance of anatomical and physiological data available at the time. In the past few years, the development of a whole range of new technical breakthroughs has boosted the availability of data with paramount importance at a breath-taking speed, and thus incorporating these recent advances to further enrich the classical model has become an urgent need.

With this idea in mind, we took advantage of Frontier's philosophy (peer-review policy, open access, web resources, and so on) to recruit a panel of leading experts in different fields of basal ganglia research to prepare focused reviews in their own areas of expertise. It gives me great pleasure to point out that they all (with only one exception) agreed immediately to participate. Besides invited reviews, eight unsolicited reviews have also been incorporated in this Special Issue after going through the standard review process. In addition to all contributors, it is also worth acknowledging the superb assistance received from all individuals reviewing the submitted manuscripts. As a part of Frontier's policy, the individuals involved in the review process are listed on the front page of each accepted manuscript. Submitted manuscripts were published immediately after acceptance without waiting for the entire Special Issue to be completed. Accordingly, the first paper to be published, prepared by Barroso-Chinea and Bezard (2010), appeared in mid-September whereas the last paper was issued by Lanciego and Obeso (2011) in July.

Although it is practically impossible to cover the whole range of new arrivals to the field with just one Special Issue, my feeling is that with all things considered we have managed to prepare a body of literature with an adequate balance between solicited and unsolicited contributions, representing a good reference for those trying to get started with the basal ganglia, particularly PhD students.

With regards to invited contributions, bearing in mind that we are dealing with basal ganglia circuits, a number of topics have been addressed in-depth. Firstly, Reiner et al. (2010) have written a comprehensive review on corticostriatal projections. Nigrostriatal and nigroextrastriatal projection systems are addressed in the contributions made by González-Hernández et al. (2010) and Rommelfanger and Wichmann (2010), respectively. Moreover, since striatal interneurons have often been neglected in the basal ganglia model, there are two contributions devoted to this topic: a review dealing with cholinergic interneurons (Bonsi et al., 2011) and another review focusing on all different types of GABAergic striatal interneurons (Tepper et al., 2010). It is also worth noting that we wanted to stress the role played by subcortical basal ganglia loops in modulating basal ganglia function. Two contributions deal with this topic: one by Redgrave et al. (2010) with a focus on the superior colliculus and the other highlighting the role of the pedunculopontine nucleus in basal ganglia circuitry (Martinez-Gonzalez et al., 2011).

The main research area that was neglected in this Special Issue is the thalamostriatal system. Although several of the invited senior authors posses an in-depth knowledge of this system, most of them were already engaged in other topics and I did not wish to overwhelm with additional commitments. For those interested in the thalamostriatal system, I would suggest reading recent reviews prepared by Smith et al. (2009, 2011). Moreover, the presence of projections arising from the subthalamic nucleus and reaching the ventral motor thalamus directly (without going through the output nuclei) has been reported by Rico et al. (2010). However, this paper was published too close to this issue to merit preparing a focused review and therefore was not considered as potential content for this Special Issue.

Two contributions emphasize a holistic view of basal ganglia function: one addresses the somatotopic organization of basal ganglia (Nambu, 2011) and the other focuses on functional anatomy and dynamic states of basal ganglia circuits (Garcia-Munoz et al., 2010). Finally, in an attempt to provide some clues on basal ganglia dysfunction in diseased states, we have incorporated two invited contributions. The first paper addresses striatal spine plasticity in parkinsonian conditions (Villalba and Smith, 2010), a hot topic that has recently attracted intensive research efforts by the scientific community. The second paper reviews the current knowledge on basal ganglia circuits underlying the generation and maintenance of levodopa-induced dyskinesia (Barroso-Chinea and Bezard, 2010), a clinical entity with devastating effects in the daily lives of parkinsonian patients.

Finally, unsolicited contributions have also played a crucial role in shaping the final outcome of the Special Issue, all of which were very much welcomed. Two manuscripts deal with subcortical striatal afferents from different sources (Day-Brown et al., 2010; Pan et al., 2010), whereas another extensively reviews the degree of segregation of striatal output pathways (Bertran-Gonzalez et al., 2010). One manuscript represents a comparative neuroanatomical work of the tree shrew striatum (Rice et al., 2011). Last but not least, two manuscripts address different aspects, such as basal ganglia circuits underlying sleep-wake regulation (Vetriveland et al., 2010) and thalamocortical integration of cognitive and emotional processes as seen with high-field fMRI.

All the aforementioned contributions have been collated here in a single e-book. I hope that this information may be useful for novice researchers approaching this exciting research field, the basal ganglia. Once again, my most sincere congratulations to all the individuals involved in any way during the preparation of this Special Issue. In addition to authors, co-authors, and reviewers, I must acknowledge the superb support received from Frontiers Team members at all times.

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Past, present, and future of the pathophysiological model of the basal ganglia

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The current model of basal ganglia (BG) was introduced two decades ago and has settled most of our current understanding of BG function and dysfunction. Extensive research efforts have been carried out in recent years leading to further refinement and understanding of the normal and diseased BG. Several questions, however, are yet to be resolved. This short review provides a synopsis of the evolution of thought regarding the pathophysiological model of the BG and summarizes the main recent findings and additions to this field of research. We have also tried to identify major challenges that need to be addressed and resolved in the near future. Detailed accounts and state-of-the-art developments concerning research on the BG are provided in the articles that make up this Special Issue.

Keywords: Parkinson's disease, dopamine, subthalamic nucleus, caudate putamen, substantia nigra

INTRODUCTION

The basal ganglia (BG) have been traditionally linked to the control of movement. This mindset was mainly derived from early clinico-pathological observations of BG lesions associated with movement disorders and, subsequently, by the profound impact that striatal dopamine (DA) depletion caused both in animals and in patients with Parkinson's disease (PD).

The first coherent model of the BG was developed in the mid-1980s (Penney and Young, 1986; Albin et al., 1989; DeLong, 1990) whereupon the BG were shown to act by integrating and processing information through a series of connections with different brain regions. This process involves the conduction of information from the cerebral cortex and thalamus to the striatum, then to the globus pallidus pars interna (GPi) and substantia nigra pars reticulara (SNr), to provide feed-back via the ventral thalamus to the cerebral cortex and the superior colliculus. This work was preceded by fundamental anatomical and physiological studies in monkeys describing the existence of segregated cortico-BG-thalamo-cortical circuits mediating different functions, as determined by the cortical area of origin. These circuits comprised the skeletomotor ("motor"), oculomotor, associative, and limbic circuits, whose general anatomical organization was similar, but whose cortical and sub-cortical component regions were distinct (Alexander et al., 1986). In simple terms, the BG were seen as a "go through" station that receives, processes and conveys information via closed and parallel loops.

THE PAST

The *model* was based on two fundamental concepts which led to the formulation of a hypothesis of how the BG function.

The first concept was settled on the preponderance of anatomical and neurochemical data available at the time, and based mainly on the fact that different populations of striatal medium spiny neurons (MSNs) project to the output of the BG (GPi and SNr) via "direct" and "indirect" pathways (Alexander et al., 1986; Penney and Young, 1986; Albin et al., 1989; DeLong, 1990). The "direct" pathway is a monosynaptic projection arising from MSNs that express D1 receptors, substance P and dynorphin, exerting a phasic and robust inhibitory effect on GPi/SNr neurons. The "indirect" pathway stems from MSNs expressing D2 receptors and enkephalin that project to the GPi/SNr through a polysynaptic ("indirect") pathway that involves relays in the external globus pallidus (GPe) and the subthalamic nucleus (STN).

The second concept was founded on physiological data. GPi/SNr neurons fire in a mostly tonic manner, keeping targeted structures in the thalamus and brainstem under tonic inhibitory control. Brief pauses in neuronal activity in the output of the BG facilitate movement (for example, saccadic eye movement) whereas neuronal discharges inhibit or halt movements (Chevalier and Deniau, 1990). Dopamine, from the nigro-striatal projection, was postulated to have a differential effect on D1/D2-expressing MSNs, facilitating and inhibiting respectively the "direct" and "indirect" pathways (Gerfen et al., 1990). In this so-called "*rate model*" it was thus postulated that in the normal state, activation of the "direct" pathway facilitates movement whereas activation of the "indirect" pathway inhibits or stops movement. Dopaminergic depletion, the key feature of the parkinsonian state, reduces the facilitation of MSNs belonging to the "direct" projection and enhances the activation of "indirect" pathway neurons, leading to increased activity of the STN which in turns overactivates inhibitory output neurons in the GPi/SNr. In this way, the combined reduction of GABA inhibition from the "direct" striatal output neurons and increased glutamatergic driving from the dis-inhibited STN led to increased activity in the GPi/SNr,

effectively reducing the likelihood of phasic inhibitory activity in these output neurons, and thereby impeding movement initiation and execution. Seminal studies in the monkey by Crossman and co-workers led to a similar understanding for the dyskinetic state (i.e., hemichorea–ballism and levodopa-induced dyskinesias), essentially considered to result from decreased GPi output and therefore representing a functional mirror of the parkinsonian state (Crossman, 1990).

The "rate" model of BG dysfunction in parkinsonism, and particularly the proposed role of increased activity in the STN and GPi was strongly supported by studies showing that subthalamotomy in MPTP-treated monkeys greatly ameliorated parkinsonian motor features and normalized BG output abnormalities (Aziz et al., 1991; Wichmann et al., 1994; Guridi et al., 1996). These findings reignited interest in the use of ablative stereotactic surgery in PD patients with levodopa-induced motor complications. Thus, in the 1990s unilateral pallidotomy firstly, and subthalamotomy subsequently, were shown to be highly effective in the treatment of patients with PD. This in turn led to the application of high-frequency stimulation ("deep brain stimulation," DBS), which was initially employed in the thalamus to treat tremor. DBS has several advantages over classic ablative surgery; for instance it can be performed bilaterally without major side effects and is potentially reversible.

The surgical experience also provided ample sources of published information and analysis, and led to two well-recognized paradoxes. The *first paradox* rests in the fact that lesion of the postero-lateral GPi, and thus interrupting BG output to the motor thalamus, does not cause dyskinesias (Obeso et al., 1997). Indeed, pallidotomy eliminates dyskinesias, which is exactly the opposite effect of the *model* prediction. The *second paradox* concerns the observation that lesion of the motor thalamus does not aggravate PD (Marsden and Obeso, 1994); furthermore, elimination of BG output nuclei by lesion of the GPi and STN is not associated with any major clinical deficit. Recently, some light has been shed on these conflicting observations. On the one hand, the "firing rate" based model has been expanded to consider that the degree of neuronal synchronization and firing patterns are also important determinants of the motor state (Brown, 2003). Normally, BG neurons discharge asynchronously, but in the parkinsonian state hypersynchrony of neuronal activity in the BG output nuclei and cortex is present. Recording local field potentials (LFPs) through DBS macro-electrodes implanted in the STN has shown enhanced beta-frequency (mean ∼18 Hz) oscillations in the "off" motor state. Such beta activity is reduced in the "on" medication state while gamma-band (>60 Hz) activity is increased (Brown et al., 2001). Subsequent studies have documented that similar oscillations also occur in the GPi and cortex in PD patients (Brown et al., 2001). Moreover, abnormal oscillatory activity in the theta band (∼6 Hz) has also been found in association with levodopa-induced dyskinesias and impulsivity induced by dopaminergic treatments (Alonso-Frech et al., 2006; Rodriguez-Oroz et al., 2011). Therefore, it is quite likely that pallidotomy eliminates dyskinesias by interrupting abnormal synchronization in BG output (Brown and Eusebio, 2008).

With regards to the *second paradox*, it is now recognized that animals with DA depletion and patients with PD demonstrate an impaired learning and execution of routines and habits and that PD patients with lesions of the BG motor output, after combined pallidotomy/subthalamotomy procedures, showed impaired acquisition of implicit learning (Obeso et al., 2009; Redgrave et al., 2010). Thus, lesions of the sensorimotor circuit of the BG are associated with deficits, but these are not apparent under normal conditions.

THE PRESENT

Several developments have occurred in the many years since the model was established, and these have had a corresponding impact on our understanding of the function(s) of the BG and their pathological derangement (Wichmann et al., 2011). Details are available in recent reviews and in the current volume of *Frontiers in Neuroanatomy*. Here, we summarize only the major areas of development. An updated version of the classical "box and arrows" model is provided in **Figure 1**.

"DIRECT" AND "INDIRECT" PATHWAYS

It is now recognized that the BG cannot be seen as a "go through" structure, whereby connectivity and functional interactions occur uni-directionally along cortico-BG-thalamo-cortical circuits (Graybiel, 2008; Obeso et al., 2008).

Revision of the original concept of the "indirect" pathway has been quite considerable in recent years. The STN is thus no longer placed in-between the GPe and GPi in the "indirect" circuit, but is now considered as another input station of the BG that receives afferents from the cerebral cortex (Inase et al., 1999; Nambu et al., 2000, 2002), thalamus (Lanciego et al., 2004) and brainstem (reviewed by Martínez-González et al., 2001, this issue). Moreover, besides the well-known STN output projections (i.e., GPe, GPi/SNr, and PPN nuclei), the presence of direct STN projections reaching the ventral thalamus has been often neglected and yet recently confirmed in the MPTP monkey model (Rico et al., 2010). Reciprocal connections between the GPe and the striatum (Sato et al., 2000) and between the striatum and DA neurons in the SNc (Haber et al., 2000) have been recognized. Finally, the STN–GPe–GPi form a microcircuit where GPe appears strategically placed to control BG output activity (Obeso et al., 2006). Accordingly, the exclusive feed-forward nature of information processing within the BG is not the only major feature of BG functional organization.

The concept of parallel cortico-BG loops has been largely confirmed in humans by functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies and also by cortical magnetoencephalography and LFPs recorded from the STN. Activation studies have revealed a topographical segregation according to the requested task and underlying functions. Activation in the posterior (i.e., sensorimotor) putamen was consistently reported for any movement and presented a somatotopical organization, with the leg lying dorsal, face ventral and arm in-between, as expected. Preparatory activation as well as finger movement sequencing was located more rostrally in the anterior putamen, while activation of the associative territory was observed during tasks such as motor internal representation, and the selection and planning of sequential actions (Lehericy et al., 2005).

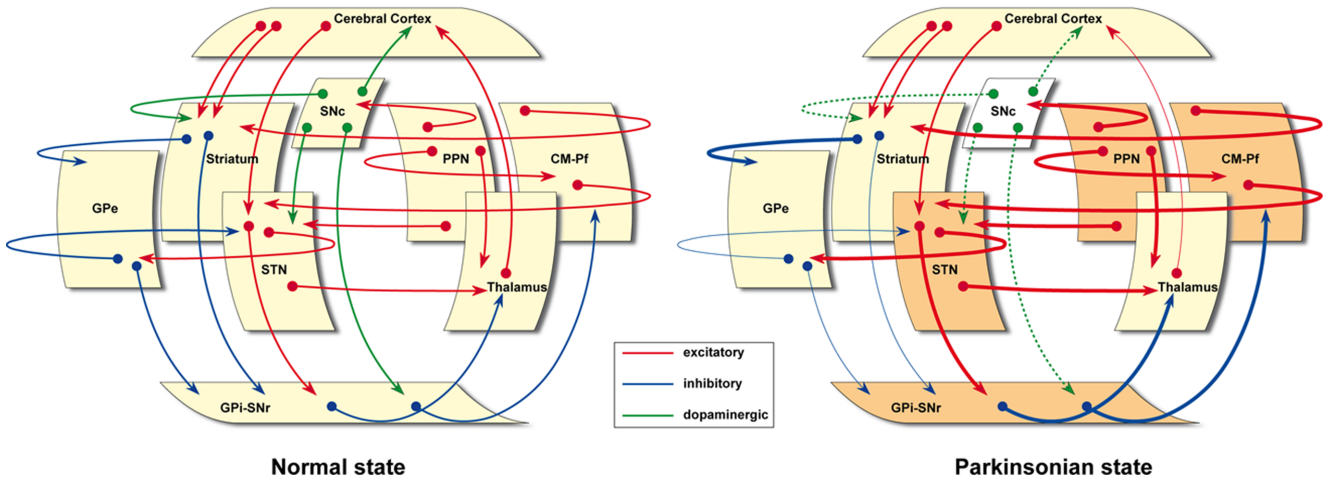

FIGURE 1 | The classical "box and arrows" basal ganglia model, updated. In the parkinsonian state, basal ganglia-related nuclei such as the STN, GPi–SNr, CM-Pf, and PPN are represented in a darker color to further illustrate that these nuclei are hyperactive following dopaminergic depletion, according to available experimental evidences. Furthermore, it is also worth noting that these cartoons emphasize the presence of a number of transverse, modulatory loops that have often been neglected in the classic BG model.

In addition to cortical input, the BG receive afferents from several sub-cortical nuclei (caudal intralaminar nuclei, PPN, superior colliculus, locus coeruleus, and raphe nuclei, among others) and, most prominently, from the thalamus (McHaffie et al., 2005).

INTERNEURONS

The original model did not take into account the role of striatal interneurons. BG nuclei are generally devoid of interneurons, the exception being the striatum which contains several types (reviewed in the accompanying manuscripts by Tepper et al., 2011 and Pisani et al., 2011, this issue). The cholinergic, tonically active neurons (TANs) and the GABAergic fast-spiking interneurons (FSIs) are the most prominent types of striatal interneurons. TANs modulate MSN excitability by pre-synaptic inhibition of cortical glutamatergic input, and mediate DAergic mechanisms leading to long-term depression (LTD). FSIs are less abundant but mediate feed-forward inhibition which together with axon collaterals from MSNs provide intrastriatal inhibition. These inhibitory effects seem particularly well suited to cause intense activation of some micro-regions of the striatum while inhibiting less active regions, i.e., they serve as a substrate for the selection process.

THE DOPAMINERGIC SYSTEM

The DAergic system projection innervates not only the striatum but also other BG structures such as the STN, GPe, and GPi, as well as the cortex, limbic system, and thalamus (Smith and Villalba, 2008). Data regarding how DA acts, particularly in the striatum, has changed considerably since the *model* was created. The nigro-striatal projection is now conceived as topographically subdivided into medial–ventral and dorso-lateral projections, which are highly divergent. DA secretion exerts a wide tonic -volume conduction- modulatory effect on striatal excitability and a more focal, SNc firing-related, phasic synaptic effect. It turns out that a majority of striatal DAergic synapses make "open" contacts with target neurons (Moss and Bolam, 2008), indicating the importance of tonic release. On the other hand, phasic changes in the dopaminergic response have been interpreted as an error signal that informs the corticostriatal system of the discrepancy between the prediction of a reward and its actual occurrence. This hypothesis sustains a major role for the DAergic system in reinforcement learning. However, it is now known that DA SNc neurons encode reward and non-reward events and are involved in other functions such as orientation, motivational driving and cognitive processing (Bromberg-Martin et al., 2010).

Another important issue under debate is represented by the D1–D2 dichotomy and its role in striatal output. DA is known to exert a dual effect on striatofugal neurons depending on the types of DA receptors present in the postsynaptic element. This reasoning represents one of the major foundations of the present BG model. Recently, anatomical, neurochemical and electrophysiological evidence (reviewed by Bertán-González et al., this issue) has supported the rather segregated distribution of D1 and D2 receptors within different types of striatofugal neurons. However, such a sharp degree of complementary expression for D1/D2 receptors in the two different populations of striatofugal MSNs still remains somewhat controversial and will probably require further elucidation. The recent introduction of BAC-transgenic mice permitted an estimation showing that only a small number of striatofugal MSNs (less than 6% in the dorso-lateral striatum) co-express D1 and D2 receptors (Bertran-Gonzalez et al., 2010). These newly available data challenge earlier results showing that roughly half of striatal MSNs co-express D1-like and D2-like receptors (as reviewed in Bertrán-González et al., this issue). It is also worth noting that anatomical data have their own limitations, including a limited number of neurons being reconstructed, the lack of information on the relative importance of each axon collateral compared to the main axonal terminal field, as well as the potential presence of tracer uptake by fibers of passage when dealing with retrograde tracing experiments.

Besides nigro-striatal projections, in the past few years accumulating evidence showing that DA also exerts an important effect on BG nuclei other than the striatum have been made available (Smith and Villalba, 2008). These projections -named the nigro-extrastriatal pathway- are likely to sustain a key role in BG pathophysiology, particularly in compensatory mechanisms underlying earlier stages of PD. As reviewed by Rommelfanger and Wichmann in this issue, BG nuclei such as the GPe, GPi, STN, and SNr are innervated by nigral DAergic terminals. Both D1 and D2 receptors (and to a lesser extent D3, D4, and D5 receptors) are found in most of these nuclei. The role played by DA (excitation or inhibition) ultimately depends on the type of receptor activated as well as on the pre or postsynaptic localization of a given DA receptor. Recently, attention has been paid to the effects of DA on extra-striatal nuclei and the implications for compensatory mechanisms in PD (see Rommelfanger and Wichmann, this issue).

STRIATAL AFFERENT SYSTEMS AND SUB-CORTICAL LOOPS

The importance of sub-cortical interactions with the BG has been extensively documented in the last decade (McHaffie et al., 2005). Special attention has been dedicated during this time to define the relationship between cortical and thalamic glutamatergic afferents (Castle et al., 2005). In addition, the presence of bilateral corticostriatal projections must also be taken into consideration. Ipsilateral corticostriatal projections arise in the rat from two different populations of corticofugal neurons, known as intratelencephalic neurons (IT neurons) and pyramidal tract neurons (PT type), whereas contralateral afferents originate only from PT type neurons. Importantly, whereas the inputs of IT type neurons mainly reach striatal MSNs giving rise to the "direct" pathway, PT neurons preferentially target MSNs projecting through the indirect pathway (reviewed in Reiner et al., 2010, this issue). Furthermore, the thalamostriatal system also represents a major source of ipsilateral glutamatergic projections to the striatum, an afferent source that has often been neglected.

PATHOPHYSIOLOGY

Animal models

The major functional sub-divisions of the BG have been further delineated by behavioral studies in monkeys. Manipulations of different functional territories of the BG nuclei are associated with emotional, learning, and motor manifestations. Specifically, focal injections of bicucculline or muscimol to block specific sub-regions of the striatum, GPe and STN, produced dyskinesias, stereotopies, and hyperactivity among other behavioral abnormalities when injected into the the postero-lateral (motor) segment, associative, and limbic regions (Karachi et al., 2009). Injections in the ventral striatum also elicited sexual responses (erection and ejaculation) and vomiting (Karachi et al., 2009). These studies indicate that interruption of different BG sub-regions gives rise to abnormal movements, behaviors, and mood changes.

Two recent studies in the rat applying optogenetics have provided strong support to the classic model. Kravitz et al. (2010) showed that selective stimulation of MSNs expressing D2 receptors ("indirect" circuit) provoked movement arrest, while activation of MSNs expressing D1 receptors led to movement activation. This essentially confirmed the notion that the "indirect" and "direct" circuits are in functional equilibrium, respectively inhibiting and facilitating movement. Furthermore, blockade of DARPP-32 to inhibit D1-expressing MSNs abolish levodopa-induced dyskinesias (Bateup et al., 2010), thus providing clear support to the key role of the "direct" circuit in mediating this complication, and well in keeping with several previous studies (Aubert et al., 2005).

Clinical studies

A number of major advances have occurred by recording LFPs via electrodes implanted in the STN (or GPi) for DBS. In the parkinsonian state there is an increment in the power at around 11–30 Hz, which is drastically reduced after taking levodopa and occurs concurrently with motor improvement (Brown, 2003; Gatev et al., 2006). Time-locked changes in STN activity have also been recorded with emotional stimuli such as the showing of emotionally laden and neutral pictures, during behavioral tasks requiring decisions between relevant or non-relevant stimuli for a given task, and during movement observation (Sauleau et al., 2009; Alegre et al., 2010). Many of these studies have been performed in the "off" (parkinsonian) medication state. In the "on" medication state when, presumably, DA deficits have been restored to some extent, the major changes consist of a marked reduction in beta power activity and enhancement of gamma activity (Brown, 2003) together with, in patients with levodopa-induced dyskinesias, a significant increment in slow (6–8 Hz) oscillations mainly from the dorsal STN (Brown, 2003).

Thus, the more recent neurophysiological data may be taken in support for activation of the BG both before and after movement onset and during cognitive and emotionally related tasks. Moreover, the motor vs. limbic, dorsal vs. ventral distribution appears substantiated, at least for the STN, as does the differential involvement of discrete circuits in abnormal movements and behaviors.

A new concept resides in the role of the BG in inhibition (Eagle and Baunez, 2010). This is applied to activities such as stopping an already ongoing order to move, halting an already initiated movement and suppressing an inconvenient behavior or desire. The STN has been particularly implicated in these inhibitory functions, which are essential for adequate performance of daily life activities (Aron and Poldrack, 2006).This function has recently been shown to be abnormal in PD patients and will be further delineated in upcoming studies.

The origin of Parkinson's disease

The real challenge at present and indeed in the future is for evidence to be uncovered to provide definitive understanding of the factors associated with the onset of the neurodegeneration process. Herein, a major effort is currently underway and will continue in the near future, to delineate the features explaining the vulnerability of dopaminergic neurons in the SNc. Although the main underlying causes sustaining this cellular degeneration remain to be fully elucidated, a number of factors have been identified, including those related to mitochondrial dysfunction, impairment of proteostasis, neuroinflammatory phenomena, glutamate-driven excitotoxicity, and even normal aging. Moreover, instead of there being a single mechanism, it is more likely that a combination of

different factors ultimately leads to the loss of DAergic neurons (Obeso et al., 2010). It is also likely that functional connectivity and physiological features will play an important role in determining selective vulnerability of ventro-lateral SNpc neurons in PD. This could become another relevant contribution of functional anatomy to improving our understanding and treatment of PD.

THE FUTURE

The *model* of the BG has paved the way toward many new developments and ignited renewed interest in the application of functional neurosurgery, which by itself may be considered a significant contribution to translational neuroscience. However, the imperfections and inaccuracies of the model are not minor and indeed, were noticed early on in the development of these concepts.

Nambu (2008) outlined in a lucid essay the seven main problems of the BG model. While major problems have been, on the whole, addressed, some of these issues have now been overshadowed by a wealth of data and information. For instance, we can now take for granted that the classic concept of "direct" and "indirect" pathways is no longer tenable, particularly because of the plethora of connections identified between the GPe and the STN with other nuclei. Similarly, the limitations of the "rate model" to explain BG pathophysiology are well accepted. However, many issues and queries remain open to study and interpretations. Among these, the following may be highlighted: (1) *Convergence.* There has to be a way whereby neuronal activity in the different loops, and even within the motor circuit, converge, so that a final coherent signal is provided for facilitating a given movement or behavior. (2) *Nigro-striatal and nigro-extrastriatal projections.* Much work needs to be done to recognize the putative topography and features of neuronal activity in register with ascending projections to the striatum and other nuclei. This is seen as particularly important regarding PD, where the most vulnerable neurons are the ones projecting to the posterior putamen. Moreover, the temporal evolution and functional implications of putative DA depletion in the nigro-extrastriatal connections awaits further clarification. (3) *Bilateral representation.* Up to now, most studies of the BG, and certainly the *Model*, contemplated connections and functions in a rather unilateral fashion. However, most movements and behaviors involve bilateral and synchronous activity. It is likely, therefore, that neuronal activity in the BG is under bilateral and reciprocal influences. (4) *Basic Functional Support.* Substantial evidence has been provided to demonstrate that inhibition or pausing of neuronal activity in the output of the BG is the primary mechanism for facilitation, while increased neuronal firing possibly sustains inhibition or arrest of actions. Whether or not the same basic principle sustains activity in the associative and limbic domains is not known and should be clarified in the near future.

In sum, a wide spectrum of future research can be envisaged, and important advances are already taking place. For instance, the ability to dissect out and study the function of specific circuits by using advanced morphological techniques and the application of optogenetics will provide even greater insight into the functional organization of the BG. Studies in awake animals, particularly in monkeys, will continue to illustrate crucial aspects of neuronal activity in relation to specific tasks. Importantly, human studies are now possible thanks to the development of powerful and sophisticated neuroimaging and neurophysiological techniques, which provide far better resolution of BG activation and distortions under normal and pathological conditions. The function(s) of the BG should no longer be considered "as dark as the basement of my house" as ironically indicated by the British neurologist Kinnier Wilson at the beginning of the twentieth century (Wilson, 1925). Nevertheless, astute experimental designs and sharp clinical observations will remain foremost priorities to further enlighten our understanding of the BG.

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Corticostriatal projection neurons – dichotomous types and dichotomous functions

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The mammalian striatum receives its main excitatory input from the two types of cortical pyramidal neurons of layer 5 of the cerebral cortex – those with only intratelencephalic connections (IT-type) and those sending their main axon to the brainstem via the pyramidal tract (PT-type). These two neurons types are present in layer 5 of all cortical regions, and thus they appear to project together to all parts of striatum. These two neuron types, however, differ genetically, morphologically, and functionally, with IT-type neurons conveying sensory and motor planning information to striatum and PT-type neurons conveying an efference copy of motor commands (for motor cortex at least). Anatomical and physiological data for rats, and more recent data for primates, indicate that these two cortical neuron types also differ in their targeting of the two main types of striatal projection neurons, with the IT-type input preferentially innervating direct pathway neurons and the PT-type input preferentially innervating indirect pathway striatal neurons. These findings have implications for understanding how the direct and indirect pathways carry out their respective roles in movement facilitation and movement suppression, and they have implications for understanding the role of corticostriatal synaptic plasticity in adaptive motor control by the basal ganglia.

Keywords: striatum, cortex, spines, synapses, projection neurons

INTRODUCTION

The so-called direct and indirect pathway model of basal ganglia function has provided a framework for understanding normal basal ganglia function, and explaining the pathophysiology of ballismus, Parkinson's disease (PD) and Huntington's disease (HD) (Albin et al., 1989; DeLong, 1990). This model, however, did not consider a number of complexities in basal ganglia organization critical to detailed understanding of its function. For example, although the cerebral cortex has a massive input to striatum, no consideration was given to how direct and indirect pathway striatal neurons might differ in their cortical input, a key issue in explaining the differing roles of these two striatal outputs in motor control. In our studies, we have found that direct and indirect pathway striatal neurons do differ in the cortical input they receive. In this paper, we review our prior findings on corticostriatal organization, present new findings, and discuss the implications of these findings for understanding the role of the basal ganglia in motor learning and movement selection.

CORTICAL PROJECTIONS TO BASAL GANGLIA

Diverse areas of cerebral cortex, including sensory, motor, and association regions, project to the striatum in all mammals studied (Kemp and Powell, 1970; Jones et al., 1977; Oka, 1980; Veening et al., 1980; Royce, 1982; Goldman-Rakic and Selemon, 1986; Tanaka, 1987; McGeorge and Faull, 1989). This input is bilateral, with an ipsilateral predominance, and it provides the striatum with the sensory and motor planning information needed for the basal ganglia to execute its role in motor control. The cortical projection is glutamatergic and ends as terminals that make asymmetric synaptic contacts, primarily with the spines of striatal projection neurons, which are by far the most abundant type of striatal neuron (Albin et al., 1989; Gerfen, 1992). The projection targets both the matrix and striosomal compartments of the striatum and is topographically organized (Kemp and Powell, 1970; Selemon and Goldman-Rakic, 1985; McGeorge and Faull, 1989). Of note with respect to the role of the basal ganglia in motor control, the dorsolateral striatum receives input from the somatosensory and somatomotor cortices, and somatotopy is preserved in this input (Jones et al., 1977; Goldman-Rakic and Selemon, 1986). While the cortical input to striatum is topographically organized, any given part of striatum receives overlapping, convergent input from multiple, often related, cortical areas (Goldman-Rakic and Selemon, 1986; Brown et al., 1998; Hoffer and Alloway, 2001). Additionally, the input to striatum from any given cortical region exhibits discontinuities (Tanaka et al., 1981; Goldman-Rakic and Selemon, 1986; Flaherty and Graybiel, 1993; Alloway et al., 1998). The discontinuity in some cases represents cortical input to striosomal patches, since cortical areas receiving prominent hippocampal and amygdaloid input (e.g., prelimbic frontal cortex) preferentially project to the striosomal compartment (Gerfen, 1984; Donoghue and Herkenham, 1986). Nonetheless, the discontinuities also represent separate terminal fields within the matrix compartment of striatum – referred to as matrisomes (Flaherty and Graybiel, 1993). These latter inhomogeneities reflect the projections of different cortical layers, or different cortical neuron types, as will be discussed subsequently.

The source of the corticostriatal projection has been of interest for many years. Ramon y Cajal (1911) suggested that the corticostriatal projection arose as a collateral projection of the corticofugal

fibers arising from pyramidal neurons of deep layer 5, as they traversed the striatum. Early retrograde labeling studies in rats, however, reported a large and widespread population of neurons in ipsilateral cortical layer 3 and in ipsilateral upper layer 5 following tracer injection into the striatum, with few of the large deep layer 5 corticobulbar and corticospinal pyramidal neurons labeled (Kitai et al., 1976; Hedreen, 1977; Hedreen and McGrath, 1977; Schwab et al., 1977; Wise and Jones, 1977; Veening et al., 1980; Arikuni and Kubota, 1986). This initially led to the view that corticostriatal input did not notably include collaterals from pyramidal tract neurons. The input from layer 3 and upper 5 was mainly thought to end in the matrix compartment, with the input to striosomes an exception arising from deep layer 5 neurons of the prelimbic cortices (Gerfen, 1989; Kincaid and Wilson, 1996). Several electrophysiological studies, however, reported that cortical neurons projecting to matrix include both pyramidal tract and non-pyramidal tract neurons. For example, Jinnai and Matsuda (1979) noted that two types of cortical neurons could be activated antidromically from striatum in cats, one type that only responded to caudate activation (60% of corticostriatal neurons detected) and one type that responded to both caudate and pyramidal tract (PT) activation (40% of corticostriatal neurons). While the conduction velocities of the PT-type and non-PT type were overlapping, the mean latency of antidromic activation from caudate was less for the PT-type, further supporting these as two separate corticostriatal neuron types. Similarly, Wilson (1986) showed that striatal EPSP response latencies to ipsilateral motor cortex stimulation in rats overlapped those to contralateral motor cortex stimulation, but ipsilateral responses included a short latency component that was absent in response to contralateral stimulation. Wilson interpreted this as evidence that striatum receives input from both more rapidly conducting PT-type cortical neurons as well as from more slowly conducting non-PT type cortical neurons. The conclusion that there was PT-type input to striatum was consistent with his prior evidence that stimulation of the pyramidal tract at midbrain levels evoked monosynaptic EPSPs in many striatal neurons (Wilson et al., 1982). Both Jinnai and Matsuda (1979) and Wilson (1986) noted that the PT collateral in striatum is thin and conducts much more slowly than does the main PT axon. The striatal projection of PT-type neurons of motor and somatosensory cortex via collaterals of the main descending extratelencephalic axon was confirmed anatomically in rats in other studies at that time by intracellular filling of PT-type neurons (Donoghue and Kitai, 1981; Landry et al., 1984).

CORTICOSTRIATAL NEURON TYPES

More recent studies in rats and monkeys employing cortical neuron-type specific labeling have made it clear that in each cortical region projecting to striatum at least two types of corticostriatal projection neuron can be distinguished by their connections within the telencephalon and their projections to other subcortical areas. One is the type whose main axon projects extratelencephalically (PT-type neurons) that was identified by Ramon y Cajal (1911), whereas the second projects to the basal ganglia and cortex but not outside the telencephalon (Wilson, 1987; Cowan and Wilson, 1994; Levesque et al., 1996a,b; Levesque and Parent, 1998; Reiner et al., 2003; Parent and Parent, 2006). We will refer to the latter as the intratelencephalically projecting type (IT-type). Note that not all IT-type neurons project to contralateral cortex and striatum, and this appears to be region-specific, since motor cortex but not somatosensory cortex projects heavily contralaterally. By contrast, PT-type neurons project only ipsilaterally to the striatum. In rats (**Figure 1**), PT-type corticostriatal neurons are typically larger than IT-type corticostriatal neurons and mainly found in lower cortical layer 5, whereas intratelencephalically projecting corticostriatal neurons are mainly found in layer 3 and upper layer 5 (Wilson, 1987; Cowan and Wilson, 1994; Levesque et al., 1996a,b; Levesque and Parent, 1998; Reiner et al., 2003; Parent and Parent, 2006). For rats, IT-type neurons have a mean diameter of 12–13 μm, while PT-type have a mean diameter of 18–19 μm (Reiner et al., 2003). These neurons differ too in their dendritic arborization – PT-type neurons have a prominent apical dendrite that ascends and branches profusely in layer 1 of cortex, while IT-type neuron dendrites are more slender and the arborization in layer 1 sparser. IT-type and PT-type corticostriatal neurons possessing these same various anatomical features have recently also been demonstrated in monkeys

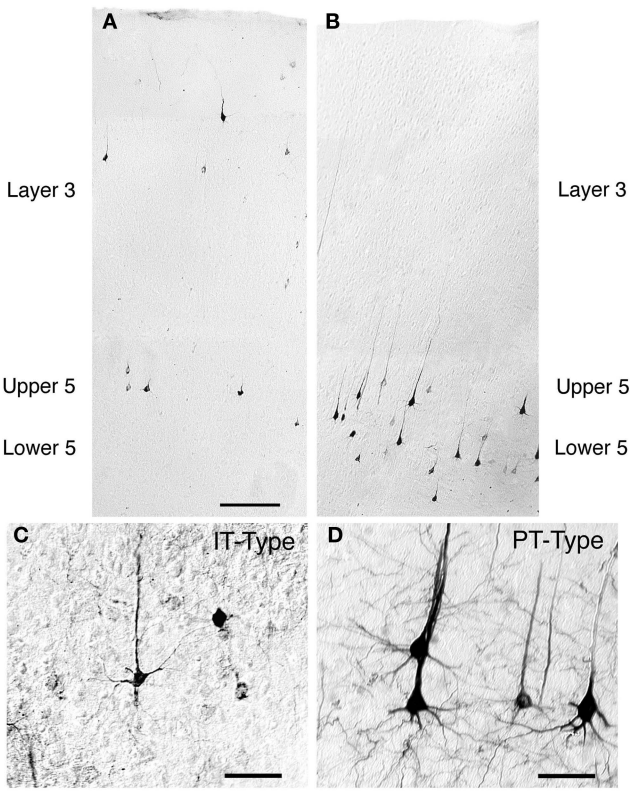

FIGURE 1 | Low-power images of the laminar distributions in primary somatosensory cortex of intratelencephalically projecting (IT)-type (A) and pyramidal tract (PT)-type (B) perikarya in the same rat. The IT-type perikarya were retrogradely labeled from the contralateral striatum with RDA3k. As is evident in **(A)**, they are 12–13 μm in size, and largely localized to layer 3 and upper layer 5. By contrast, the PT-type perikarya retrogradely labeled by BDA3k injection into the ipsilateral pontine pyramidal tract are largely localized to deep layer 5 (**B**) and are larger (18–19 μm) than the IT-type perikarya. Scale bar = 200 μm in A (applies to **A** and **B**). Images **C** and **D** show high power views of IT-type **(C)** and PT-type perikarya **(D)** in cortex. The PT-type perikarya are larger and possess a more prominent apical dendrite than the IT-type perikarya. Scale bars = 50 μm in **(C)** and **(D)**.

(Parent and Parent, 2006). It should be noted that layer 5 of cerebral cortex broadly consists of two neurochemically, morphologically, and physiologically distinct pyramidal neuron types matching the description of IT-type and PT-type neurons (Molnar and Cheung, 2006). Thus, IT-type and PT-type corticostriatal neurons do not merely represent a subset of layer 5 pyramidal neurons. Rather, layer 5 pyramidal neurons fall into two types – an intratelencephalically projecting type and an extratelencephalically projecting type, with each possessing a projection to striatum.

The laminar distribution of IT-type and PT-type perikarya differs slightly among cortical regions, and among species. In the somatosensory cortex in rats, the vast majority of IT-type perikarya are in layer 3 and upper layer 5, with the neurons being comparably abundant in the two (Wilson, 1987; Cowan and Wilson, 1994; Reiner et al., 2003). By contrast, in motor cortex of rats, the predominant location of IT-type perikarya is in upper layer 5, with additional IT-type perikarya being more abundant in lower layer 5 than in layer 3. In cats, layer 3 seems to be the more prevalent location of ipsilaterally projecting IT-type neurons (Oka, 1980; Royce, 1982). Monkeys, however, appear to be more similar to rats, with upper layer 5 the predominant location of IT-type neurons revealed by ipsilateral intrastriatal retrograde tracer injection (Jones and Wise, 1977; Jones et al., 1977; Goldman-Rakic and Selemon, 1986), and in single neuron tracing studies (Parent and Parent, 2006). Using retrograde labeling from the pontine pyramidal tract to identify PT-type neurons in rats (**Figure 1**), we observed that about 90% of the PT-type neurons of somatosensory cortex were in deep layer 5, but only 65% of the PT-type neurons of motor cortex were in deep layer 5 (Reiner et al., 2003). Most of the PT-type neurons not in deep layer 5 were located in upper layer 5 in rats.

Using intracellular filling of electrophysiologically identified PT-type neurons in rats, Cowan and Wilson (1994) found that individual neurons of this type give rise to an intrastriatal arborization that consists of scattered small, dense focal clusters of terminals (about 250 μm in diameter per focal cluster) spread over a 1–2-mm expanse of striatum (Cowan and Wilson, 1994). Using single axon tracing, a similar result was reported for the striatal PT-type input in monkeys (Parent and Parent, 2006). The discontinuous arborization pattern of PT-type neurons would explain why individual regions of cerebral cortex have a discontinuous projection to striatum. Moreover, part of the terminal field of each PT-type neuron of motor and somatosensory cortices has a discrete ending in dorsolateral striatum, which appears to account for the somatotopically ordered input of these cortical areas to motor striatum (Wright et al., 1999). Additionally, PT-type neurons of prelimbic cortex appear to account for the cortical input to striosomes (Kincaid and Wilson, 1996; Levesque and Parent, 1998). More recently, however, Zheng and Wilson (2001) used juxtacellular labeling to study the intrastriatal arborization of PT-type neurons of motor and cingulate cortex in rats, and reported that the PT-type neurons identified in that study possessed a broader and more diffuse striatal arborization pattern than reported in prior studies. It is uncertain whether PT-type neurons vary in their intrastriatal arborization, with cortical areas perhaps differing in the PT-type neuron varieties they possess, or if the differences observed stems from methodological differences.

IT-type corticostriatal neurons project to the ipsilateral and in many cases contralateral cortex and striatum, and neurons of the bilaterally projecting type are numerous in motor cortex (Wilson, 1987; Cowan and Wilson, 1994; Gerfen and Wilson, 1996; Kincaid and Wilson, 1996; Wright et al., 2001; Parent and Parent, 2006). In contrast to the scattered focal arborization of the PT-type neuron, the intrastriatal axon of individual IT-type neurons gives rise to an arborization that has sparse en passant terminals over a wide (about 1.5 mm in diameter) striatal expanse (Cowan and Wilson, 1994; Kincaid and Wilson, 1996). Recent LM studies suggest that primate striatum as well receives input from IT-type and PT-type cortical neurons possessing similar laminar location and extracortical projection patterns as in rats (Parent and Parent, 2006).

PT-type and IT-type neurons convey different signals to striatum. For example, the PT-type neurons of motor cortex in primates fire during movement and the IT-type neurons more typically fire in relation to movement planning (Bauswein et al., 1989; Turner and DeLong, 2000; Beloozerova et al., 2003). In addition, the conduction velocities of the parent PT-type axons are about three to four times more rapid than those of the parent IT-type axons (Wilson, 1986, 1987; Bauswein et al., 1989; Cowan and Wilson, 1994; Turner and DeLong, 2000). Even with the conduction velocity slowing for the thin PT-type collateral in striatum, PT-type signals reach their striatal target a few milliseconds before IT-type signals do upon their co-activation.

ULTRASTRUCTURE OF CORTICAL INPUT TO STRIATUM

Because of their differing neuronal morphologies, laminar location, and physiologies, we sought to determine if the ultrastructure of IT-type and PT-type terminals in striatum also differed (Reiner et al., 2003). IT-type intrastriatal terminals were selectively labeled anterogradely by biotinylated dextran amine (BDA)-10k injection into the contralateral motor or primary somatosensory cortex. Because IT-type but not PT-type neurons have crossed projections, BDA10k-labeled terminals in striatum contralateral to cortical injection are all IT-type. We selectively labeled PT-type terminals by BDA3k injections into pontine pyramidal tract, which yielded retrograde labeling of intrastriatal collaterals of PT-type cortical neurons. Both IT-type and PT-type terminals were seen to make asymmetric synaptic contact with spine heads and less frequently with dendrites (**Figure 2**). The IT-type terminals tended to be round and relatively small, and the postsynaptic density (PSD) at their axospinous contacts was rarely perforated (about 3.3%). By contrast, PT-type terminals were more variable in shape and nearly twice as large as IT-type terminals, and the PSD at their axospinous contacts was commonly perforated (about 40%). We recently re-measured the diameters of 240 IT-type and 220 PT-type axospinous synaptic terminals from our prior study, consistently measuring the diameter of the terminal parallel to the PSD and 0.1 μm behind the presynaptic membrane. We found that the mean diameters for axospinous synaptic IT-type and PT-type terminals measured in this standardized way were 0.52 and 0.91 μm, respectively. Because these measurements were made in random sections that did not necessarily pass through the widest point of each terminal, they underestimate the peak size of IT-type and PT-terminals. For the IT-type terminals, this underestimate is likely to be small, since the terminals themselves are relatively small. To

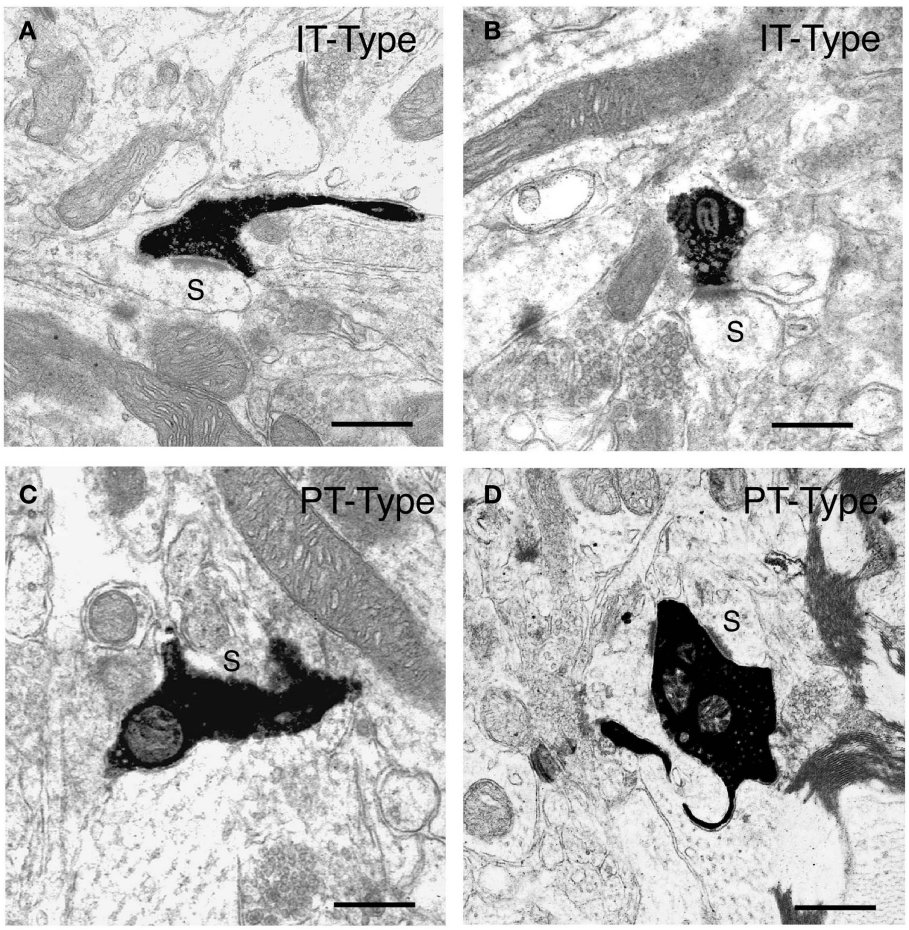

FIGURE 2 | Examples of the BDA10k-labeled intrastriatal terminals of IT-type corticostriatal neurons (A, B) and of PT-type corticostriatal neurons (C, D) at the electron microscopic level. The IT-type terminals and PT-type terminals shown each make asymmetric synaptic contact with a spine (s), as revealed by their size and the presence of spine apparatus, presumably belonging to striatal projection neurons. Note that the IT-type terminals are round, largely regular in shape, and about 0.5 μm in diameter, while the PT-type terminals shown are typically large, irregular in shape, and in some cases envelop their postsynaptic target structure. Scale bars = 0.5 μm in (**A–D**).

address the underestimate for PT-type terminals, we have analyzed several PT-type terminals in semi-serial sections, and found that their peak size is about 1 μm. Thus, PT-type axospinous terminals in rats labeled retrogradely collaterally from the pons are about twice the size of the IT-type axospinous terminals labeled anterogradely from contralateral cortex.

Concerns can be raised that our IT-type labeling of contralateral IT-type axons may not be representative of those that project ipsilaterally, and that the phenomenon of retrograde collateral labeling may be selective for axons that are not representative of the PT-type population as a whole. The work of Wright et al. (1999, 2001) on the intrastriatal terminals of IT-type and PT-type corticostriatal neurons of the primary somatosensory cortex of rat addresses these concerns, and is consistent with our findings. They used two anterograde pathway tracers, PHA-L and BDA, and found two distinct types of corticostriatal pathways: a non-topographic projection to the striatum with an intrastriatal arborization that was termed a "diffuse" system (Wright et al., 1999), and a topographically ordered projection that was termed the "discrete" pathway. The "discrete pathway" was seen to arise as collaterals of corticofugal axons descending through the striatum, it was only ipsilateral, and it gave rise to scattered patches of dense focal innervation. The authors concluded that the diffuse system arose from the IT-type corticostriatal neurons, and the discrete system from PT-type corticostriatal neurons (Wright et al., 2001). Wright et al. (1999) noted the terminals of the diffuse pathway had a mean diameter of about 0.55 μm and made asymmetric synaptic contacts with striatal projection neuron dendritic spines, and the contacts rarely possessed a perforated PSD. By contrast, the discrete system gave rise to large terminals with a mean diameter of 0.89 μm, and they made complex asymmetric synaptic contacts with striatal projection neuron spines that frequently possessed a perforated PSD. The findings of Wright et al. (1999, 2001) are thus consistent with our own, and obviate concerns that our techniques labeled atypical subsets of IT-type and PT-type terminals.

We further assessed the size of IT-type and PT-type axospinous striatal terminals by comparing their size frequency distributions to that of axospinous striatal terminals immunolabeled

for VGLUT1. Cortical projection neurons use the vesicular glutamate transporter VGLUT1 for packaging glutamate in synaptic vesicles, while excitatory thalamic neurons use VGLUT2 (Fremeau et al., 2001, 2004; Herzog et al., 2001; Varoqui et al., 2002; Fujiyama et al., 2004). Thus, VGLUT1 is a marker of corticostriatal terminals, and VGLUT2 a marker of thalamostriatal terminals (Raju et al., 2006; Lacey et al., 2007). Using the same approach for measurement as in the case of our IT-type and PT-type axospinous endings in rats, we studied 423 VGLUT1 immunolabeled axospinous terminals in rats. Counts of random striatal fields indicated that about 70% of axospinous synaptic terminals immunolabeled for VGLUT1 and are thus corticostriatal. We also found that about 90% of VGLUT1+ terminals synapsed on spines and the remainder on dendrites. Combining the IT-type and PT-type size frequency distributions in a 1:1 ratio gave an approximate, but not exact, match to the VGLUT1 size frequency distribution. Using curve-fitting with SPSS software to more rigorously compare the size frequency distributions of IT-type and PT-type axospinous terminals to VGLUT1 axospinous terminals in rat, we found that a 58% IT-type and 42% PT-type frequency combined to give the best fit to the VGLUT1 size frequency distribution (**Figure 3A**).

Thus, IT-type and PT-type axospinous terminals in rat dorsolateral striatum appear to occur in about a 3:2 ratio. Note, however, that this analysis suggests that our methods for labeling IT-type terminals were biased slightly toward smaller terminals, and that we did miss about 5% of IT-type terminals, which were in the 0.5 μm size range. Similarly, our PT-type terminal labeling was slightly biased toward larger terminals, and we missed about 5% of PT-type terminals, which were in the 0.7 μm size range. If we correct for this by adjusting the IT-type and PT-type distributions to sum to match the VGLUT1 distribution (**Figure 3B**), with no change in their relative frequencies, then the mean predicted IT-size is 0.547 μm and the mean predicted PT-size is 0.862 μm, which

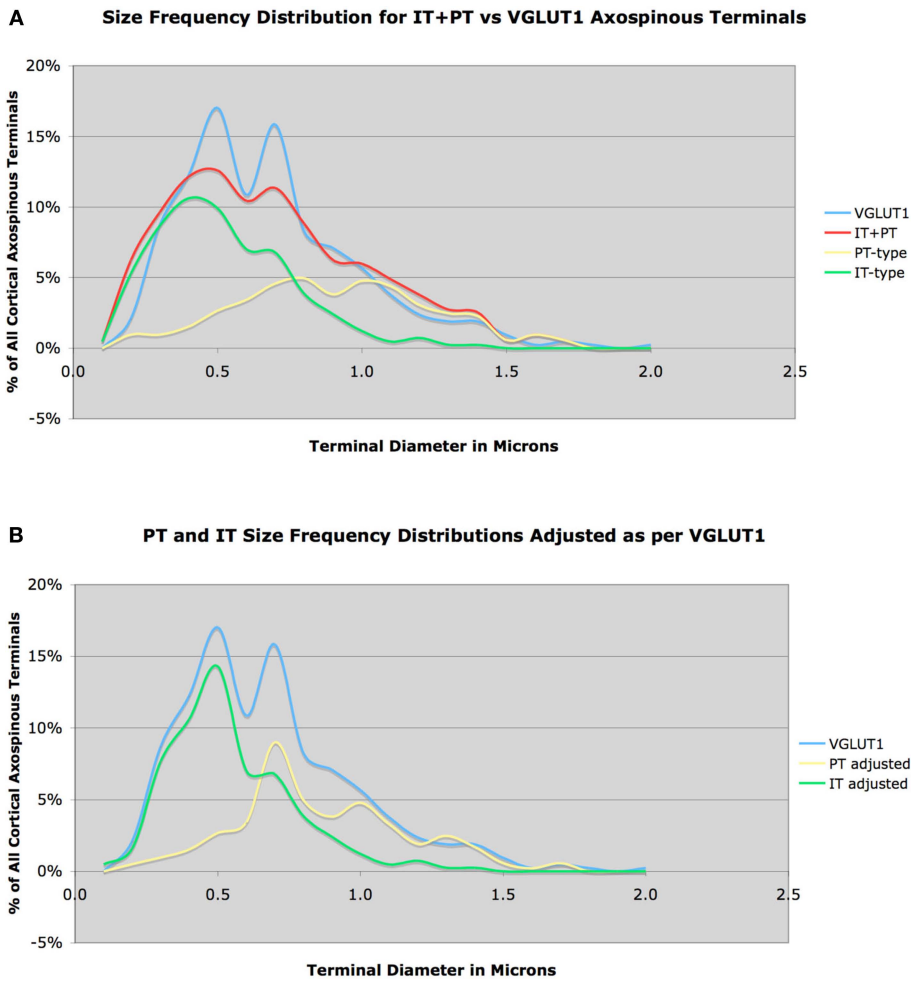

FIGURE 3 | Size frequency distribution of VGLUT1+ corticostriatal axospinous terminals in rat, compared to the measured size frequency distributions for IT-type and PT-type axospinous terminals in striatum, with IT-terminals graphed as 58% and PT-type 42% of all corticostriatal axospinous terminals (A). Note that the IT-type and PT-type distributions sum to closely approximate the VGLUT1 distribution, but there is a short fall in IT-type and PT-type terminals at 0.5 and 0.7 μm, respectively. In image **(B)**, we have corrected for this shortfall, by adjusting the IT-type and PT-type distributions slightly to fit the VGLUT1 distribution, again with the IT-terminals graphed as 58% and the PT-type 42% of all corticostriatal axospinous terminals.

yet more closely matches the sizes for these in Wright et al. (1999, 2001), and is thus more likely to accurately represent their size frequency distributions.

Recent LM studies suggest that PT-type corticostriatal terminals are larger than IT-type in monkeys as well (Parent and Parent, 2006). We have examined this issue in more detail at the EM level. We injected rhodamine dextran amine (RDA) into motor cortex and examined corticostriatal terminals in contralateral striatum (Reiner et al., 2008). As IT but not PT terminals in monkeys as well possess a contralateral striatal projection, the RDA-labeled contralateral M1 terminals were exclusively IT-type. The RDA+ IT-type axospinous synaptic terminals had a mean diameter of 0.62 μm, and tended to be rounded (**Figure 4**). To characterize all corticostriatal terminals, we examined striatum in tissue that had been immunolabeled for VGLUT1. We found that 74% of VGLUT1+ corticostriatal terminals ended on spines (151 VGLUT1 axospinous terminals), and included two subpopulations by size and morphology. One type had a peak size of about 0.7 μm, and formed simple round terminals, and thus resembled the RDA+ IT-type terminals (**Figure 4**). The second type had a peak size of about 1.4–1.5 μm. As true of PT-type terminals in rats, these larger VGLUT1+ terminals often enveloped the postsynaptic spine and the PSD of the contacted spine was often perforated. These results suggest that, as in rats, IT-type and PT-type corticostriatal axospinous terminals are morphologically distinct. Subtracting the IT-type size frequency distribution from the VGLUT1 size frequency distribution to derive the IT-type and

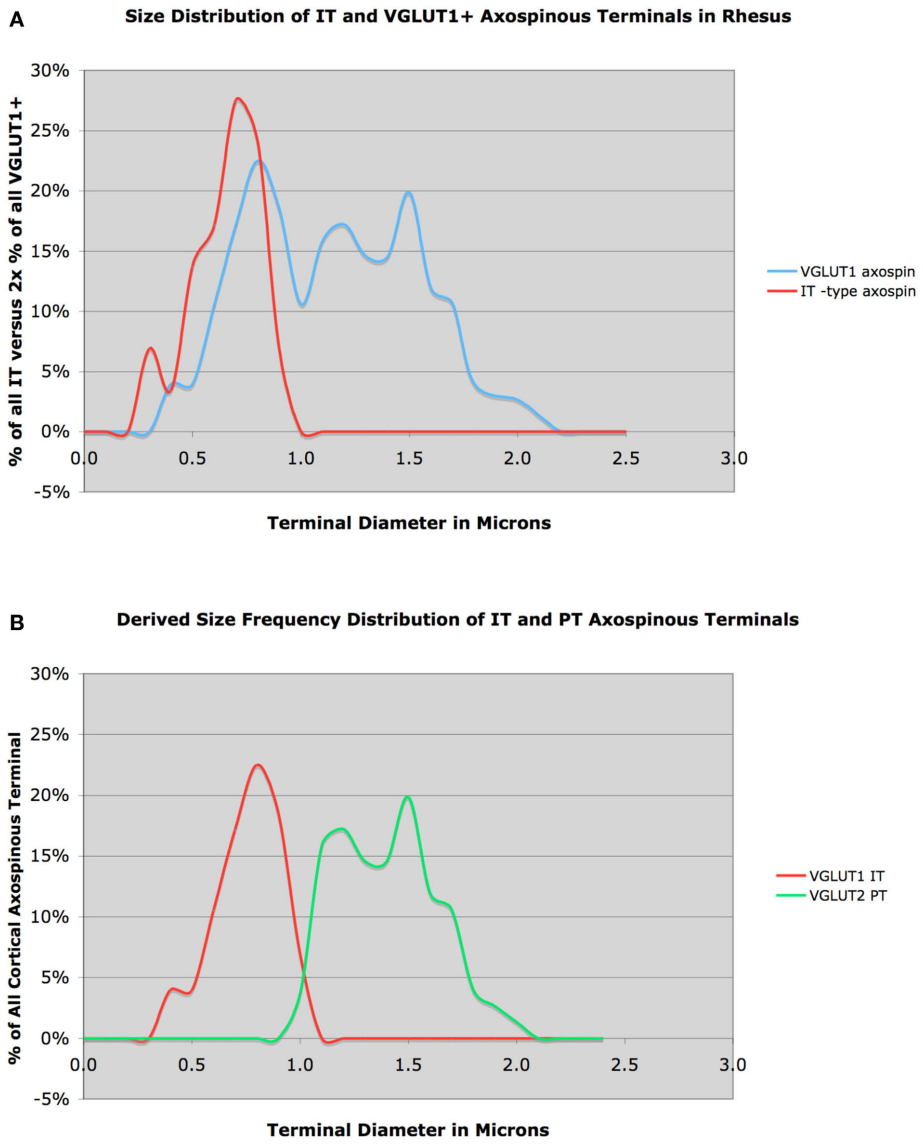

FIGURE 4 | Graph (A) shows the size frequency distribution of VGLUT1+ corticostriatal axospinous terminals (blue) in rhesus monkey, compared to the size frequency distributions for IT-type axospinous terminals (red) in striatum. Note that the IT-type distribution largely coincides with the VGLUT1 peak at about 0.7 μm. Graph **(B)** shows the inferred size frequency distributions of IT-type (red) and PT-type axospinous terminals (green) in rhesus monkey **(B)**, derived from the relationship of IT-type terminals to VGLUT1 terminals shown in **(A)**.

PT-type distributions, our preliminary results suggest that IT-type account for 42% of VGLUT1 axospinous terminals in monkeys and are 0.76 μm in size, and that 58% of VGLUT1+ axospinous endings are PT-type and are 1.40 in mean size.

DIFFERENTIAL INPUT OF CORTEX TO STRIATAL NEURONS

PROJECTION NEURONS OF STRIATAL MATRIX

We have assessed if the two types of corticostriatal neurons project differentially to the D2 receptor-rich enkephalinergic indirect pathway striato-GPe neurons and the D1 receptor-rich substance P (SP)-containing direct pathway striato-GPi/SNr neurons. Since axospinous IT-type terminals differ from PT-type in size, we examined in rats if axospinous synaptic terminals differed in size on these two striatal projection neuron types. We identified direct pathway neurons either by BDA3k retrograde labeling from the substantia nigra or by immunolabeling for the D1 dopamine receptor, and we identified indirect pathway neurons by BDA3k retrograde labeling from the GPe or by immunolabeling for the D2 dopamine receptor (Lei et al., 2004). Since striato-GPi neurons in rats have a collateral in SNr, BDA3k injection into substantia nigra yields retrograde labeling of both striato-SNr and striato-GPi neurons. We thus refer to the neurons BDA3k-labeled from substantia nigra as striato-GPi/SNr. Note that while D1+ versus D2+ neurons in striatum largely represent striato-GPi/SNr versus striato-GPe projection neurons, respectively, this labeling approach is preferential but not selective due to some colocalization of these two receptor types (Surmeier et al., 1996; Deng et al., 2006). We measured the diameter of asymmetric axospinous synaptic terminals on either striatal projection neuron type in dorsolateral striatum. The values presented here are from recent re-measurements taken parallel to the PSD and 0.1 μm behind the presynaptic membrane (Deng et al., 2010), and are thus revised from those presented in Lei et al. (2004). We found that asymmetric synaptic terminals on BDA3k-labeled striato-GPi/SNr neuron spines were characteristically small (0.54 μm, based on 340 terminals) and rounded (**Figure 5**).

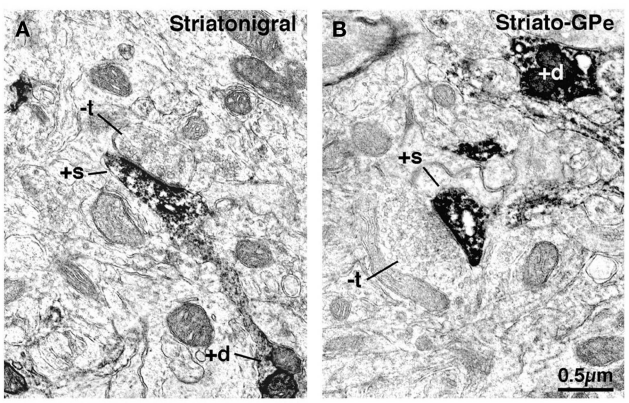

FIGURE 5 | Electron microscopic images of dendrite (+d) and spine (+s) labeling of striatonigral (A) and striato-GPe (B) neurons that had been retrogradely labeled with BDA3k from their target areas. Note that striatonigral **(A)** spines receive asymmetric synaptic contact from smaller unlabeled terminals (–t) than do striato-GPe neuron spines **(B)**.

Similarly, we found that the mean size of terminals making asymmetric synaptic contact with D1+ spines was 0.55 μm, based on 1004 terminals. The close match in size frequency distributions for axospinous terminals on striato-GPi/SNr neurons compared to IT-type axospinous terminals indicates that IT-type greatly predominates over PT-type in the cortical input to the direct pathway neuron spines (**Figure 6A**). Because our VGLUT1 data suggested that our IT-type terminal labeling method may have undercounted axospinous terminals in the 0.5 μm size range, we also compared the VGLUT1-adjusted IT-type axospinous terminal size frequency distribution to the size frequency distributions for axospinous terminals on striato-GPi/SNr neurons (**Figure 6B**). Note the yet closer fit of the adjusted IT-type distribution to the striato-GPi/SNr distribution. Thus, IT-type terminals appear to account for the vast majority of the axospinous input to striato-GPi/SNr neurons. Determining from this approach precisely how much of the axospinous input is IT-type requires, however, also knowing the size frequency distribution of the axospinous thalamic input to striato-GPi/SNr, thought to be about 20–30% of the axospinous input (Chung et al., 1977; Smith et al., 2004). While we have found that thalamostriatal axospinous terminals immunolabeled for VGLUT2 in rats have a mean size of about 0.6 μm, we do not know the size frequency distribution of those ending specifically on striato-GPi/SNr neurons. In any event, these considerations suggest that striato-GPi/SNr neuron spines receive mainly IT-type input from cortex, and a less common thalamic axospinous input of largely similar size, but relatively little PT-type input from cortex (Deng et al., 2010). It will be important, however, to determine if the size frequency distribution of axospinous PT-type input to direct pathway neurons is the same as for axospinous PT-type terminals as a group. It is possible that direct pathway neuron spines receive more PT-type input than suggested by the congruence of the size distributions of axospinous IT-type terminals and axospinous terminals on direct pathway neurons, if putative PT-type input to direct pathway spines is skewed toward the smaller end of the PT-type size range. We have, however, no evidence this is the case from our prior study (Lei et al., 2004).

In contrast to direct pathway spines, asymmetric synaptic terminals on BDA3k-labeled striato-GPe neuron spines tended to be notably larger (0.71 μm, based on 212 terminals), irregular in shape, and in many cases associated with a perforated postsynaptic density (Deng et al., 2010). Given the sparseness of the intra-GPe collateral of SP+ striato-GPi/SNr neurons (Kawaguchi et al., 1990; Wu et al., 2000), and given that we previously found that only 20–25% of neurons retrogradely labeled from GPe possess direct pathway neuron neurochemistry (i.e., D1+ and ENK-negative) (Deng et al., 2006; Wang et al., 2006), the majority but not all of the striatal neurons labeled from GPe with BDA3k must have been ENK+ striato-GPe neurons. Consistent with this, the mean size and size frequency distribution of the axospinous terminals on striato-GPe neurons cannot be accounted for by input from IT-type axospinous terminals (**Figure 7A**). While a prominent input from PT-type terminals better explains the size of axospinous terminals on striato-GPe neurons, the size frequency distribution of PT-type axospinous terminals, nevertheless, also does not match that for axospinous terminals on striato-GPe neurons. Several factors are likely to contribute to this.

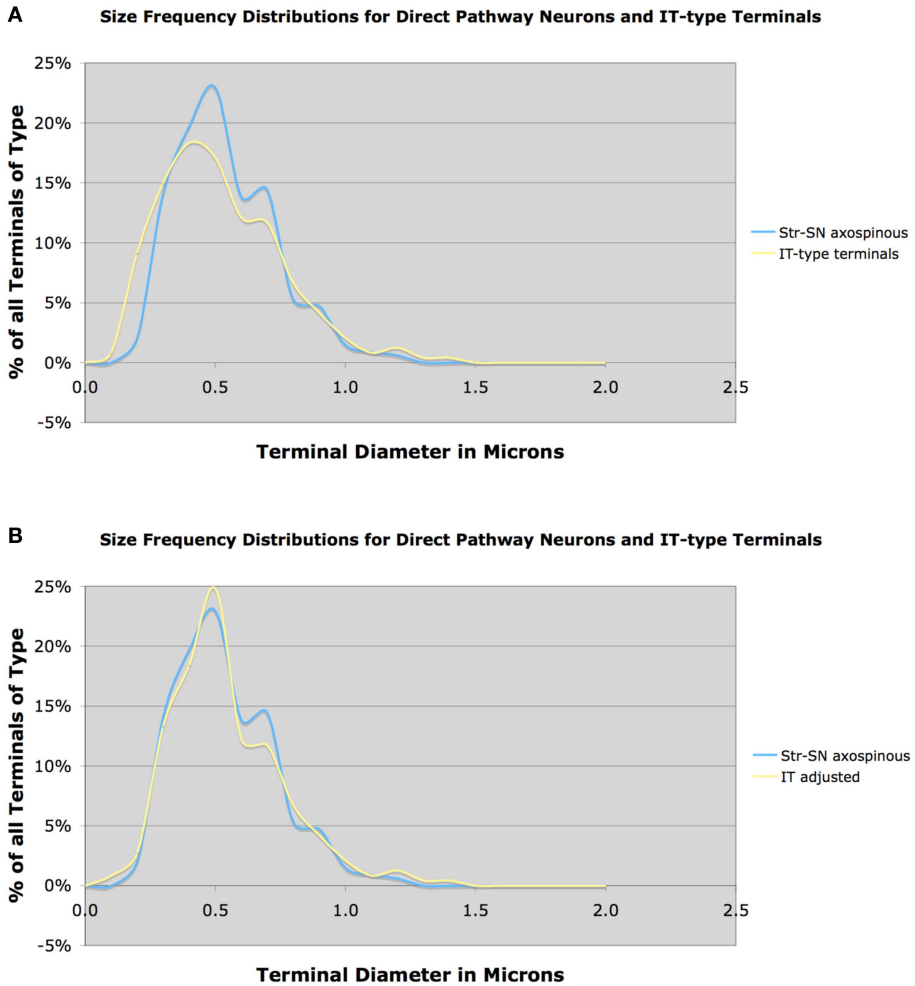

FIGURE 6 | Graph (A) shows the size frequency distribution of IT-type corticostriatal axospinous terminals in rat compared to the size frequency distribution of axospinous terminals on striato-GPi/SNr neurons neurons, while graph **(B)** shows the size frequency distribution of axospinous terminals on striato-GPi/SNr neurons compared to the VGLUT1-adjusted IT-type size frequency distribution shown in **Figure 3B.** Note that the size frequency distribution of axospinous IT-type terminals in A closely matches that of axospinous terminals on striato-GPi/SNr neurons, indicating that IT-type input (plus thalamic input of a similar size) to direct pathway neurons dominates. The fit between IT-type axospinous terminals and axospinous terminals on striato-GPi/SNr neurons is even closer for the adjusted IT-type distribution.

First, our above noted comparison of the VGLUT1 size frequency distribution to the IT-type and PT-type size frequency distributions suggests that mean PT-type axospinous size and size frequency distribution may actually have their peak at a slightly smaller size than the distribution measured from anterogradely BDA3k-labeled PT-type terminals. When we compare the VGLUT1-adjusted PT-type size distribution to the striato-GPe axospinous distribution, in fact, a much better match is obtained, with PT-type terminals seeming to account for about 75% of the input (**Figure 7B**). Nonetheless, the PT-adjusted distribution does not account for the many axospinous terminals in the 0.4–0.6 μm size range. These additional terminals must represent IT- and/or thalamic inputs to the striato-GPe neurons. Note also that some neurons retrogradely BDA3k-labeled from the GPe may actually be direct pathway neurons labeled via their fibers of passage, which may also account for some of the smaller terminals. The contribution of each of these factors needs to be known to determine the relative abundance of PT-type axospinous input to indirect pathway projection neurons. In any event, the mean size and size frequency distribution of the axospinous terminals on striato-GPe neurons indicate that they receive predominantly PT-type axospinous input from cortex (Deng et al., 2010). In the case of D2 immunolabeling to preferentially label indirect pathway spines, the mean size of terminals making asymmetric synaptic contact with D2+ spines was 0.65 μm, based on 497 terminals. Thus, this approach too indicates indirect pathway neuron spines to be the target of the large PT-type terminals. Nonetheless, the results for asymmetric terminals on D2+ spines is likely to understate the selectivity of PT-type input for striato-GPe neurons, since we showed that about 30% of D2+ neurons are striatonigral (Deng et al., 2006). This is likely to explain why the mean size of axospinous terminals for D2+ spines is slightly less than that for striato-GPe spines.

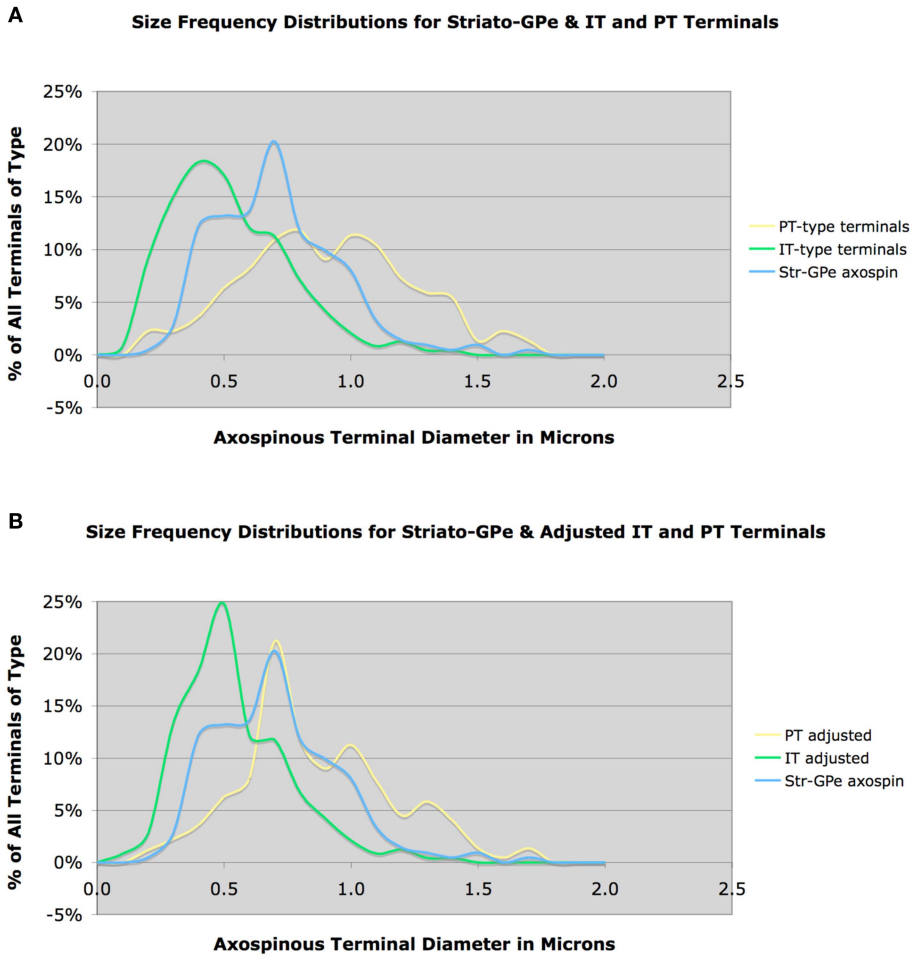

FIGURE 7 | Graph (A) shows the size frequency distributions for IT-type and PT-type axospinous terminals compared to that for striato-GPe neurons, while graph **(B)** shows the size frequency distributions for axospinous terminals on striato-GPe neurons compared to the VGLUT1-adjusted PT-type size frequency distribution shown in **Figure 3B (B).** Note that neither IT-type nor PT-type size distributions precisely match that for striato-GPe neurons in graph **(A)**. The adjusted PT-type distribution does show a better fit, but still does not account for the abundance of terminals <0.7 μm. This suggests that striato-GPe neurons receive a combination of IT- type and PT-type input, and the PT-type input predominates.

Similar analysis of axospinous terminals on striatonigral neurons in rhesus monkey indicated that mean axospinous terminal size on striatonigral direct pathway neurons was smaller than that on indirect pathway striato-GPe neuron spines (**Figure 8A**). The mean size for 81 axospinous terminals on striatonigral neurons from one rhesus monkey was 1.01 μm, while for 118 striato-GPe axospinous terminals from two monkeys was 1.39 μm. Given the size frequency distributions for IT-type and PT-type terminals evidenced in our VGLUT1 analysis, we could reach conclusions regarding the pattern of IT-type and PT-type inputs to these two striatal neuron types. The large striatonigral axospinous terminal peak at about 0.8 μm clearly corresponds to the IT-type terminal peak, and indicates that IT-type input to this neuron type predominates over PT-type input. The relative thalamic versus PT-type input to this neuron type is uncertain, in part because the size frequency distribution of thalamic input is unknown for rhesus monkey.

Nonetheless, the peak at about 1.1 μm is likely to represent thalamic input, since the two major PT-type peaks are greater than 1.1 μm. An approximately 60:30 ratio of IT-type and thalamic input seems likely for striatonigral neurons in rhesus monkey, with the small peak at 1.5 μm perhaps reflecting some PT-type input. Whether striato-GPi neurons are similar to striato-SNr neurons is yet uncertain. In the case of the striato-GPe neuron input, except for the peak at 1.1 μm, the size frequency distribution for striato-GPe axospinous terminals is closely matched by the PT-type terminal distribution inferred from the VGLUT1 data (**Figure 8B**). Thus, a 70:30 ratio of PT-type input and some combination of thalamic and IT-type input to striato-GPe neurons appears reflected in these distributions.

To directly assess the cortical input to the two striatal projection neuron types, we combined BDA-labeling of IT-type or PT-type terminals with D1 or D2 immunolabeling in rats (Lei et al., 2004). We found that of all axospinous IT-type synaptic terminals labeled with BDA10k in tissue immunolabeled as well for D1, 50.9% made synaptic contact with D1+ spines. By contrast, of all axospinous IT-type synaptic terminals labeled with BDA10k in tissue immunolabeled for D2, only 12.6% made synaptic contact with D2+ spines. Double-labeling for PT-type terminals and D1+ (striato-GPi/SNr) or D2+

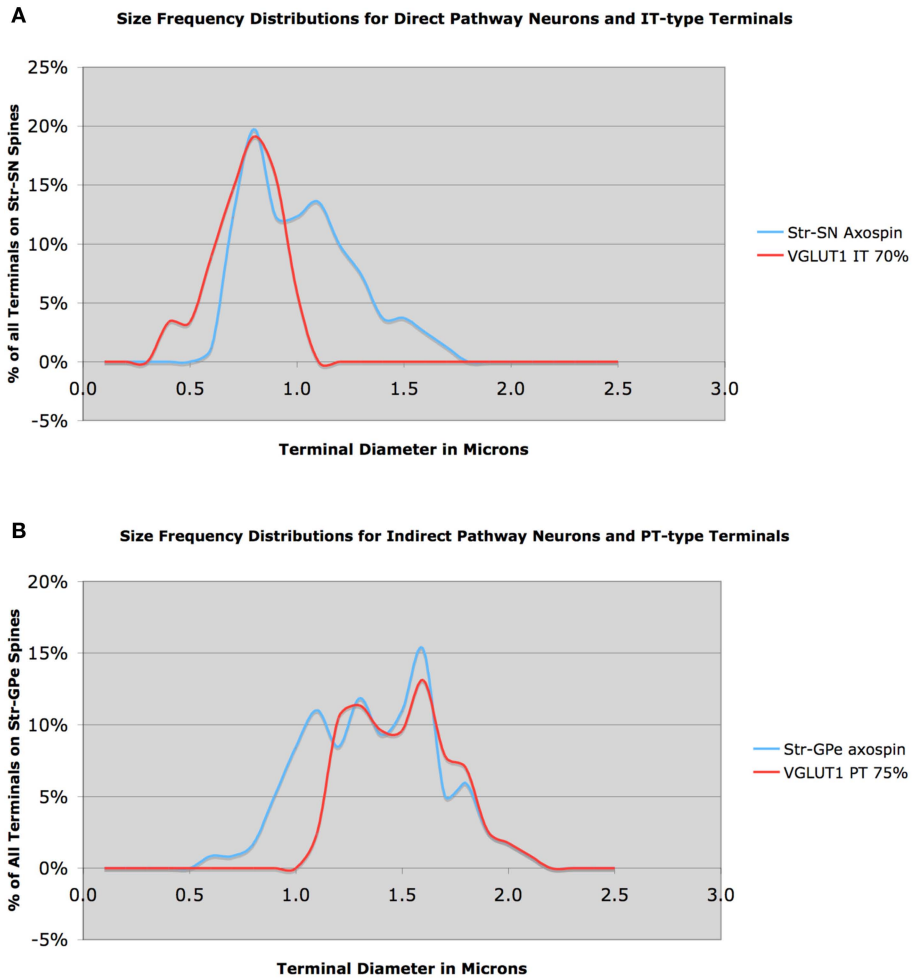

FIGURE 8 | Size frequency distribution of IT-type corticostriatal axospinous terminals in rhesus monkey compared to the size frequency distributions of axospinous terminals on striato-SNr neurons (A), and size frequency distribution of PT-type corticostriatal neurons compared to the size frequency distribution of axospinous terminals on striato-GPe neurons **(B).** Note that the size distribution of IT-type terminals closely matches that of terminals on striato-SNr neurons, except for a peak at 1.1 μm. Similarly, note that the size distribution of axospinous PT-type terminals closely matches that of axospinous terminals on striato-GPe neurons, except for a peak at 1.1 μm. The peak at 1.1 μm is likely to include thalamic terminals.

(striato-GPe) spines showed the opposite trend – of all axospinous PT-type synaptic terminals labeled with BDA3k in tissue immunolabeled as well for D1, only 21.3% synaptically contacted D1+ spines, while of all axospinous PT-type synaptic terminals labeled with BDA3k in tissue immunolabeled for D2, 50.5% synaptically contacted D2+ spines. Thus, these differences as well, which are statistically significant, show that IT-type terminals preferentially contact D1+ spines whereas PT-type terminals preferentially contact D2+ spines. Note, however, that these results for direct pathway neurons suggest a greater PT-type input to direct pathway neuron spines than indicated by comparisons of size frequency distributions for the different terminal types in **Figure 6**. Since the size frequency data clearly indicate meager PT-type input to direct pathway spines, it may be that our double-labeling resulted in some intensification of D1 immunolabeling of otherwise weakly D1+ indirect pathway neurons. The presence of IT-type terminals on D2+ spines indicated by our double-label studies is, however, consistent with our comparisons of size frequency distributions for the different terminal types in **Figure 7**. Nonetheless, some D2+ neurons contacted by IT-terminals in our double-label study may be direct pathway neurons with D2 receptors (Deng et al., 2006). GENSAT mice with labeling restricted to direct versus indirect pathway neurons may be ideal for resolving these uncertainties about the relative extent of IT-type versus PT-type input to the two striatal projection neuron types (Gong et al., 2007). In preliminary studies, we have found that mean axospinous synaptic terminal size on D2+ spines in D2 GENSAT mice is greater (0.61 μm) than on D2-negative spines (0.42 μm).

PROJECTION NEURONS OF STRIOSOMES

One study has used BDA anterograde labeling to examine the input of motor and cingulate cortex to striosomes as identified by mu opiate receptor (MOR) immunolabeling in rats (Wang and Pickel, 1998). They found that the BDA-labeled corticostriatal terminals ending on MOR+ spines tended to be large, and

many exhibited a perforated postsynaptic density. Based on our measurements of the terminals shown in that study, the mean size of nine terminals making axospinous synaptic contact on MOR+ spines (thus establishing them as within striosomes) was 0.88 μm and a third of these possessed perforated PSDs. These results are similar to those we have found for BDA-labeled PT-type terminals in the matrix compartment of dorsolateral striatum, and thus the results of Wang and Pickel (1998) are consistent with the view that striosomes are innervated by PT-type input ending as large axospinous terminals.

PHYSIOLOGICAL EVIDENCE FOR DICHOTOMOUS CORTICAL PROJECTIONS TO STRIATAL PROJECTION NEURONS

Our findings that IT-type terminals preferentially target direct pathway neurons and PT-type terminals preferentially target indirect pathway neurons are consistent with several electrophysiological studies. For example, indirect pathway neurons have a lower paired-pulse ratio and a higher mEPSC frequency than do direct pathway neurons in mouse striatum (Kreitzer and Malenka, 2007; Cepeda et al., 2008; Ding et al., 2008). These results suggest that excitatory synapses on indirect pathway neurons have a higher probability of transmitter release than do those on direct pathway neurons, consistent with the larger cortical terminals on the indirect pathway neurons. The larger terminals also may explain, in part, why indirect pathway striatal neurons have higher basal firing rates (Mallet et al., 2006). Cepeda et al. (2008) reported several additional findings they noted as consistent with our observation that indirect pathway neurons preferentially receive PT-type terminals and direct pathway neurons the smaller IT-type terminals: (1) D2+ but not D1+ neurons displayed prominent inward currents and large, long-lasting depolarization with increased cortical firing induced by bath application of GABAA antagonist; and (2) direct electrical activation of cortical input more readily elicited D2+ neuron responses than D1+ neuron responses at low stimulating current intensities. Our results are also consistent with the finding that activation of cortex *in vivo* tends to preferentially induce immediate early gene expression in ENK+ striatal neurons (Berretta et al., 1997; Parthasarathy and Graybiel, 1997), and diminished cortical activation of striatum preferentially reduces ENK expression (Uhl et al., 1988). Nonetheless, it should be emphasized that striatal projection neuron firing rate and cortical responsivity are also affected by intrinsic membrane properties and local circuit connections.

PHYSIOLOGICAL EVIDENCE AGAINST DICHOTOMOUS CORTICAL PROJECTIONS TO STRIATAL PROJECTION NEURONS?

One study reported evidence they viewed as rebutting the notion that PT-type input ends preferentially on indirect pathway type striatal neurons (Ballion et al., 2008). In one line of study, they found that the earliest ipsilateral spikes in response to the second cortical pulse in a 100-ms pair were similar in latency for the two striatal projection neuron types (distinguished by antidromic activation from nigra). In a second approach, they found that the two striatal projection neuron types responded equally commonly to the second pulse in a 100-ms pair delivered to contralateral cortex. Since neither outcome matched their simple prediction for a dichotomous projection of IT-type and PT-type neurons to striatum from Lei et al. (2004), Ballion et al. (2008) concluded that IT-type input did not preferentially end on direct pathway neurons but rather ended equally on both direct and indirect pathway neurons, and PT-type input was meager to indirect pathway neurons as well as to direct pathway neurons. Note, however, the prediction being tested is overly strong given the data of Lei et al. (2004). That study indicated that striato-GPe neurons receive significant IT-type input, as does our more recent size frequency curve-fitting approach presented here. Thus, while it is valuable to demonstrate that striato-GPe neurons can spike in response to IT-type input, the findings of Ballion et al. (2008) are not inconsistent with the idea that IT-type input preferentially targets striato-GPI/SNr neurons and PT-type input preferentially targets striato-GPe neurons. It should be noted that in a prior study in which they assessed direct and indirect pathway striatal neuron responses to the first of a 100-ms pair of stimulus pulses to ipsilateral cortex, indirect pathway striatal neurons responded significantly more rapidly than did direct pathway striatal neurons (Mallet et al., 2006), thus suggesting that PT input does preferentially target indirect pathway striatal neurons.

The work of Ballion et al. (2008) and Mallet et al. (2006), however, raises the issue of the relative abundance of the IT-type and PT-type inputs to the striatum. The data of Jinnai and Matsuda (1979) suggests that about 40% of the overall cortical input to dorsolateral striatum from motor and sensory cortex is PT-type. This interpretation is consistent with our VGLUT1 immunolabeling for rats noted above. Given their preferential input to indirect pathway neurons, a lesser PT-type input to striatum in rodents would be consistent with the sparser dendritic trees of indirect pathway type striatal projection neurons (Gertler et al., 2008). More detailed studies are needed to determine if this is also the case in primates. PT-type neurons projecting to the pons are present, however, throughout cortex, although the extent of their overall projection to striatum and the nature of the signal that they convey from non-motor or non-somatosensory cortices to striatum is uncertain. Genes have been identified that are uniquely expressed by either PT-type or IT-type neurons, and mice have been engineered that express green fluorescent protein (EGFP) in one or the other of these neuron types (Gong et al., 2007; Molyneaux et al., 2007). Such mice will be useful for assessing the relative abundances of the PT and IT inputs to the entire striatum.

FUNCTIONAL CONSIDERATIONS

The finding of a differential cortical input to striatal projection neurons may have implications for understanding how the cortical input contributes to the role of the direct and indirect pathway striatal projection neurons in motor control (**Figure 9**). In the case of direct pathway striatal neurons, convergence of IT-type input from diverse cortical areas providing information on movement planning, body position and the environment, and reward-prediction-related information from dopaminergic midbrain neurons onto individual striato-GPi/SNr neurons (Wilson, 1987; Cowan and Wilson, 1994; Zheng and Wilson, 2001), may provide the coherent input required to activate individual direct pathway neurons so that they facilitate movement. Because they are inherently less excitable and because their IT-type inputs are relatively ineffective at producing postsynaptic depolarization, more temporally correlated activation may be needed for direct pathway neurons than

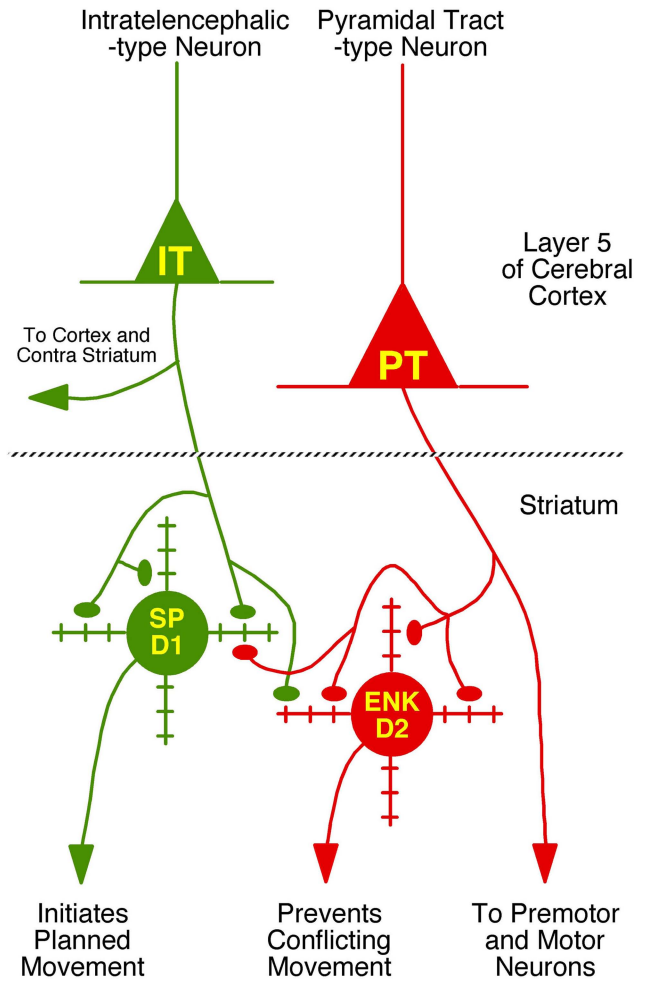

FIGURE 9 | Schematic illustration of the differential projections of IT-type and PT-type cortical neurons to the two main types of striatal projection neurons – the direct pathway type containing substance P (SP) and possessing D1-type receptors and the indirect pathway type containing enkephalin (ENK) and possessing D2-type dopamine receptors. The major functions and/or projection targets of each of the two main types of cortical pyramidal and striatal neurons is indicated. The degree of selectivity of IT-type input for direct pathway neurons, and PT-type input for indirect pathway neurons, as well as the extent of thalamic input to these two striatal neuron types is not yet known. Moreover, in our analysis, we have only considered axospinous input, and the axodendritic input to these neuron types from these three sources also needs to be determined.

indirect pathway neurons and suited to the role of direct pathway neurons in motor sequence selection and initiation. Thalamic input related to attentional mechanisms may provide further excitatory drive needed to push the direct pathway neuron activation over the threshold for motor initiation (Smith et al., 2004).

Our findings also raise the possibility that striato-GPe neurons use an efference copy of movement commands provided by the PT-type input, which enables their role in suppressing movements that would otherwise conflict with ongoing selected movements. The somewhat more rapid conduction velocity of the PT-type input to striatum seems suited to such a role. The preferential PT-type input to striato-GPe neurons might also explain why movement-related activity exhibited by striatal projection neurons typically occurs during but not before movement (Jaeger et al., 1995; Mink, 1996) – most of the active striatal neurons are indirect pathway type neurons responding to collaterals of cortical pyramidal neuron axons. Nonetheless, the PT signal will reach premotor and motor neurons before the PT feedback signal reaches motor cortex via the striato-GPe-STN-GPi-motor thalamus loop, and thus be too late to prevent movements conflicting with the already initiated movement. This implies that the movement suppression caused by the PT signal to striato-GPe neurons may serve to suppress movements that would conflict with the next desired movement in the action sequence. The topographic organization of the PT-type input from somatosensory and somatomotor cortex to dorsolateral striatum may facilitate this role. Graybiel (2005) has also suggested the possibility that the PT-type input to ENK+ neurons may serve to terminate a specific act in the sequence initiated by SP+ neurons.

BASAL GANGLIA, MOTOR LEARNING AND THE DIFFERENTIAL CORTICAL INPUT TO STRIATUM

Motor learning is a key part of the role of the basal ganglia (Graybiel, 2005). Considerable evidence supports the view that dopamine released from the intrastriatal terminals of substantia nigra both acts as a reward signal that sculpts the activity of striatal neurons during motor learning (Schultz et al., 2003; Graybiel, 2005), and instructs striatal neurons on the likelihood that a given circumstance can lead to reward (Ljungberg et al., 1992; Satoh et al., 2003; Morris et al., 2004; Tobler et al., 2005). The means by which motor learning occurs appears to be, in large part, changes in the efficacy of cortical synapses on striatal projection neurons. For example, to facilitate the onset of a specific motor routine, the efficacy of the cortical input to direct pathway neurons controlling that onset must be increased while the efficacy of the cortical input to the indirect pathway neurons suppressing that same routine must be reduced. Similarly, for those movements potentially conflicting with the desired routine, the efficacy of the cortical input to direct pathway neurons controlling the onset of such competing routines must be decreased, while the efficacy of the cortical input to the indirect pathway neurons suppressing those competing routines must be enhanced. The facts that D1-dependent LTP has been demonstrated in direct pathway neurons and D1 receptors are preferentially localized to direct pathway neurons suggest that the rewarding effects of dopamine on behavior are mediated via facilitation of IT-type inputs to direct pathway striatal neurons that control behaviors that obtain the reward (Kreitzer and Malenka, 2008; Shen et al., 2008). In this manner, the coincident activation of the convergent cortical inputs to the direct neurons mediating the rewarded behavior becomes more able to fire those neurons. This phenomenon may explain the emergence of striatal activity in response to a go cue during procedural learning (Ljungberg et al., 1992; Satoh et al., 2003; Morris et al., 2004; Tobler et al., 2005). The striatal activity in this sense reflects a motor go cue when the combination of exteroceptive and interoceptive circumstances are appropriate. The observation that dopamine depletion converts LTP to LTD in direct pathway neurons is consistent with the notion that absence of a dopaminergic reward signal to the IT-type inputs projecting to those striatal neurons initiating the unrewarded behaviors makes those synapses less likely to initiate the unrewarded response (Kreitzer and Malenka, 2008).

If the indirect pathway neurons function to serve as a stop feedback signal to the PT-type neurons that provide them an efference copy of their discharge to motor or premotor neurons, then plasticity at the PT-type synapse would serve to modulate the stop signal. In the case of desirable behaviors, the stop signal might be inappropriate. Reward-mediated depression of the PT-type input would then help adjust the pyramidal neuron control of the movement so its duration is better suited to achieve a rewarded outcome. Conversely, PT-type neuronal activity that brings about unrewarded behaviors would send a corollary discharge to indirect pathway neurons that becomes strengthened by the absence of dopamine reward, leading to a heightened tendency of the indirect pathway neurons to suppress these same PT-type cortical neurons, and reduce likelihood of the occurrence of the unsuccessful behavior. Because of the efficacy of this synaptic contact and the high responsivity of indirect pathway neurons to their cortical input, basal firing of the PT input that is subthreshold for movement may be adequate to maintain sufficient indirect pathway neuron output to keep pyramidal tract neuron firing below movement threshold. This suggests that motor learning in basal ganglia may involve learning which movements to suppress and which not to suppress, and the PT-type input to striato-GPe neurons may thus be an important neural substrate by which the basal ganglia learns to refine motor sequences during procedural learning.

Loss of basal dopamine levels due to dopaminergic neuron degeneration in Parkinson's disease (PD) results in loss of basal dopamine inhibition of indirect pathway neurons and basal dopamine excitation of direct pathway neurons (Kreitzer and Malenka, 2008). As a consequence, the basal ganglia output is abnormal and yields increased movement suppression and decreased movement initiation. The loss of striatal dopamine, however, impairs corticostriatal plasticity as well. LTD at the PT-type inputs to indirect pathway neurons may be diminished, further contributing to the excess inhibition of movement in PD. Similarly, loss of dopamine may impair LTP at IT-type input to direct pathway neurons, and impair the corticostriatal facilitation that underlies new motor learning. Thus, impairments in both learning and carrying out motor routines may be impaired in PD (Arbuthnott and Wickens, 2006).

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Vulnerability of mesostriatal dopaminergic neurons in Parkinson's disease

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The term vulnerability was first associated with the midbrain dopaminergic neurons 85 years ago, before they were identified as monoaminergic neurons, when Foix and Nicolesco (1925) reported the loss of neuromelanin containing neurons in the midbrain of patients with post-encephalitic Parkinson's disease (PD). A few years later, Hassler (1938) showed that degeneration is more intense in the ventral tier of the substantia nigra compacta than in its dorsal tier and the ventral tegmental area (VTA), outlining the concept of differential vulnerability of midbrain dopaminergic (DA-) neurons. Nowadays, we know that other neuronal groups degenerate in PD, but the massive loss of nigral DA-cells is its pathological hallmark, having a pivotal position in the pathophysiology of the disease as it is responsible for the motor symptoms. Data from humans as well as cellular and animal models indicate that DA-cell degeneration is a complex process, probably precipitated by the convergence of different risk factors, mediated by oxidative stress, and involving pathogenic factors arising within the DA-neuron (intrinsic factors), and from its environment and distant interconnected brain regions (extrinsic factors). In light of current data, intrinsic factors seem to be preferentially involved in the first steps of the degenerative process, and extrinsic factors in its progression. A controversial issue is the relative weight of the impairment of common cell functions, such as energy metabolism and proteostasis, and specific dopaminergic functions, such as pacemaking activity and DA handling, in the pathogenesis of DA-cell degeneration. Here we will review the current knowledge about the relevance of these factors at the beginning and during the progression of PD, and in the differential vulnerability of midbrain DA-cells.

Keywords: Parkinson's disease, neurodegeneration, aging, differential vulnerability, nigrostriatal, mesolimbic, mitochondrion, proteostasis

INTRODUCTION

The level of interest that mesostriatal dopaminergic (DA-) neurons have attained within the brain motor circuits in the last 30 years is mostly because of the relevance of this cell group in the pathophysiology of Parkinson's disease (PD). PD is a neurodegenerative disorder characterized by neuronal loss and the presence of intraneuronal inclusions known as Lewy bodies (LBs) in different brain centers (Braak et al., 2003), which in its sporadic form affects more than 1% of people over the age of 60 (Nussbaum and Ellis, 2003; de Lau and Breteler, 2006). Besides midbrain DA-cells, this neuronal loss affects the locus coeruleus, dorsal nucleus of the vagus, hypothalamus, nucleus basalis of Meynert, olfactory bulb, and the peripheral and enteric systems, although they do not all display the same susceptibility to degeneration and clinical impact (Pearce et al., 1995; Forno, 1996). Post-mortem studies show that at the end-stage of the disease, the cell loss in non-dopaminergic nuclei ranges between 30 and 50%, while in the SN it reaches 80% (Victorof et al., 1996; Zarow et al., 2003; Thannickal et al., 2007), with most DA-cells dying during the preclinical period (Greffard et al., 2006). So, the massive loss of SN DA-cells is considered the hallmark of PD, and responsible for most, if not all, motor symptoms characterizing parkinsonian patients (Hornykiewicz, 2006). Nowadays, basic research is mainly focused on elucidating the cause and cellular mechanisms underlying DA-cell degeneration. Despite these efforts, the etiology and pathogenesis of PD remain unclear. Current data point to it being more of a heterogeneous entity, arising from different causes and pathogenic mechanisms, and converging in a relatively uniform phenotype. A number of studies support the relevance of the impairment of common cellular functions, such as mitochondrial metabolism and protein degradation, and pathogenic mechanisms, such as inflammation and excitotoxicity, while others highlight the role of metabolic and functional aspects specific to DA-cells in the degenerative process. However, the relative weight of each of these factors, and the reason why midbrain DA-cells are particularly susceptible remain unknown. Our knowledge is based on data from different sources, including patients with familial forms of PD, whose clinical and pathological features in many cases differ from those in sporadic PD, and from animal and cellular models based on the manipulation of cellular functions probably involved in the pathogenesis of PD. The aim of this review is to summarize and discuss the current data in favor of and against the relevance of these factors in the sequence of events leading to DA-cell degeneration.

COMMON CELLULAR FACTORS AND PATHOGENIC MECHANISMS

MITOCHONDRIAL DYSFUNCTION

Mitochondria are organelles where a number of important cellular functions occur, including oxidative energy metabolism, calcium homeostasis, and control of cell death (Kroemer and Blomgren, 2007; Celsi et al., 2009; Galluzzi et al., 2009). Furthermore, deficient mitochondrial metabolism may generate reactive oxygen species with catastrophic consequences for the cell. These facts make mitochondrial dysfunction an attractive candidate for playing a key role in neurodegeneration and aging.

The relationship between mitochondrial dysfunction and DA-cell degeneration was reported for the first time by Schapira et al. (1990) who found reduced activity of mitochondrial complex I (NADH-quinone oxidoereductase) in the substantia nigra of parkinsonian patients. The enzymatic deficiency was later confirmed in the cerebral cortex (Keeney et al., 2006; Parker et al., 2008), and in blood platelets (Krige et al., 1992; Haas et al., 1995; Schapira, 2008), suggesting that a number of patients can undergo a systemic decrease in mitochondrial complex I activity. In addition, cells transfected with mitochondrial DNA from platelets of PD patients show complex I deficiency and other features of PD brains (Swerdlow et al., 1996; Borland et al., 2009).

The involvement of mitochondrial dysfunction was also supported by the finding in the early 80s of an acute and persistent parkinsonism in humans after intravenous injection of an illicit drug which contained 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) (Langston et al., 1983). MPTP is converted by the monoamine oxidase enzyme of glial cells into its active metabolite MPP^+ and taken up by DA-cells through the dopamine transporter (DAT). Once inside the cell, MPP^+ can exert its neurotoxic effect by means of different mechanisms, including the inhibition of the mitochondrial complex I (Nicklas et al., 1985; Di Monte et al., 1986a; Mizuno et al., 1988). Taking advantage of this effect, MPTP has been widely used for inducing models of PD (DA-cell degeneration) in mice and monkeys (Przedborski and Jackson-Lewis, 1998; Blum et al., 2001).

The structural similarity between MPP^+ and the herbicide paraquat raised the possibility of a relationship between exposure to environmental toxins, impairment of oxidative phosphorylation and PD. This idea was supported by epidemiological studies reporting a positive association between the incidence of PD and exposure to pesticides (Semchuk et al., 1992; Liou et al., 1997; Dhillon et al., 2008), although others could not confirm such a relationship (Firestone et al., 2005). Experimental data show that paraquat, subchronically administrated in mice, induces midbrain DA-cell loss (McCormack et al., 2002) and α-synuclein accumulation (Manning-Bog et al., 2003), but its cytotoxic effect is due to the production of reactive oxygen species at cytosolic level, with potential indirect effects on mitochondria, but not to the direct inhibition of the mitochondrial complex I (Di Monte et al., 1986b; Miller, 2007). Rotenone is another pesticide widely used to induce an animal model of PD (Betarbet et al., 2000; Bove et al., 2005). Its adequacy to reproduce PD features has been a matter of controversy (Cicchetti et al., 2009). Some authors argue a high variability in the lesion pattern, including brain regions unaffected in PD as well as the liver and gastrointestinal tract, suggesting that it acts as a systemic unspecific toxin (Hoglinger et al., 2003; Lapointe et al., 2004). In contrast, others maintain that, by using the appropriate administration schedule, rotenone only produces PD specific brain lesions, including dopaminergic degeneration, alpha-synuclein-positive inclusions, and impairment of the ubiquitin–proteasome system (UPS) (Sherer et al., 2003; Cannon et al., 2009). Because of its high lipophilicy, rotenone can cross all cell membranes and accumulates in cellular organelles. Its main cytotoxic effect comes from the interaction with the ND1 and PSST subunits of the mitochondrial complex I, resulting in the loss of its enzymatic activity (Schuler and Casida, 2001). Interestingly, although the complex I impairment is evident at the systemic level, degeneration only affects nigrostriatal neurons (Betarbet et al., 2000), supporting the idea that a systemic mitochondrial dysfunction together with the special susceptibility of midbrain DA-cells may be the origin of DA-cell degeneration in sporadic PD.

Convincing evidence for the involvement of mitochondrial dysfunction in the pathogenesis of PD also arises from the discovery of mutations in genes which encode the Parkin, PINK1, DJ-1, α-synuclein, and LRRK2 proteins.

Mutations in the Parkin gene (PARK2) were first associated with PD in a Japanese family (Kitada et al., 1998). Today, they are recognized as the most frequent cause of juvenile-onset parkinsonism, although later-onset cases have also been reported (Lohmann et al., 2003). Parkin is a ubiquitously expressed protein, present in different subcellular fractions (Shimura et al., 1999), with several domains for protein–protein interactions and E3 ubiquitin protein ligase activity (Zhang et al., 2000). Parkin-associated endothelin-receptor (Pael-R) is one of the substrates of parkin ligase activity which has been shown to be accumulated in the brain of patients with PARK2 mutations. When this receptor is overexpressed in cells it tends to become unfolded and insoluble, and induces cell death (Imai et al., 2001). Parkin has also been recognized as a mitochondrial protection factor. Its overexpression in cells prevents the mitochondrial swelling induced by ceramide (Darios et al., 2003), upregulates the expression of complex I subunits and reduces the accumulation of reactive oxygen species in mitochondria (Kuroda et al., 2006). While its deficiency leads to a decrease in the levels of complex I and IV subunits and mitochondrial respiratory capacity in mice (Palacino et al., 2004).

PTEN-induced putative kinase 1 (PINK1) is a serine/threonine kinase with autophosphorylation capacity which is transcribed in the nucleus, and imported to the mitochondria (Silvestri et al., 2005). Its mutations are associated with hereditary early-onset PD, although heterozygous mutations have also been found in sporadic early-onset forms (Valente et al., 2004). PINK1 regulates the activity and phosphorylation of TRAP1 (TNF-receptor-associated protein 1), a protein which acts as a chaperone to prevent protein misfolding (Pridgeon et al., 2007), HtrA2/Omi, which acts as a protease involved in the degradation of misfolded proteins (Alnemri, 2007), and Parkin. PINK1 regulates the mitochondrial localization of Parkin through its phosphorylation (Kim et al., 2008). Neurons from PINK1 knockout mice develop mitochondrial dysfunction, including reduced membrane potential, increased production of oxygen radicals and sensitivity to apoptosis (Wood-Kaczmar et al., 2008). Furthermore, the striatum of PINK1 knockout mice shows an increase in the number of large mitochondria and impairment

of complex I and II activity (Gautier et al., 2008). So, mitochondrial dysfunction induced by PINK1 mutations may in part be due to defects in Parkin functions.

DJ-1 is a 189 amino acid/20 kDa multifunctional protein with antioxidant and transcription modulatory activity (Takahashi et al., 2001; Taira et al., 2004). Under normal conditions, DJ-1 preferentially localizes in the cytosol, but under conditions of oxidative stress it translocates to the mitochondria and nucleus (Zhang et al., 2005). This redistribution ability has been related to its neuroprotective capacity (Canet-Aviles et al., 2004; Ashley et al., 2009). Mutations in its encoding gene (PARK7) are a rare cause of recessively inherited PD (Bonifati et al., 2003). DJ-1 deficient mice show motor abnormalities and changes in DA handing, but not spontaneous DA-cell degeneration (Goldberg et al., 2005). However, they become more susceptible to MPTP than their wild-type congeners (Kim et al., 2005).

Alpha-synuclein is a presynaptic protein whose relevance in PD comes from the fact that it is present in LBs, and because mutations and triplications of its gene locus (PARK1) are a cause of autosomal dominant PD (Polymeropoulos et al., 1997; Singleton et al., 2003). The mechanism by which α-synuclein exerts its toxicity has been related to the tendency of the mutated and overexpressed protein to aggregate in form of fibrils (Conway et al., 2000; Fredenburg et al., 2007). Oligomeric intermediates of the α-synuclein fibrillation pathway, called protofibrils, can form annular pores in cell membranes, including those of mitochondria, leading to their permeabilization. This phenomenon may be enhanced in DA-cells because cytosolic DA interacts with α-synuclein slowing the conversion of protofibrils to fibrils (Conway et al., 2000; Rochet et al., 2004). Overexpression of mutant and wild-type α-synuclein can induce damage at different cell levels, including proteasome, endoplasmic reticulum, and mitochondria (Hsu et al., 2000). Mitochondrial impairment includes oxidation of mitochondria-associated proteins (Poon et al., 2005), mitochondrial DNA damage and reduced complex IV activity (Martin et al., 2006). In this respect, higher α-synuclein levels have been found in mitochondria from substantia nigra of PD patients than in those from healthy individuals (Devi et al., 2008).

Leucine-rich repeat kinase 2 (LRRK2) is a widely distributed protein with a multidomain structure which includes a serine/threonine kinase domain (Giasson et al., 2006). Mutations in the gene encoding LRRK2 (PARK8) are a common cause of autosomal dominant PD, and are also present in about 1% of patients with sporadic PD (Paisan-Ruiz et al., 2004; Di Fonzo et al., 2005). The toxicity of several of these mutations appears to be associated with an increase in its kinase activity (Gloeckner et al., 2006). Although the involvement of mitochondria in the pathogenesis of LRRK2 mutant-associated PD remains unclear, the facts that LRRK2 partially colocalizes with mitochondrial markers (Biskup et al., 2006), and that overexpression of mutant LRRK2 proteins induces Apaf1 dependent apoptosis (Iaccarino et al., 2007), suggest that LRRK2 mutations can compromise mitochondrial function.

In sum, a large body of evidence supports the idea that mitochondrial dysfunction plays a key role in the pathogenesis of DA-cell degeneration. However, this idea is challenged by certain facts. For example, diseases associated with mitochondrial DNA mutations are typically multisystemic, involving heart, skeletal muscle, and brain, but not specifically DA-cells. Furthermore, DA-cell degeneration is not relevant in most neurodegenerative diseases associated with mutations in the nuclear genes which encode proteins that are targeted to mitochondria (Schon and Manfredi, 2003). Thus, although mitochondria impairment may be involved in the degenerative process in many cases of PD, other factors must contribute to making DA-cells particularly vulnerable in this disorder.

PROTEIN HOMEOSTASIS

Cells control the quantity and quality of intracellular proteins through the balance between the rate of their synthesis, maturation and degradation (Shah and Di Napoli, 2007). Folding is part of this process converting newly synthesized proteins into functional molecules. The endoplasmic reticulum contains most of the machinery responsible for maintaining an appropriate protein folding through a coordinated signaling program known as unfolded protein response (UPR) (Hosoi and Ozawa, 2009). The response of the endoplasmic reticulum includes translational attenuation, induction of chaperones, and degradation of misfolded proteins. The two principal pathways of intracellular protein degradation are the UPS, responsible for the bulk of the turnover of short-lived cytosolic proteins, and the autophagy–lysosome pathway (ALP), involved in the degradation of long-lived stable proteins as well as in the recycling of organelles (Jellinger, 2009). The capacity of this proteostasis network is enough to maintain protein homeostasis under normal conditions and under short-term stressful circumstances. However, this seems to be overwhelmed by chronic stress as probably occurs in aging, cancer, or metabolic and neurodegenerative diseases. In these cases, proteins lose their normal configuration and activity, and become misfolded with a propensity to aggregate. The structural instability of some mutated or postranslationally modified proteins makes them particularly inclined to aggregate to each other as well as to other normal proteins. These protein aggregates may interfere with critical intracellular processes including UPR function, and induce cytotoxicity.

A number of findings indicate that these cellular functions are compromised in DA-cells in PD. For example, proteinaceous aggregations, containing ubiquitin, and α-synuclein and known as Lewy bodies (LBs), are abundant in the SN and other brain centers affected by degeneration in sporadic PD (Braak et al., 2003). The enzymatic activity and expression of some α-subunits of the proteasome and the proteasomal activator PA700 have been found to be low in the SN of PD patients, while in unaffected brain regions they are high, suggesting that neurons in these regions, but not those in the SN, can activate compensatory responses against the proteolytic stress (McNaught et al., 2001, 2003). Even in healthy subjects, the levels of the proteasome activator PA28 in the SN pars compacta (SNc) have been reported to be lower than in other brain regions (McNaught et al., 2002a, 2003). In addition, a recent immunohistochemistry study showed that under normal conditions, PA28 and PA700 expression are very low in the rat SNc, and that different stressful stimuli induce PA28/PA700 overexpression in a variety of cells but not in midbrain DA-neurons (McNaught et al., 2010). Therefore, it is possible that the UPS is constitutively weak in midbrain DA-cells, contributing to their particular vulnerability in sporadic PD.

The relevance of proteolytic stress is also supported by data arising from familial PD. As mentioned above, although the mechanism by which Parkin mutations induce degeneration is not well known, it has been mainly related to a defect in its ubiquitin ligase activity. Parkin interacts with the 26S proteosome through its ubiquitin ligase domain facilitating the recognition of ubiquitinated substrates by the proteasomal activator PA700 (Pickart, 2004). Parkin acts in conjunction with other enzymes to ubiquitinate a variety of substrates, such as Pael-R, which have been found to be accumulated and non-ubiquinated in LBs (Murakami et al., 2004). In addition, Parkin prevents cell death induced by overexpression of Pael-R (Imai et al., 2001). Therefore, Parkin mutations could impair ubiquitination and degradation of substrate proteins which might be aggregated and cause cytotoxicity.

Ubiquitin C-terminal hydrolase L1 (UCH-L1) is a 230 amino acid/26 kDa protein only expressed in neurons, which acts as a deubiquinating enzyme removing ubiquitin from protein adducts before their entry in the proteosome (Leroy et al., 1998). A missense mutation in the UCH-L1 gene (PARK5) has been found in two German siblings with PD, and is considered today to be a rare cause of familiar PD (Olanow and McNaught, 2006). It has been reported that the *in vitro* deubiquinating activity of mutated UCH-L1 form is low (Nishikawa et al., 2003), and that inhibition of the ubiquitin hydrolase activity leads to the formation of LB-like inclusions and DA-cell degeneration in cell cultures (McNaught et al., 2002b). So, it is possible that, in these patients, deubiquitination failure blocks the proteasomal processing of proteins, leads to the formation of aggregates and interferes with the supply of ubiquitin monomers to additional unwanted proteins (Olanow and McNaught, 2006).

The protective role of DJ-1 has been mostly associated to its ability to translocate to the mitochondria and nucleus where it can exert antioxidant and transcriptional modulatory actions (Zhang et al., 2005). However, its molecular structure and *in vitro* properties indicate that it can also work as a molecular chaperone and protease (Abou-Sleiman et al., 2003; Lee et al., 2003). In addition, such proteolytic activity is lost when it is mutated (Olzmann et al., 2004; Shendelman et al., 2004). So, mutations in DJ-1 can compromise UPS function and promote protein aggregation.

Under physiological conditions, α-synuclein is monomeric and unfolded, and preferentially degraded by the proteasome in an ubiquitin-independent manner (Bennett et al., 1999). But in high concentration or as a mutated form, it resists proteasomal degradation (Stefanis et al., 2001; Tanaka et al., 2001), becomes misfolded and oligomeric, and tends to self-aggregate and aggregate to other proteins (Bennett, 2005). As mentioned above, the toxicity of α-synuclein has been related to the ability of α-synuclein intermediate aggregates to form ring-like pores in cellular membranes, increasing their permeability (Lashuel et al., 2002). *In vitro* studies reveal that α-synuclein aggregates can also bind to the proteasomal protein S6', a subunit of the 19S cap of the 26S proteasome, resulting in the inhibition of its function (Snyder et al., 2003).

On the other hand, similar to other protein complexes and membrane proteins too large in size to pass through the proteasome barrel, α-synuclein oligomers and aggregates can also be cleared by the ALP (Pan et al., 2008). The principal pathways for α-synuclein clearance through lysosomes are the macroautophagy, which is also involved in the recycling of damaged organelles, and the chaperone-mediated autophagy (CMA) (Cuervo et al., 2004). In CMA, α-synuclein couples to the chaperone Hsc70 forming a protein–molecular chaperone complex that binds to the lysosomal membrane (Mak et al., 2010). The A30P and A53T α-synuclein mutants, which are associated with familial PD, remain firmly attached to the chaperone, blocking their lysosomal translocation and degradation, and the binding of other CMA protein substrates (Cuervo et al., 2004; Pan et al., 2008). In addition, missense mutations of the gene encoding lysosomal ATPase 13A2 (PARK9), which are associated with an atypical form of juvenile-onset PD, lead to deficient autophagy activity with α-synuclein aggregation (Ramirez et al., 2006).

In spite of the unequivocal evidence for the impairment of proteostasis in the pathogenesis of DA-cell degeneration, it is still not clear whether degeneration starts with this phenomenon, whether the proteolytic stress does or does not play a determinant role in the relative specificity of DA-cell loss in PD, and whether LB-like proteinaceous inclusions are toxic entities promoting neuron death or whether they represent protective mechanisms by which neurons sequester unwanted proteins (Harrower et al., 2005). Based on the regional distribution of LBs, Braak et al. (2003) proposed a neuropathological staging scheme according to which PD starts in non-dopaminergic structures of the medulla oblongata and progresses rostrally, affecting nigral DA-cells later on in the course of the disease. However, this idea contrasts with quantitative studies reporting that most SN cell loss and striatal denervation occurs during the 5–10 year preclinical period (Gibb and Lees, 1988; Greffard et al., 2006), suggesting that cell loss and α-synuclein deposition are not parallel processes (Burke et al., 2008). It should also be noted that LBs and α-synuclein immunoreactive inclusions are present in different brain regions in different neurodegenerative diseases encompassed by the term LB disorders, including PD with dementia and dementia with LBs (Lippa et al., 2007), and in normal aging (Hindle, 2010); that the density of LBs in neurodegenerative diseases does not correlate with the intensity of cell degeneration, and that LBs are virtually absent in juvenile-onset PD associated with Parkin mutations (Olanow and McNaught, 2006).

NEUROINFLAMMATION

The participation of inflammatory reaction in the degeneration of midbrain DA-cells was suggested for the first time by the report of an increased number of microglial cells immunoreactive for the major histocompatibility complex (MHC) antigen II in the SN of PD patients (McGeer et al., 1988). The presence of activated microglia was thereafter confirmed using other markers of microglial activation (Mogi et al., 1994a; Banati et al., 1998; Imamura et al., 2003). The fact that DA-cells express the receptors for some of the cytokines found in the SN of PD patients, such as the tumor necrosis factor (TNF) α, interleukin (IL)-1β and interferon γ (Boka et al., 1994), and that the expression of some of these, e.g., TNFα receptor 1, increases in PD (Mogi et al., 2000), indicates that DA-cells may be sensitive to the direct effect of cytokines. However, cytokines can also affect DA-cells by inducing enzymes such as nitric oxide synthase, cyclo-oxygenase 2, and NADPH-oxidase which produce toxic reactive species (Hunot et al., 1996; Knott et al., 2000).

Interestingly, an analysis of cerebrospinal fluid in PD patients showed an increase in TNFα, IL-1β, and IL-6 concentration (Mogi et al., 1994b; Blum-Degen et al., 1995), and an analysis of

peripheral blood showed an increase in activated CD4(+) CD25(+) lymphocytes (Bas et al., 2001). Furthermore, the population of circulating CD3+ CD4 bright+ CD8 dull+ lymphocytes is significantly greater in PD patients than in age-matched control subjects (Hisanaga et al., 2001). Since the count of these lymphocytes usually increases after a viral infection, the authors proposed that postinfectious immune abnormalities could be associated with the pathogenesis of PD.

Animal and cellular models of PD have reproduced most cellular and molecular inflammatory events observed in the brain of PD patients, but have also provided additional insight for understanding the role of inflammation in DA-cell degeneration. For example, microglial activation has been found in the SN of mice and monkeys after peripheral injection of MPTP (Liberatore et al., 1999; Hurley et al., 2003; McGeer et al., 2003). The addition of α-synuclein or neuromelanin, two products released by degenerating DA-cells, to microglial cultures induces activation of proinflammatory pathways, and the injection of α-synuclein protofibrils in the rat SN induces microglial activation and loss of adjacent neurons (Wilms et al., 2003, 2009). Furthermore, the administration of steroidal and non-steroidal anti-inflammatory drugs reduce the toxicity of 6-OHDA and MPTP in rodents (Kurkowska-Jastrzebska et al., 2002; Di Matteo et al., 2006; Hirsch and Hunot, 2009). Taken together, these findings suggest that damaged DA-cells might upregulate and/or release products which activate microglial cells, and these lead to a local neuroinflammatory reaction which helps to perpetuate degeneration. Interestingly, recent *in vivo* and *in vitro* studies show that DA-degeneration induces an increase in the activity of angiotensin converting enzyme and angiotensin II (AII) levels, that the addition of AII to midbrain cell cultures increases 6-OHDA toxicity, and that angiotensin type-1 receptor antagonists inhibit both DA-cell degeneration and NADPH-oxidase and early microglial activation (Muñoz et al., 2006; Rodriguez-Pallares et al., 2008; Joglar et al., 2009). So, the brain angiotensin system may play a key role in the inflammatory process in PD. The fact that inflammatory changes are also present in other neurodegenerative disorders, such as Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis (McGeer and McGeer, 2004), suggests a common phenomenon in the progression of neuronal degeneration.

GLUTAMATE EXCITOTOXICITY

Glutamate is the major excitatory neurotransmitter in the mammalian brain, and is also a potent neurotoxin when it reaches high extracellular concentrations. The levels of extracellular glutamate are regulated by neuronal and glial transporters, and by its metabolism and recycling via glutamine synthetase in astrocytes, which prevent the extracellular glutamate concentration from rising to neurotoxic values (Plaitakis and Shashidharan, 2000; Dervan et al., 2004). Glutamatergic actions are exerted through two families of glutamate receptors, ionotropic and metabotropic. According to their agonist selectivity, ionotropic receptors have been classified into: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA), and kainic acid (KA) receptors (Burnashev et al., 1992; Hollmann and Heinemann, 1994; Geiger et al., 1995). Metabotropic receptors are coupled by G-proteins to different secondary messenger systems, and have also been classified into different groups (Groups I–III; Conn and Pin, 1997). Extracellular glutamate comes from two different sources, the neuronal Ca^{2+}-dependent pool, and the glial Ca^{2+}-independent pool. Under basal conditions, the glial pool provides 60% of extracellular glutamate, although at present its functional relevance is controversial. After K^+-induced depolarization, the neuronal pool augments from 30% to 60% of the total extracellular glutamate (Xue et al., 1996; Baker et al., 2002; Cavelier and Attwell, 2005). Current data show that the glutamatergic input to the SN comes from the pedunculopontine tegmental nucleus (PPN), and particularly from the subthalamic nucleus (STN) (Charara et al., 1996; Iribe et al., 1999; Forster and Blaha, 2003). Curiously, hodological and electron microscopic studies indicate that the bulk of subthalamic afferents to the SN reach the pars reticulata and form asymmetrical synapses with TH-negative (presumably GABAergic) dendrites, with those making contact with TH-positive being more than 10% (Kita and Kitai, 1987). So, depending on the target cell and the receptor type, glutamate can directly excite DA-cells, indirectly inhibit them by exciting GABAergic neurons, and directly inhibit or excite them by activating metabotropic receptors on DA-cells (Lee and Tepper, 2009).

Glutamate signaling may be affected by alterations in its homeostasis, which leads to an increase in the extracellular concentration, or by alterations in the energy metabolism of its target cells, which lead to a decrease in the activation threshold for their receptors, particularly those of the NMDA type (Novelli et al., 1988). Experimental data indicate that glutamate might contribute to DA-cell degeneration by means of both mechanisms. In a model of PD in mice, the loss of striatal DA-terminals is accompanied by an increase in the size and number of astrocytes, but the density of transporters per astrocyte is low, suggesting that the astrocyte function is compromised (Dervan et al., 2004). On the other hand, NMDA antagonists reduce the loss of DA-neurons due to the inhibition of the cellular metabolism in midbrain cell culture (Zeevalk et al., 1998), and the loss of DA-cells induced by MPP^+ and MPTP in rodents and in non-human primates (Turski and Lachowicz, 1991). STN activity has been found to be enhanced in both the experimental models of PD (Bergman et al., 1994) and in the PD patients (Benazzouz et al., 2002), and its modulation results in an improvement of motor symptoms (Limousin et al., 1998; Garcia et al., 2005). Hyperactivity of STN has even been detected before the appearance of clinical signs in MPTP-treated monkeys, suggesting that it may be a compensatory mechanism for the maintenance of striatal dopaminergic homeostasis (Bezard et al., 1999a), but it could also contribute to the progression of DA-cell loss from the first stages of the disease.

AGING

The relationship between aging and DA-cell degeneration is based on several facts. PD in its sporadic form is an age-related degenerative disorder, with a mean onset age of 55, and the incidence increases from 20/100.000 to 120/100.000 at age 70 (Dauer and Przedborski, 2003). Lewy bodies, the pathological hallmarks of PD (Braak et al., 2003), are also present in normal aged brains (Saito et al., 2004; Hindle, 2010). Oxidative stress is currently considered to play a central role in both aging (Harman, 1956; Squier, 2001) and PD (Dauer and Przedborski, 2003; Jenner, 2003). Mitochondrial DNA deletions occurring during normal aging, probably as a consequence

of oxidative stress, are particularly abundant in midbrain DA-cells (Bender et al., 2006; Kraytsberg et al., 2006). In addition, morphological studies in monkeys (Pakkenberg et al., 1995; Gerhardt et al., 2002) and humans (McGeer et al., 1977; Fearnley and Lees, 1991; Pakkenberg et al., 1995; Cabello et al., 2002; Gerhardt et al., 2002) have reported a decrease in the number of midbrain DA-cells with age, suggesting that midbrain DA-neurons undergo a low intensity degenerative process during normal aging. Based on these findings, it has been proposed that everyone should get PD if they live long enough (Chan et al., 2007; Surmeier, 2007). However, this idea is challenged by other studies performed on monkeys (Irwin et al., 1994; McCormack et al., 2004; Collier et al., 2007) and humans (Kubis et al., 2000) according to which the number of midbrain DA-cells does not change during normal aging, suggesting that the age-related loss of striatal DA levels (Kish et al., 1992; Haycock et al., 2003) is due to a progressive chemical and functional decline rather than to degeneration. Furthermore, biochemical studies performed on humans show that the regional pattern of striatal DA loss during normal aging substantially differs from that typically observed in PD (Kish et al., 1992). Consistent with these data, we have recently observed that the loss of DA in aged rats is higher in the ventral striatum than in the dorsal striatum (Cruz-Muros et al., 2007). In addition, two different age-related processes were detected. One is characterized by DOPA decarboxylase decrease involving both the nigrostriatal and the mesolimbic compartments, and is responsible for the moderate DA decrease in the dorsal striatum. The other process is characterized by axonal degeneration and aggregation of α-synuclein in their original somata, restricted to mesolimbic regions and responsible for the decline of tyrosine hydroxylase activity and the greater decrease in DA levels in this compartment. Therefore, although both the nigrostriatal and the mesolimbic systems are vulnerable to aging, in contrast to what occurs in PD, the mesolimbic system seems to be more vulnerable to normal aging than the nigrostriatal system. Furthermore, although several age-related aspects, such as oxidative stress or impairment of the energy metabolism, make aging the most important risk factor for PD, anatomical and biochemical features of normal aging in the mesostriatal system seem to be more probably related to age-associated behavioral and mood disturbances than to parkinsonian motor signs.

SPECIFIC DOPAMINERGIC FACTORS

The aforementioned studies indicate that intrinsic (mitochondrial dysfunction and proteostasis impairment), extrinsic (neuroinflammation and excitotoxicity) and age-related factors participate in the degenerative process of DA-cells in PD. However, a question of critical importance remains to be solved: why are midbrain DA-neurons, and especially some of these neurons, particularly vulnerable to degeneration? It is known that in PD, DA-cells in the SN are more vulnerable than those in the VTA, and that within the SN, neurons lying in its ventrolateral and caudal region (SNcv) are more vulnerable than those in the rostromedial and dorsal region (SNrm) (Hassler, 1938; German et al., 1989; Damier et al., 1999). However, there is no evidence for substantial differences in the ER, mitochondria or other structural organelles between DA-cells and non-DA cells, or between midbrain DA-cells that show different susceptibility to degeneration. So, differential vulnerability should be linked to physiological, neurochemical, and/or metabolic aspects which are specific to DA-cells and also differ from one DA-subpopulation to another, making some of them more vulnerable or resistant.

PHYSIOLOGICAL PROFILE OF DA-NEURONS

A characteristic of midbrain DA-cells is that, unlike most neurons in the brain, they display two electrophysiological patterns. One phasic pattern in the form of bursts of action potentials with a mean frequency of 20 Hz and triggered by excitatory inputs from different sources (Schultz, 2002; Omelchenko and Sesack, 2005), and the other tonic pattern, with a mean frequency of 2–4 Hz and which is generated in absence of afferents (Sulzer and Schmitz, 2007). This intrinsic pacemaking activity is believed to be important in maintaining basal DA levels in the striatum, and may be detected from the first postnatal days in mice. During the first weeks, this is driven by sodium and hyperpolarization-activated and cyclic nucleotide-gated cation (Na^+/HCN) channels. Thereafter, the expression of L-type Ca^{2+} channels increases in SN DA-cells, and the pacemaking is driven by Ca^{2+} currents. Adult VTA DA-cells also have L-type Ca^{2+} channels, but as opposed to SN DA-cells, their pacemaking continues to be driven by sodium channels. The L-type Ca^{2+} channels used by SN DA-cells have a Ca_v 1.3 pore-forming subunit which allows them to open at a relatively hyperpolarized potential (Striessnig et al., 2006; Chan et al., 2007). While in most cells, Ca^{2+} channel opening is a rare and brief event, the sustained used of Ca_v 1.3 Ca^{2+} channels by SN DA-cells results in a large Ca^{2+} influx which requires a high metabolic cost to be handled. Cytosolic Ca^{2+} should be pumped back across the plasma membrane against a concentration gradient or rapidly sequestered. The two organelles most directly involved in Ca^{2+} handling are ER and mitochondrion. Cytosolic Ca^{2+} is moved into ER by high-affinity ATP-dependent transporters. Thereafter, it is released to the cytosol to be used in modulatory functions or pumped to the extracellular space (Berridge, 2002; Choi et al., 2006). Ca^{2+} released from the ER can also enter mitochondria through apposition points between both organelles (Rizzuto and Pozzan, 2006). This ER-mitochondrial system contributes to Ca^{2+} buffering but also to ATP production (McCormack et al., 1990). However, it may be overburdened by the sustained use of Ca_v1.3 Ca^{2+} channels. Both depletion and increase in intraluminal ER Ca^{2+} concentration induce ER stress and compromise proteostasis (LaFerla, 2002; Paschen and Mengesdorf, 2005). Furthermore, the high demand of ATP needed to move Ca^2 out of the cell increases the electron transport chain activity leading to the production of reactive oxygen species (Sulzer and Schmitz, 2007; Chan et al., 2009). The relevance of the Ca^{2+}-dependence of pacemaking activity in the vulnerability of SN DA-cells is based on the finding that blockade of Ca_v1.3 Ca^{2+} channels in adult SN DA-cells induces a reversion of the pacemaking pattern to that of adult VTA and juvenile SN DA-cells, also protecting them against rotenone and MPTP (Chan et al., 2007). Reinforcing the role Ca^{2+} homeostasis in the differential vulnerability of midbrain DA-cells, DA-cells in the dorsomedial region of SN and VTA express calbindin-D_{28k} (Gerfen et al., 1987; McRitchie and Halliday, 1995; Liang et al., 1996; McRitchie et al., 1996). The ability of this protein to buffer intracellular Ca^{2+}, together with its localization in DA-cell subpopulations resistant to degeneration, have suggested

that calbindin-D_{28k} confers neuroprotection (Gerfen et al., 1985; Yamada et al., 1990; German et al., 1992). However, some findings contrast with this hypothesis. For example, no differences have been observed between the neurotoxic effect of MPTP in midbrain-DA cells of calbindin-D_{28k}-deficient mice and their wild-type littermates (Airaksinen et al., 1997). Furthermore, DA-cells in the lateral region of the SN which express calbindin-D_{28k} are more sensitive to the neurotoxic effect of 6-OHDA than other SN DA-cells which do not express calbindin-D_{28k} (Rodriguez et al., 2001).

METABOLIC PHENOTYPE OF DA-NEURONS

Another peculiarity of DA-cells implicated in their vulnerability concerns DA itself. Under normal conditions, most DA acting as a neurotransmitter is contained inside synaptic vesicles of DA-cells (Jonsson, 1971). When DA concentration increases in the cytosol, DA is rapidly metabolized via monoamino oxidase or by autooxidation. Both enzymatic and non-enzymatic catabolisms of DA are important sources of reactive oxygen species which have been implicated in DA-cell degeneration. Through its enzymatic metabolism, DA is converted into 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylacetic acid, also generating hydrogen peroxide. This may be neutralized by antioxidant enzymes, or, in the presence of iron, generate high toxic hydroxyl radicals. Through autooxidation, DA is converted into reactive quinones and superoxide anion. This can be converted into hydrogen peroxide by superoxide dismutase, or react with nitric oxide to generate the highly toxic radical peroxynitrite (Barzilai et al., 2001). Reactive quinones may be oxidized to cyclized aminochromes, which are thereafter polymerized to form neuromelanin, or interact with cysteine residues of different molecules inhibiting their activity (Miyazaki and Asanuma, 2009). On the basis of this interaction, the formation of quinones by DA oxidation has gained interest in the pathogenesis of PD. There are studies supporting the notion that DA-quinone, or some of its metabolic products, can inhibit the ubiquitin ligase activity of parkin (LaVoie et al., 2005), the permeability of mitochondrial pores (Berman and Hasting, 1999) and the proteasomal function (Zafar et al., 2006), as well as induce microglial activation (Kuhn et al., 2006).

Neuromelanin is another product from the DA metabolism that has been implicated in the pathogenesis of PD. Neuromelanin is a complex polymeric molecule which probably arises from both the enzymatic (Hasting, 1995; Sanchez-Ferrer et al., 1995) and non-enzymatic (Fornsted et al., 1986; Sulzer et al., 2000) metabolism of catecholamines, and is particularly abundant in DA-cells in the SN and noradrenergic cells in the locus coeruleus of humans, being responsible of their dark color (Fasano et al., 2006). Neuromelanin was first proposed as playing a role in PD on the basis of morphological studies reporting a correlation between neuromelanin concentration and DA-cell degeneration (Mann and Yates, 1983; Hirsch et al., 1988). However, this idea was challenged by the report that DA-cells in the dorsal tier of the SN contain higher levels of neuromelanin than those in the ventral tier which are more vulnerable to degeneration (Gibb, 1992). Experimental studies performed during the last two decades indicate that neuromelanin may play a dual role, protecting from or contributing to DA-cell degeneration, depending on the environment (Ben-Shachar et al., 1991; Sulzer et al., 2000). On the one hand, neuromelanin can bind MPP+ (D'Amato et al., 1986) and paraquat (Lindquist et al., 1988), reducing their neurotoxicity, and accumulate dopaminergic drugs, regulating their intracellular concentration (Salazar et al., 1978). Neuromelanin also has the capability to sequester redox-active ions, including iron, thereby reducing the formation of free hydroxyl radicals (Enochs et al., 1994; Zecca et al., 2003). However, under oxidative conditions, neuromelanin can interact with hydrogen peroxide, becoming a source of free radicals and releasing toxic metals which accelerate cell death (Swartz et al., 1992; Shima et al., 1997). In addition, as mentioned above (Wilms et al., 2003), neuromelanin released from degenerated DA-cells can induce microglial activation, also perpetuating the degenerative process.

It should be also noted that the cytosolic levels of DA are closely related to two transport processes: DA uptake from the extracellular space, and once inside the cell, DA packing into small synaptic vesicles. DA uptake is performed by the DAT, a 12-transmembrane domain glycoprotein with three *N*-glycosylation sites in the second extracellular loop (Horn, 1990; Giros and Caron, 1993; Uhl et al., 1994). DA vesicular storage is performed by the vesicular monoamine transporter type 2 (VMAT2), also present in other monoaminergic cells (Miller et al., 1999). DAT may contribute to the vulnerability of DA-cells serving as an entrance for DA and its metabolites, while VMAT2 may serve as a neuroprotective factor by sequestering DA into vesicles, and preventing interaction with their catabolic enzymes. So, the functional balance between both transporters may affect the differential vulnerability between different midbrain DA-cell subpopulations. This idea is supported by the fact that VMAT2 deficient mice are more susceptible to MPTP than their homologous wild-type (Takahashi et al., 1997). Furthermore, they display spontaneous and progressive DA-cell degeneration (Caudle et al., 2007), and non-motor parkinsonian symptoms (Taylor et al., 2009), suggesting that VMAT2 may also be involved in the degeneration of other monoaminergic cells in PD. Studies carried out in our laboratory show that during normal aging, in parallel with the decline in vesicular DA uptake, VMAT2 is deglycosylated and moved from the vesicle membrane to the soluble compartment (Cruz-Muros et al., 2008), probably contributing to the increase of the vulnerability of DA-cells with age.

On the other hand, the damaging role of DAT is supported by the fact that its expression and functional integrity are required for making DA-cells susceptible to DA analog neurotoxins (Kopin, 1992; Gainetdinov et al., 1997; Bezard et al., 1999b), and by the report of an anatomical correlation between the distribution of DA-neurons expressing high DAT mRNA levels and those showing high vulnerability to degeneration in PD and animal models of PD (Cerruti et al., 1993; Hurd et al., 1994; Uhl et al., 1994). However, this correlation does not apply in some DA-cell groups. DA-cells in the parabrachialis pigmentosus region of the A10 cell group, which contain higher levels of DATmRNA than those in the dorsal tier of SN in monkeys (Haber et al., 1995) and rats (Shimada et al., 1992) are also more resistant to MPTP (Varastet et al., 1994) and 6-OHDA (Rodriguez et al., 2001) respectively. In addition, a comparative analysis of DAT and VMAT2 mRNA expressions in the ventral midbrain of rats revealed no differences between both mRNA expression patterns, with the highest levels in the SNrm, followed by the SNcv, and the lowest ones in VTA (Gonzalez-Hernandez et al., 2004). So, the involvement of DA transporters

in the differential vulnerability of DA-cells could be due to aspects other than differences in their mRNA levels. Given that DAT activity depends on its glycosylation status and membrane expression, we then explored a possible relationship between DAT glycosylation and function and the differential vulnerability of DA-cells (Afonso-Oramas et al., 2009). Glycosylated-DAT expression, DA uptake and DAT V_{max} were significantly higher in nigrostriatal neuron terminals than in those of mesolimbic neurons. These findings are consistent with those reporting that the number of ^{3}H-DA uptake sites in the dSt is higher than in the vSt (Missale et al., 1985; Marshall et al., 1990; Cass et al., 1992), and correlate regional differences in DA uptake with the glycosylation status of DAT in both striatal compartments. Differences in DAT regulation may contribute to defining the pattern of DA time signaling and functional specialization in the dSt and vSt (Wickens et al., 2003). Interestingly, the expression pattern of glycosylated-DAT in the human midbrain and striatum showed a close anatomical relationship with DA-degeneration in parkinsonian patients. In addition, this relationship was confirmed in rodent and monkey models of PD, and in HEK-cells expressing the wild-type and a partially deglycosylated DAT form.

CONCLUDING REMARKS

Nearly two centuries after PD was first described by James Parkinson, its etiopathogenesis is still unknown. There is a tendency to consider PD as a multicausal disorder resulting from the convergence of environmental-, genetic-, and age-related factors. In recent years, mitochondrial dysfunction and proteostasis impairment have been proposed as having a leading role in the degenerative process. However, this idea contrasts with the selectivity of neuronal populations affected in PD. In spite of the large body of evidence supporting the participation of mitochondria and ER in the pathogenesis of PD, it is difficult to accept that a failure in functions common to all cells is the main factor responsible for degeneration of particular neuronal groups. Ca^{2+}-dependent pacemaking and DA handling are two oxidative stress generating activities specific to midbrain DA-cells. The reliance on $Ca_v1.3$ Ca^{2+} channels to drive pacemaking and the pattern of DAT maturation and DA uptake also explain the differential susceptibility between nigrostriatal and mesolimbic neurons. Morphological and molecular studies performed in the last 20 years, with the aid of laser capture microdissection and more recently microarray analysis, show differences in the expression levels of more than 100 genes between vulnerable (nigrostriatal) and resistant (mesolimbic) DA-cells (Chung et al., 2005; Greene et al., 2005). These genes belong to different categories, including Ca^{2+} buffering proteins, mitochondrial proteins, L-type Ca^{2+} and inward rectifier K^+ channels, axon guidance and transcription factors, etc. (for review see Gonzalez-Hernandez et al., 2009). But the function and relevance of most of these in the differential vulnerability of DA-cells is still unknown.

Finally, we should not forget that, to a varying degree, other neuronal populations, such as noradrenergic neurons in the locus coeruleus, and cholinergic neurons in the dorsal nucleus of the vagus are also affected in PD, and that Ca^{2+}-dependent pacemaking and DA handling cannot explain their vulnerability. It would be interesting to elucidate whether there is a vulnerability factor common to all cell populations involved in PD, or whether independent factors determine the start of the degenerative process in each neuronal group for their posterior convergence in a common pathogenic route.

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Extrastriatal dopaminergic circuits of the basal ganglia

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The basal ganglia are comprised of the striatum, the external and internal segment of the globus pallidus (GPe and GPi, respectively), the subthalamic nucleus (STN), and the substantia nigra pars compacta and reticulata (SNc and SNr, respectively). Dopamine has long been identified as an important modulator of basal ganglia function in the striatum, and disturbances of striatal dopaminergic transmission have been implicated in diseases such as Parkinson's disease (PD), addiction and attention deficit hyperactivity disorder. However, recent evidence suggests that dopamine may also modulate basal ganglia function at sites outside of the striatum, and that changes in dopaminergic transmission at these sites may contribute to the symptoms of PD and other neuropsychiatric disorders. This review summarizes the current knowledge of the anatomy, functional effects and behavioral consequences of the dopaminergic innervation to the GPe, GPi, STN, and SNr. Further insights into the dopaminergic modulation of basal ganglia function at extrastriatal sites may provide us with opportunities to develop new and more specific strategies for treating disorders of basal ganglia dysfunction.

Keywords: subthalamic nucleus, globus pallidus, substantia nigra, Parkinson's disease, basal ganglia, dopamine, GABA, glutamate

INTRODUCTION

The basal ganglia consist of the striatum, the external and internal segment of the globus pallidus (GPe and GPi, respectively), the subthalamic nucleus (STN), and the substantia nigra pars compacta and reticulata (SNc and SNr, respectively). It is well known that the functions of these structures are strongly modulated by the neuromodulator dopamine. The striatum has long been the focus of investigation into dopamine's effects on basal ganglia function, as the dopamine concentration in this nucleus surpasses that in other basal ganglia nuclei. Recent research has demonstrated that dopamine is also present in basal ganglia areas outside of the striatum, and has strong effects on the neuronal activities in these nuclei. In this review we will first briefly describe relevant features of the general circuit anatomy of the basal ganglia, followed by a summary of the current state of our knowledge of the anatomical and functional features of the dopamine supply to the extrastriatal basal ganglia.

CIRCUIT ANATOMY OF THE BASAL GANGLIA

The basal ganglia are components of larger functionally and anatomically segregated circuits that also involve the cerebral cortex and thalamus (Alexander et al., 1986, 1990; Hoover and Strick, 1993; Middleton and Strick, 1997, 2002; Kelly and Strick, 2004; Mallet et al., 2007; Wichmann and Delong, 2007). The "motor" circuit originates in the frontal cortical motor areas and involves motor portions of the striatum, GPe, STN, GPi, SNr, and thalamus, returning to the frontal cortex. "Associative" and "limbic" circuits originate from the prefrontal associative and limbic cortices and involve related areas in the basal ganglia and thalamus separate from those occupied by the motor circuit. While the motor circuit is thought to be involved in the control of movement, the associative circuit may play a role in the control of executive functions, and the limbic circuit in the control of emotions and motivation. Dysfunction in elements of these circuits may contribute to diseases ranging from classical movement disorders, such as Parkinson's disease (PD), to neuropsychiatric conditions, such as Tourette syndrome or addiction.

The anatomy of individual connections within these circuits has been described in considerable detail. Within the basal ganglia, the striatum and STN serve as input stations, while GPi and SNr serve as output stations. Glutamatergic efferents from cortex and thalamus project to the striatum and STN in a topographically organized manner (Alexander et al., 1986, 1990; Middleton and Strick, 2002; Kelly and Strick, 2004; Mallet et al., 2007; Wichmann and Delong, 2007). The input and output nuclei of the basal ganglia are connected through two main pathways, i.e., the monosynaptic GABAergic "direct" pathway and polysynaptic "indirect" pathway. The indirect pathway involves GABAergic projections from the striatum to GPe and from GPe to the STN, as well as excitatory glutamatergic projections from the STN to GPe, GPi, and SNr.

At the most basic level of analysis, the polarities of the connections within the direct and indirect pathways oppose one another. Activation of the striatal neurons of the direct pathway have predominately net inhibitory effects on GPi/SNr activity, while activation of the striatal neurons of the indirect pathway have net excitatory effects on them. This scheme is too simplistic, however, as the interactions between and within the two pathways may shape firing patterns independent of firing rates (e.g., oscillatory and burst patterns of discharge, in the GPi/SNr; Galvan and Wichmann, 2008).

The GPi and SNr send topographically organized GABAergic projections to the thalamus and brainstem. Motor circuit output from the GPi and SNr reaches the anterior portion of the ventrolateral thalamic nucleus (VLa), which then project back

to motor areas of the frontal cortex. In contrast, associative circuit output from the SNr and GPi, reaches the thalamic ventral anterior (VA) nucleus, which sends efferents to the dorsolateral prefrontal cortex and the lateral orbitofrontal cortices (Hoover and Strick, 1993; Haber et al., 1995; Kaneda et al., 2002; Romanelli et al., 2005). Collaterals of the GPi/SNr projection to the ventral thalamus reach the intralaminar thalamic centromedian and parafascicular nuclei (CM/PF), as well as brain stem targets such as the pedunculopontine nucleus, and the reticular formation (Smith et al., 2009).

STRIATAL ACTIONS OF DOPAMINE

It has been known for many decades that the neurotransmitter dopamine is present in high concentrations in the basal ganglia. The dopamine supply to these structures originates in the midbrain dopaminergic nuclei, the SNc and ventral tegmental area. The striatum is the most prominent release site for dopamine in the basal ganglia, influencing the overall balance of activity along the direct and indirect pathways via different types of dopamine receptors (Gerfen et al., 1990). D1-like receptors (D1LR, including D1- and D5-receptors; Clark and White, 1987; Neve, 1997) are found on striatal neurons that give rise to the direct pathway, while D2-like receptors (D2LRs, including D2-, D3-, and D4-receptors; Neve, 1997) are found on striatal neurons that give rise to the indirect pathway (see, for instance, recent studies in transgenic mice; Heintz, 2001; Day et al., 2006; Wang et al., 2006). Activation of D1LRs on direct pathway neurons is thought to facilitate corticostriatal transmission, while activation of D2LRs on indirect pathway neurons appears to reduce corticostriatal transmission (Gerfen et al., 1990; Gerfen, 1995). According to traditional models of the basal ganglia, the dopamine-mediated increase in activity of the inhibitory direct pathway, in conjunction with the dopamine-mediated reduction of activity in the net excitatory indirect pathway leads to an overall reduction of activity of GPi/SNr neurons, acting to disinhibit thalamocortical projection neurons. In addition to the regulation of transmission along direct and indirect pathways, striatally released dopamine is also implicated in the modulation of learning and neuronal plasticity through processes such as long-term depression (LTD) or potentiation (LTP), acting at glutamatergic synapses (Aosaki et al., 1994; Cragg, 2003; Picconi et al., 2003; Wang et al., 2006; Calabresi et al., 2007; Kreitzer and Malenka, 2007; Schultz, 2007; Flajolet et al., 2008; Kreitzer and Malenka, 2008; Pawlak and Kerr, 2008).

The duration of action and diffusion of dopamine are to some extent regulated by dopamine transporter- (DAT-) mediated uptake (Blakely and Bauman, 2000; Cenci and Lundblad, 2006; Rice and Cragg, 2008). In rodent studies, it has been shown that DAT concentrations and dopamine clearance rates differ among striatal territories, with a dorso-ventral gradient (Missale et al., 1985; Kuhr et al., 1986; Stamford et al., 1988; Letchworth et al., 2001). Given the topographical organization of the striatum, such differences may affect the physiologic role and significance of dopamine in different behavioral domains. For instance, physiological data indicate that the time course of DA signaling may determine the pattern of dopamine-glutamate interaction in different areas of the striatum (Calabresi et al., 2000; Wickens et al., 2003).

EXTRASTRIATAL ACTIONS OF DOPAMINE

EXTERNAL PALLIDAL SEGMENT

Anatomical studies

The GPe is a component of the indirect pathway, receiving GABAergic inputs from the striatum (Chang et al., 1981; Filion and Tremblay, 1991; Sidibe and Smith, 1996; Raz et al., 2000), and sending GABAergic projections to STN, GPi, and SNr (Moriizumi et al., 1992; Parent and Hazrati, 1995a,b). Several studies have shown that the primate globus pallidus (GP) receives dopaminergic inputs that are differentially distributed in GPe and GPi, with dopamine fibers arborizing profusely in the GPi and more sparsely in dorsal portion of the GPe (Parent and Smith, 1987; Lavoie et al., 1989; Parent et al., 1989; Hedreen, 1999). Some of these fibers are passing through the pallidum *en route* to the striatum. However, retrograde and anterograde labeling studies in rats and monkeys have shown that at least some of these fibers arise as a nigropallidal projection that is separate from the nigrostriatal projection (Fallon and Moore, 1978; Lindvall and Bjorklund, 1979; Smith et al., 1989; Gauthier et al., 1999; Jan et al., 2000; Smith and Kieval, 2000; Anaya-Martinez et al., 2006). Low levels of dopamine (Pifl et al., 1990) as well as DAT immunoreactivity and DAT ligand binding have also been detected in postmortem studies on human GPe tissue (Ciliax et al., 1999; Porritt et al., 2005) and rodent GP, the rodent homologue of the primate GPe (Ciliax et al., 1995; Coulter et al., 1995) indicating the presence of terminals of a dopaminergic projection in the GPe.

Dopamine receptors are found at pre- and postsynaptic locations in GPe (**Table 1**). Most of the presynaptic dopamine receptors are thought to be D2LRs, and are located on terminals of the GABAergic striatopallidal projection (Parent and Smith, 1987; Gerfen et al., 1990; Deng et al., 2006). Using electron microscopy we recently confirmed the presence of presynaptic D2-receptors in the monkey GPe on putatively GABAergic axons and terminals, with sparse labeling of putatively glutamatergic terminals (unpublished observation). A previous rat study identified D4-receptors primarily on axons and on a few putatively glutamatergic terminals in GP (Rivera et al., 2003).

There is also evidence for postsynaptic expression of D2LRs in GPe. For example, D2- and (less) D3-receptor mRNA has been found in the human GPe (Murray et al., 1994). However, another study did not confirm D3-receptor mRNA expression in monkeys (Quik et al., 2000). In rats, D2-receptor mRNA was found, particularly in pallidal cells projecting to the striatum (Marshall et al., 2001; Hoover and Marshall, 2004). Low levels of D2-receptor protein labeling have been detected in human GPe (Levey et al., 1993) and in postsynaptic structures in the rat (Yung et al., 1995). Scattered cell bodies in the rat GP showed immunoreactivity for D2-, D3-, and D4-receptor (Khan et al., 1998). In the monkey, both D3- (Quik et al., 2000) and D4-receptors (Mrzljak et al., 1996) have been found. The latter are associated with the parvalbumin-positive GABAergic neurons which project predominantly to the basal ganglia output nuclei (Mrzljak et al., 1996).

In addition to these D2LRs, lower levels of D1LRs have been detected, in axons and terminal boutons forming symmetric, putatively GABAergic synapses in the rodent GP (Levey et al., 1993; Yung et al., 1995). D5-receptors were identified in the rodent GP and monkey GPe (Ciliax et al., 2000; Khan et al., 2000).

Table 1 | Dopamine receptor localization in the extrastriatal basal ganglia.

		D1LRs		D2LRs		
Structure	**Synaptic location**	**D1**	**D5**	**D2**	**D3**	**D4**
GPe	Pre	Axons, GABA terminals **R** (Levey et al., 1993; Yung et al., 1995)		GABA terminals **R** (Levey et al., 1993), **M** (unpublished observations) Glutamate terminals **M** (unpublished observations)		Axons, terminals **R** (Rivera et al., 2003)
	Post		Perikarya **R** (Ciliax et al., 2000; Khan et al., 2000), **M** (Ciliax et al., 2000)	Dendrites **R** (Yung et al., 1995) Perikarya **R** (Khan et al., 1998)	Perikarya **R** (Khan et al., 1998)	Perikarya **R** (Khan et al., 1998), **M** (Mrzljak et al., 1996)
STN	Pre	Terminals **M** (Rommelfanger et al., 2010)	Terminals **M** (Rommelfanger et al., 2010)	Terminals **M** (Rommelfanger et al., 2010)		
	Post		Dendrites **R** (Baufreton et al., 2003), **M** (Rommelfanger et al., 2010) Perikarya **R** (Ciliax et al., 2000), **M** (Ciliax et al., 2000)			
GPi	Pre	Axons **R** (Yung et al., 1995), **M** (Kliem et al., 2010) GABA terminals **R** (Yung et al., 1995)	Axons **M** (Kliem et al., 2010)	GABA and glutamate terminals **M** (unpublished observations)		GABA terminals **R** (Rivera et al., 2003)
	Post		Dendrites **M** (Kliem et al., 2010) Perikarya **R** (Ciliax et al., 2000), **M** (Ciliax et al., 2000; Kliem et al., 2010)			Perikarya **M** (Mrzljak et al., 1996)
SNr	Pre	GABA terminals **R** (Levey et al., 1993; Yung et al., 1995; Caille et al., 1996) Axons **M** (Kliem et al., 2010), **R** (Caille et al., 1996)		GABA and glutamate terminals **M** (unpublished observations)		Axons, glutamate terminals **R** (Rivera et al., 2003)
	Post		Dendrites **M** (Kliem et al., 2010), **R** (Khan et al., 2000) Perikarya **M** (Ciliax et al., 2000; Kliem et al., 2010), **R** (Ciliax et al., 2000; Khan et al., 2000)	(Ventral SNr) neurons **R** (Yung et al., 1995)		Perikarya **M** (Mrzljak et al., 1996) Dendrites **R** (Rivera et al., 2003)

Only studies verifying the presence of receptor protein are included in this table.
H, human; M, monkey; R, rodent.

Functional studies

Substantia nigra pars compacta lesions in rodents and monkeys reduce dopamine levels in GPe (Parent et al., 1990; Jan et al., 2000; Fuchs and Hauber, 2004). Furthermore, *in vivo* microdialysis studies in rats have shown that dopamine is released in the GP, that local administration of high-potassium solutions increases dopamine concentrations in pallidal dialysates, and that the release is inhibited by reverse dialysis of the sodium channel blocker tetrodotoxin, or by the use of low-calcium-medium, supporting the notion that dopamine is released in a spike-dependent fashion at this site (Dewar et al., 1987; Pifl et al., 1990; Hauber and Fuchs, 2000).

Given the predominance of D2LRs in GPe, it is likely that most actions of dopamine in GPe are mediated via D2LRs. Activation of pallidal D2LRs has been shown to increase the activity of GPe neurons (see **Table 2**). For instance, activation of D2LRs in the rat GP increases the expression of the immediate early gene c-fos (Billings and Marshall, 2003), and infusions of the non-specific dopamine receptor agonist apomorphine into the rat GP increases pallidal neuron activity (Napier et al., 1991). Our recent studies in primates have also demonstrated that the neuronal activity in GPe was increased after intra-GPe infusions of the D2LR agonist quinpirole (Hadipour Niktarash et al., 2008), and that infusions of the D2LR antagonist sulpiride lowered pallidal firing rates, suggesting that the pallidal D2LRs are occupied by endogenous dopamine under normal conditions (unpublished observations).

While some of the pallidal effects of D2LR ligands may be mediated by postsynaptic D4-receptors (Shin et al., 2003), most of them are likely due to presynaptic modulation of GABAergic transmission. GABA release in GPe originates from terminals of the "indirect" striatopallidal pathway, and from local axon collaterals of pallidal neurons (Parent et al., 1999, 2000; Kita et al., 2004). Given the high activity levels of pallidal neurons (DeLong, 1971; Anderson and Horak, 1985; Miller and DeLong, 1987; Tremblay et al., 1989; Matsumura et al., 1995; Nambu et al., 2000; Raz et al., 2001; Kita et al., 2004; Starr et al., 2005), it is likely that most of the pallidal GABA stems from local axon collaterals. To what extent collateral interactions influence pallidal activities remains unclear. Early studies in anesthetized rats showed that iontophoresis of dopamine or of amphetamine, a dopamine releasing agent, reduces GABAergic transmission in the pallidum (Bergstrom and Walters, 1984). Microdialysis studies showed that activation of D2LRs decreased GABA release in the rat GP while activation of D1LRs increased GABA release (Floran et al., 1990, 1997). Subsequent patch clamp recordings of GP neurons in rat brain slice demonstrated that activation of presynaptic D2LRs decreases GABA-A receptor-mediated currents in the pallidum (Cooper and Stanford, 2001).

Dopamine receptor activation may also modulate the glutamatergic inputs to the GPe from the STN (Kita and Kitai, 1987; Robledo and Feger, 1990; Smith et al., 1990; Hazrati and Parent, 1992; Shink et al., 1996; Nambu et al., 2000) or CM/PF (Mouroux et al., 1997; Yasukawa et al., 2004). *In vitro* patch clamp studies in rodent brain slices have suggested that activation of presynaptic D1LRs facilitates glutamate release (Hernandez et al., 2007) while activation of D2LRs reduces it (Hernandez et al., 2006). These effects are not mutually exclusive, indicating that the involved receptors may be located on different axon terminals.

Studies of behavioral effects

In general, activation of D1LRs or D2LRs in the rodent GP appears to facilitate movement. In support of this notion, local intra-pallidal infusions of D1LR or D2LR antagonists were found to induce akinesia in rats, likely by blocking the effects of endogenous dopamine on these receptors (Hauber and Lutz, 1999). Similarly, intra-pallidal infusion of D1LR agonists (Sanudo-Pena and Walker, 1998) increased general movement. Other studies have demonstrated that infusion of D1LR agonists, D2LR agonists (Koshikawa et al., 1990), or amphetamine (Costall et al., 1972a,b) induces stereotypic jaw movements. The behavioral effects of dopamine receptor activation in the GPe have not been examined in other species.

SUBTHALAMIC NUCLEUS

Anatomical studies

The STN consists of glutamatergic neurons that send most of their projections to GPe, GPi, and SNr (Smith and Parent, 1988). The activity of STN cells is strongly regulated by its afferents, including inhibitory GABAergic inputs from the GPe and glutamatergic inputs from the cerebral cortex (Mink, 1996; Nambu et al., 1996, 2002; Takada et al., 2001). Smaller projections from the intralaminar nuclei of the thalamus to the STN have also been described (Sugimoto et al., 1983; Lanciego et al., 2004).

Anatomical studies have demonstrated that the STN receives sparse collaterals from the nigrostriatal pathway which pass the nucleus at its dorsal surface (Lavoie et al., 1989; Hedreen, 1999; Augood et al., 2000; Francois et al., 2000). These inputs form symmetric synapses on dendrites of STN neurons in rats (Cragg et al., 2004) and monkeys (Smith and Kieval, 2000). In postmortem human brains, terminal-like tyrosine hydroxylase-positive elements were identified along the STN's dorsal surface (Cossette et al., 1999). Anatomical studies using retrograde (Rinvik et al., 1979; Campbell et al., 1985; Francois et al., 2000) or anterograde tracers (Hassani et al., 1997; Gauthier et al., 1999; Francois et al., 2000) in rats and monkeys support the existence of an SNc–STN projection. Others have detected DAT binding in the rodent STN (Coulter et al., 1995) and found that DAT blockade in rodent slices of STN increases dopamine release as measured by voltammetry (Cragg et al., 2004). We have also noted low levels of DAT immunoreactivity in the monkey STN (unpublished observations) suggesting that dopamine terminals may be found in the STN.

Dopamine receptors exist in the STN (Smith and Kieval, 2000; Smith and Villalba, 2008), but their distribution and relative expression level need further investigation (**Table 1**). Receptor binding studies have demonstrated D1LRs in the rat and human STN (Boyson et al., 1986; Dawson et al., 1986, 1988; Mansour et al., 1992; Parry et al., 1994; Augood et al., 2000). Similarly, binding studies using ligands for D2LRs (Bouthenet et al., 1987; Johnson et al., 1994), or ligands preferring D1-, D2-, D3-, or D4-receptors (Flores et al., 1999) have detected binding targets in the rat STN. Using electron microscopy, we have recently identified presynaptic D1- and D2-receptors in the monkey STN (Rommelfanger et al., 2010).

The available data on dopamine receptor mRNA is contradictory, but suggest that a portion of the dopamine receptors in the STN are postsynaptically expressed. Several authors have described the presence of the mRNA for D1-, D2-, and D3-receptors (Flores et al.,

Table 2 | Functional effects of dopamine receptor agonists.

Structure	Effects of dopamine or non-specific D1LR/ D2LR agonists	D1LR agonist effects	D2LR agonist effects
GPe	Increases firing rate (Napier et al., 1991) Decreases GABA transmission (Bergstrom and Walters, 1984) Increases GABA release (Floran et al., 1990)	Increases glutamate release (Hernandez et al., 2007) Increases GABA release (Floran et al., 1990)	Increases firing rate (Hadipour Niktarash et al., 2008; unpublished observations) Increased c-fos (Billings and Marshall, 2003) Decreases GABA release (Floran et al., 1997) Decreases GABA-A currents (Cooper and Stanford, 2001; Shin et al., 2003) Decreases glutamate release (Hernandez et al., 2007)
STN	Increases firing rate (Ni et al., 2001; Zhu et al., 2002; Cragg et al., 2004) Decreases GABA transmission (Shen and Johnson, 2000; Cragg et al., 2004; Baufreton and Bevan, 2008) Increases oscillations (Shen and Johnson, 2000; Cragg et al., 2004) Decreases bursting (Baufreton and Bevan, 2008) Decreases glutamate transmission (Shen and Johnson, 2000) Decreases firing rate (Campbell et al., 1985; Hassani and Feger, 1999)	Increases firing rate (Mintz et al., 1986; Ni et al., 2001; Rommelfanger et al., 2010) Increases bursting (D5) (Baufreton et al., 2003) Decreases firing rate (Hassani and Feger, 1999)	Increases firing rate (Rommelfanger et al., 2010) Decreases GABA release (Floran et al., 2004) Decreases GABA-A currents (Shen and Johnson, 2000; Cragg et al., 2004; Baufreton and Bevan, 2008) Decreases firing rate (Hassani and Feger, 1999)
GPi	Increases GABA release (Floran et al., 1990)	Decreases firing rate (Kliem et al., 2007a) Increases GABA release (Ferre et al., 1996; Kliem et al., 2007a) Increases oscillations (Kliem et al., 2007a) Increases bursting (Kliem et al., 2007a)	Decreases firing rate (Hadipour Niktarash et al., 2008; unpublished observations)
SNr	Decreases multiunit activity (Timmerman and Abercrombie, 1996) Increases GABA release (Floran et al., 1990)	Decreases firing rate (Timmerman and Abercrombie, 1996; Kliem et al., 2007a) Increases GABA release (Timmerman and Westerink, 1995; Rosales et al., 1997; Matuszewich and Yamamoto, 1999; Trevitt et al., 2002; Acosta-Garcia et al., 2009) Increases oscillations (Kliem et al., 2007a) Increases bursting (Kliem et al., 2007a) Increased firing rate (Waszczak, 1990; Martin and Waszczak, 1994) Decreases GABA transmission (Miyazaki and Lacey, 1998; Radnikow and Misgeld, 1998) Increases glutamate release (Rosales et al., 1997) Increases glutamate transmission (Ibanez-Sandoval et al., 2006)	Decreases firing rate (unpublished observations) Inhibits GABA transmission (Martin and Waszczak, 1996) Decreases GABA release (Matuszewich and Yamamoto, 1999), (D4) (Acosta-Garcia et al., 2009) Decrease GABA transmission (Waszczak, 1990)

1999), and for D5-receptors in the rat STN (Svenningsson and Le Moine, 2002; Baufreton et al., 2003). Other studies have confirmed the expression of modest amounts of mRNA for D2-receptors, but not of D1-receptors (Mansour et al., 1992; Hurd et al., 2001) or D3-receptors (Quik et al., 2000). Neither D1- nor D2-receptor mRNA expression was found in the human STN (Augood et al., 2000). Postsynaptic D5-receptor protein expression has been identified at the light and electron microscope level in rats and monkeys (Ciliax et al., 2000; Baufreton et al., 2003; Rommelfanger et al., 2010).

Functional studies

Early studies of dopamine receptor activation in the STN suggested that dopamine receptor activation in the STN may act to decrease STN neuronal activity. Campbell et al. (1985) showed that iontophoretic application of dopamine or apomorphine *in vivo* decreased the activity of most STN neurons, while the non-specific dopamine receptor antagonist haloperidol increased neuronal firing. Other *in vivo* studies showed that microinjections of apomorphine or agonists acting at D1LRs or D2LRs into the STN reduced STN firing (Hassani and Feger, 1999). *In vitro* brain slice recording studies showed that dopamine reduces glutamatergic currents in the STN (Shen and Johnson, 2000).

However, more recent studies have supported the view that dopamine facilitates rather than inhibits neuronal firing in the STN (**Table 2**), via actions on D1LRs and D2LRs. Thus, activation of postsynaptic D1LRs were shown to increase STN activity (Baufreton et al., 2005a). This was specifically demonstrated for postsynaptic D5-receptors whose activation appears to potentiate burst firing in a subgroup of STN neurons (Baufreton et al., 2003).

There is also strong evidence for D2LR-mediated facilitation of STN activity. These may involve postsynaptic effects, as demonstrated by Zhu et al. (2002), but also prominent activation of presynaptic D2LRs. For example, activation of D2LRs in the STN was shown to reduce GABA-A receptor-mediated currents in STN neurons by reducing GABA release (Shen and Johnson, 2000; Floran et al., 2004). Studies by Cragg et al. (2004) showed that dopamine release occurs in the STN, and that dopamine depolarizes neurons, increases spontaneous spike generation, and reduces the magnitude and frequency of evoked GABA-A receptor-mediated inhibitory postsynaptic potentials in the STN. More recent *in vitro* brain slice recording studies confirmed that D2LR activation increases STN activity via a reduction of GABA release, and that this may result not only in firing rate changes, but also in a reduction of rebound bursting activities in this nucleus (Baufreton and Bevan, 2008; Johnson, 2008). Baufreton et al. (2005b) have proposed that the combined actions of dopamine on D1LRs and D2LRs on STN cells leads to increased firing and reduced bursting in most STN neurons (see also section on the effects of dopamine depletion below).

Facilitatory effects of dopamine receptor activation have also been demonstrated in several *in vivo* studies. For example, intra-subthalamic infusions of dopamine or of the D1LR agonist SKF38393 activated STN neurons in rats (Mintz et al., 1986; Ni et al., 2001). We have recently carried out preliminary studies indicating that activation of D1LRs or D2LRs in the monkey STN increases the firing rates of STN neurons, and that D1LR activation decreases bursting activities of these neurons (Rommelfanger et al., 2010).

The discrepancy between studies demonstrating inhibitory and facilitatory effects of dopamine in the STN may in part be explained by differences in the location of the recorded neurons within the STN, the choice of anesthetics, or the pharmacological properties of the drugs used in these studies. For example, dopamine binds with higher affinity to D3-, D4-, and D5-receptors than to D1-receptors (Sunahara et al., 1991; Missale et al., 1998), and SKF38393 is a partial rather than full agonist at D1LRs (Twery et al., 1994; Kreiss et al., 1996; Gleason and Witkin, 2004), complicating the interpretation of some of the earlier studies.

Studies of behavioral effects

Few studies have investigated the behavioral effects of dopamine receptor ligands in the STN. The available studies suggest that agents acting at D1LRs have stronger behavioral effects than agents acting at D2LRs. Activation of D1LRs in the STN resulted in orofacial dyskinesias in normal and dopamine-depleted rats (Parry et al., 1994; Mehta et al., 2000). In normal animals, bilateral STN infusions of D1LR- but not D2LR antagonists induced catalepsy in one study (Hauber, 1998). No such information is available from primate experiments.

INTERNAL PALLIDAL SEGMENT

Anatomical studies

GPi activity is indirectly under the control of dopamine released in the striatum, via the direct and indirect pathways. In addition, the primate GPi receives its own diffusely arborizing dopaminergic input (Parent and Smith, 1987; Lavoie et al., 1989; Parent et al., 1989; Hedreen, 1999), as demonstrated through the detection of dopamine in GPi (Pifl et al., 1990; Hornykiewicz, 1998), by the presence of DAT in ligand binding studies, and through immunohistochemical investigations on human postmortem tissue (Marcusson and Eriksson, 1988; Ciliax et al., 1999; Porritt et al., 2005), monkey (Gnanalingham et al., 1995) GPi and in the rodent entopeduncular nucleus, the rat homologue to the monkey GPi (Ciliax et al., 1995; Coulter et al., 1995). Retrograde and anterograde tract tracing studies in rodents and monkeys have demonstrated that dopaminergic terminals in GPi do not arise from collaterals of the nigrostriatal tract, but from a separate population of SNc neurons that directly innervate the GPi (Fallon and Moore, 1978; Lindvall and Bjorklund, 1979; Smith et al., 1989; Parent et al., 1990; Schneider and Dacko, 1991; Gauthier et al., 1999; Jan et al., 2000).

Most of the available evidence suggests that dopaminergic effects in GPi are primarily mediated via D1LRs (**Table 1**). In rats, D1LR binding (Fremeau Jr. et al., 1991) and D1-receptor protein (Levey et al., 1993; Yung et al., 1995) were found in the entopeduncular nucleus (the rat homologue to the monkey GPi), predominately at presynaptic locations, on axons and putatively GABAergic terminals. In primates, receptor binding studies have demonstrated the presence of D1LRs in GPi (Richfield et al., 1987; Besson et al., 1988), which was recently confirmed by our ultrastructural studies (Kliem et al., 2010). Most of the D1- and D5-receptor labeling was found in unmyelinated pre-terminal axons, with additional postsynaptic D5-receptor labeling in dendrites and glial processes in rodents and monkeys (Ciliax et al., 2000; Kliem et al., 2010).

Receptor binding studies have demonstrated that the level of D2LRs is much lower than that of D1LRs in the primate GPi (Richfield et al., 1987; Besson et al., 1988). D3-receptor binding (but not mRNA) was also demonstrated in the monkey GPi (Quik et al., 2000), and D2- and D3-receptor mRNA and binding sites have been identified in the human GPi (Gurevich and Joyce, 1999). Studies in rats and monkeys have documented D4-receptor protein in the GPi (Mrzljak et al., 1996; Rivera et al., 2003). Using electron microscopy, we recently found that D2-receptors in the monkey GPi are almost exclusively presynaptic, with some receptors at presumably glutamatergic (i.e., forming asymmetric synapses) terminals (unpublished observations). Experiments in D2-receptor knockout mice have suggested that at least some of the presynaptic D2-receptors are

autoreceptors (Mercuri et al., 1997; Koeltzow et al., 1998). Presynaptic D4-receptors in the rat entopeduncular nucleus may be located on GABAergic striatopallidal terminals (Rivera et al., 2003).

Functional studies

Overall, dopamine appears to decrease the neuronal activity in the GPi, likely via activation of D1LRs (**Table 2**). We found that microinjections of a D1LR agonist into the monkey GPi reduces GPi firing rates, and increases neuronal burst discharges and oscillatory firing in the 3–15 Hz range of frequencies. Interestingly, blockade of D1LRs in these studies resulted in increased spontaneous neuronal activity, suggesting that the D1LRs are occupied by endogenous dopamine under normal conditions (Kliem et al., 2007a). Because most D1LRs are found presynaptically on putatively GABAergic terminals (see above), it is likely that the D1LR agonist infusions into GPi acted through a facilitation of GABA release. Microdialysis studies have, in fact, directly shown that GABA levels in the entopeduncular nucleus (in rats) or GPi (in monkeys) increase in response to activation of D1LRs (Ferre et al., 1996; Kliem et al., 2007a), and that GABA release is reduced upon D1LR antagonist administration (Floran et al., 1990). Increased GABA levels may act to hyperpolarize GPi cells, lowering GPi firing and triggering rebound bursts, as in GPe and STN (Nambu and Llinas, 1994; Overton and Greenfield, 1995; Beurrier et al., 1999; Bevan et al., 2002; Kass and Mintz, 2006). The source(s) of the GABAergic inputs whose activity is regulated via D1LRs in GPi is not entirely certain, but it is likely that these fibers originate largely from the striatal medium spiny neurons that give rise to the direct pathway. Activation of postsynaptic D5-receptors and subsequent activation of GPi cells may also occur, counteracting some of the changes in GABA release induced by presynaptic D1LR activation.

There is relatively little evidence supporting D2LR-mediated effects in GPi. Peripheral administration of D2LR agonists decreases neuronal firing in human GPi cells (Hutchinson et al., 1997). Peripheral exposure to D2LR antagonists increases Fos-like immunoreactivity in the entopeduncular nucleus in normal rats (Wirtshafter and Asin, 1995), and reduces firing abnormalities in the entopeduncular nucleus in parkinsonian animals (Ruskin et al., 2002). It is likely that these drug effects are in large part secondary to activation or inactivation of striatal D2LRs, and are transmitted to GPi via the indirect pathway. In our recent experiments in monkeys, local activation of D2LRs in the GPi also resulted in decreased firing rates (Hadipour Niktarash et al., 2008), and blockade of these receptors increased firing rates (unpublished observations). The results of these local microinjection studies can perhaps be explained through activation of presynaptic D2LRs on glutamatergic terminals, although other mechanisms of action cannot be excluded.

Studies of behavioral effects

Very few studies have examined the behavioral effects of dopamine receptor activation in the GPi. Studies in human subjects have indicated that decreased dopamine levels in GPi, as measured with raclopride displacement positron emission tomography (PET), were associated with faster motor learning (Garraux et al., 2007). Studies using (18)F-DOPA PET in patients with PD suggested that pallidal (18)F-DOPA uptake may be increased in early stages of the disease, perhaps as a compensatory change (Whone et al., 2003).

SUBSTANTIA NIGRA PARS RETICULATA

Dopamine release in the SNr

Studies in the 1970s showed that dopamine release in the substantia nigra differs from that in the other basal ganglia nuclei in that the release is dendritic, rather than axonal (Korf et al., 1976; Leviel et al., 1979). Dendrites of SNc neurons may supply dopamine to SNr neurons from up to several hundred microns away, (Bjorklund and Lindvall, 1975; Nieoullon et al., 1978; Arsenault et al., 1988; Hausser et al., 1995). There continues to be debate regarding some of the characteristics of dendritic dopamine release. For instance, some studies have documented that dendritic release can be reduced by blockade of sodium channels with tetrodotoxin (Araneda and Bustos, 1989; Santiago and Westerink, 1991; Westerink et al., 1994; Cragg and Greenfield, 1997), and increased by depolarizing agents (Rice et al., 1994), and that release is calcium-dependent (Ford et al., 2010), suggesting that it may be mediated by action potentials. However, other authors concluded that the dendritic release of dopamine in the SNr is independent of action potentials (Robertson et al., 1991) and not stimulated by amphetamine (Bernardini et al., 1991; Robertson et al., 1991; Hoffman and Gerhardt, 1999; Gerhardt et al., 2002).

Another area of disagreement pertains to the question whether nigral dopamine release is vesicular. Early studies did not identify storage vesicles for dopamine in SNc dendrites (Reubi and Sandri, 1979; Wassef et al., 1981), but more recent studies have reported otherwise. Pleiomorphic vesicles have been detected in the symmetrical dendrodendritic synapses of SNc neurons (Groves and Linder, 1983) and have been shown more recently to express the vesicular monoamine transporter (Nirenberg et al., 1996). Vesicular storage of dopamine at the level of the SN is also supported by evidence that the nigral release of dopamine is sensitive to reserpine, a compound that depletes vesicular dopamine pools (Elverfors et al., 1997), as well as compounds that interfere with vesicular fusion and release (i.e., the 25 kDa synaptosome-associated protein SNAP-25; Bergquist et al., 2002). In addition, dendritic dopamine signaling can be terminated via the DAT as DAT blockade can enhance nigral dopamine levels (Robertson et al., 1991; Santiago and Westerink, 1992; Cragg et al., 1997; Cragg et al., 2001). Dendritic DAT immunoreactivity in SNc and SNr has been detected in rodent (Nirenberg et al., 1996) and human tissue (Ciliax et al., 1999).

Anatomical studies

Receptor binding studies (Richfield et al., 1987) and immunohistochemical studies of the distribution of dopamine receptor protein (Levey et al., 1993; Yung et al., 1995; Kliem et al., 2010) have shown that the rat and monkey SNr contains predominantly pre- and postsynaptic D1LRs (**Table 1**). D1-receptor immunoreactivity associated with the SNr was shown to extend into the ventral SNc (Yung et al., 1995). These receptors have been described as being expressed mostly on putative GABAergic terminals of the direct striatonigral pathway (Levey et al., 1993; Bergson et al., 1995; Yung et al., 1995; Caille et al., 1996; Kliem et al., 2010), supported by the finding that striatal lesions reduce or abolish D1LR binding in the SNr (Beckstead, 1988; Berger et al., 1991). Postsynaptic D1-receptor localization has been suggested, on the basis of the detection of D1-receptor mRNA (Fremeau Jr. et al., 1991). D5-receptors have also been identified in rodents and monkeys in postsynaptic locations (Ciliax et al., 2000; Khan et al., 2000; Kliem et al., 2010).

There are fewer reports of D2LRs in the SNr (**Table 1**). D2-receptor protein has been identified in neurons in the ventral SNr in rats (Yung et al., 1995). Furthermore, mRNA distribution and receptor binding studies have shown D2- and D3-receptors in human SNr neurons (Gurevich and Joyce, 1999). D2-receptors were also detected in cell bodies and dendrites of SNc neurons extending into the SNr (Yung et al., 1995) suggesting that D2 autoreceptors in the SNc may regulate dopamine release within the SNr. We have recently demonstrated the presence of presynaptic D2-receptors in the monkey SNr on putatively GABA- and glutamatergic synapses (unpublished observations). In addition, D4-receptor protein has been identified with electron microscopy in neurons of the monkey SNr (Mrzljak et al., 1996), and at pre- and postsynaptic locations in the rat SNr (Rivera et al., 2003).

Functional studies

Most of the available studies in rodents agree that dopamine in the SNr acts primarily at presynaptic D1LRs, and that activation of these receptors reduces SNr firing via facilitation of GABA transmission from striatonigral (i.e., direct pathway) fibers (**Table 2**). Our recent primate recording experiments have confirmed that the local activation of D1LRs in the SNr reduces the activity of SNr neurons (Kliem et al., 2007a). Furthermore, local D1LR activation influenced the discharge patterns of SNr neurons, increasing oscillations in the low frequency ranges (3–15 Hz range of frequencies) and increasing bursting (Kliem et al., 2007a,b), perhaps through the induction of rebound bursts due to GABA-mediated hyperpolarization of SNr cells, as has been demonstrated to occur in GPe and STN (Nambu and Llinas, 1994; Overton and Greenfield, 1995; Beurrier et al., 1999; Bevan et al., 2002; Kass and Mintz, 2006). Increased GABA release upon activation of D1LRs was shown in microdialysis studies, and is also supported by electrophysiologic experiments (Floran et al., 1990; Timmerman and Westerink, 1995; Rosales et al., 1997; Radnikow and Misgeld, 1998; Matuszewich and Yamamoto, 1999; Trevitt et al., 2002; Acosta-Garcia et al., 2009). Although it has also been shown that endogenous dopamine inhibits SNr neurons in anesthetized (Timmerman and Abercrombie, 1996) and awake, behaving rats (Windels and Kiyatkin, 2006), we did not find convincing pharmacological evidence for a significant dopaminergic "tone" in our recent primate experiments (Kliem et al., 2007a; unpublished observations).

Not all studies have agreed that the activation of D1LRs increases GABA release and reduces the activity of neurons in the SNr. Presynaptic *inhibition* of GABA release upon exposure to D1LR agonists was seen by some authors (Martin and Waszczak, 1994; Miyazaki and Lacey, 1998). These data corroborate previous *in vivo* studies in which the activity of SNr neurons was increased by iontophoretic application of D1LR agonist in anesthetized rats (Waszczak, 1990). It is possible that some of these excitatory effects of D1LR agonists arose from actions at non-GABAergic sites. For example, there is evidence that D1LR activation in the SNr may increase glutamate released by terminals originating from the STN (Rosales et al., 1997; Ibanez-Sandoval et al., 2006). It remains unclear why such effects were not seen in other studies.

In contrast to the large body of evidence describing the effects of D1LR activation in the SNr, there are few studies examining the effects of D2LR activation. These studies have come to contradictory conclusions (see **Table 2**). In rodents local application of agonists at D2LRs was shown to block the inhibitory effects of striatal stimulation on SNr neurons (Waszczak, 1990; Martin and Waszczak, 1996) and to reduce GABA release (Matuszewich and Yamamoto, 1999). Other studies have shown that activation of nigral D4-receptors inhibits dopamine-induced GABA release in rat brain slices, an effect that was reversed by lesions of the pallidum (Acosta-Garcia et al., 2009). In contrast, we have recently found that injections of the D2LR agonist quinpirole decreases firing rates of neurons in the monkey SNr, which may be explained through an inhibitory effect on glutamatergic afferents from the STN (Hadipour Niktarash et al., 2008).

There is also limited evidence that dendritic dopamine release may inhibit SNc neuron activity, and may, thus, indirectly affect dopamine release in the striatum, presumably resulting in secondary effect on the basal ganglia via direct and indirect pathways (Lacey et al., 1987; Pucak and Grace, 1994).

Studies of behavioral effects

The activation of dopamine receptors in the rat SNr has been shown to increase movement. Thus, infusions of agonists at D1LRs into the rodent SNr result in increased movement, drug-seeking behaviors, and an enhanced startle response (Meloni and Davis, 2004). Bilateral infusions of D1LR antagonists into the SNr were also shown to decrease lever-pressing and general locomotor activity (Jackson and Kelly, 1983a,b; Kelly et al., 1987; Trevitt et al., 2001), while unilateral injection of the D1LR antagonist inhibited amphetamine-induced stereotypies (Yurek and Hipkens, 1993; Lee et al., 1995; Timmerman and Abercrombie, 1996) and induced contralateral circling (Asin and Montana, 1988). In contrast, D1LR and D2LR antagonists impaired rod-balancing performance in normal rats (Bergquist et al., 2003). Irreversible blockade of dopamine receptors in the rat SNr was shown to increase electromyographic (EMG) activity and may contribute to the development of rigidity in parkinsonism (Crocker, 1995; Hemsley and Crocker, 1998) likely mediated via effects on D1LRs (Hemsley and Crocker, 2001). Depletion of dopamine release from SNc neurons through local intranigral administration of the VMAT2 inhibitor tetrabenazine was shown to impair motor performance in rats without altering striatal dopamine release (Andersson et al., 2006).

EXTRASTRIATAL DOPAMINE LOSS IN PARKINSONISM

While the degeneration of the dopaminergic nigrostriatal tract is the hallmark pathology of PD, substantial dopamine loss also occurs in basal ganglia areas outside of the striatum in patients with PD and in animal models of the disorder. For instance, a study of dopamine loss in monkeys rendered severely parkinsonian by injections of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) demonstrated that striatal dopamine loss of >99% was accompanied by dopamine loss in the extrastriatal basal ganglia of up to 90% (Pifl et al., 1990). Significant reductions of dopamine in the GP, SNr, and STN were also detected in the post-mortem studies on brain tissue from PD patients (Hornykiewicz, 1998). The dopamine loss in PD is accompanied by significant DAT loss in the striatum and, to a lesser extent, extrastriatal regions (Leenders et al., 1990; Porritt et al., 2005).

GPe

Loss of the nigropallidal projection has been demonstrated in patients with PD and in animal models of the disorder (Jan et al., 2000). The nigropallidal system may be more strongly affected in MPTP-treated vervet monkeys (Bergman et al., 1994; Jan et al., 2000) than in MPTP-treated macaques (Parent et al., 1990; Schneider and Dacko, 1991). This difference may contribute to the differences in the sensitivities of these species to the effects of MPTP (Pifl et al., 1992). Intra-pallidal infusions of dopamine were shown to partially restore motor deficits in rats whose midbrain dopaminergic system was damaged through infusions of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (Galvan et al., 2001). Interestingly, despite the predominance of D2LRs in the GP, intra-pallidal injections of agonists at D2LRs had no effect (El-Banoua et al., 2004). In 6-OHDA-treated rats, grafts of fetal mesencephalic cells into the GP were shown to result in behavioral recovery (Bartlett and Mendez, 2005).

SUBTHALAMIC NUCLEUS

There is substantial dopamine loss in the STN in MPTP-treated monkeys (Pifl et al., 1990; Rommelfanger et al., 2010) and in human PD patients (Hornykiewicz, 1998) which may contribute to the expression of motor signs. In addition, unilateral 6-OHDA lesions of the rat STN may result in contralateral muscle rigidity (Flores et al., 1993).

Recent studies in rodent brain slice preparations have suggested that the reduction of dopaminergic transmission in the parkinsonian state results in a lack of activation of D2LRs and D1-receptors which, in turn, contributes to the development of irregular discharges in the STN (see section on STN above). As D5-receptors are constitutively active, even in the absence of dopamine (Tiberi and Caron, 1994; Demchyshyn et al., 2000), D5-receptor activation in the dopamine-depleted state may contribute to the development of burst discharges in the STN (Baufreton et al., 2005b), a feature of parkinsonism in monkeys (Bergman et al., 1994) and humans (Hutchison et al., 1998).

The STN may be a target for dopaminergic drug treatments. For instance, intra-STN infusions of D1LR agonists reduced the motor asymmetry in rats with ipsilateral 6-OHDA lesions of the SNc (El-Banoua et al., 2004). The contribution of D5-receptor activation to neuronal bursting in the STN has been exploited in recent experiments in which the dopamine receptor antagonist flupenthixol reduced bursting activities of STN neurons in the dopamine-depleted state, presumably through actions on constitutively active D5-receptors (Chetrit et al., 2010).

Transplantation of dopaminergic tissue or stem cells into the STN alone (Anderson and Caldwell, 2007) or in combination with striatal or nigral transplants has resulted in improved forepaw use or rotational behaviors in dopamine-depleted rats (Mukhida et al., 2001; Pavon-Fuentes et al., 2002; Inden et al., 2005), although this effect has been questioned by others (Pavon-Fuentes et al., 2002). The behavioral studies exploring the effects of injections or tissue grafts in rodents need to be replicated in monkeys before firm conclusions regarding their significance can be drawn. Because of the very small size of the rat STN, agents or cells injected in this nucleus may inadvertently diffuse to the neighboring SNr. Other than the results listed above for the STN, there is little experience with the effects of stem cells into extrastriatal regions. Given the relatively low yield of dopaminergic cells from traditional graft sources, the use of higher yield stem cell therapies aimed at replacing dopamine in extrastriatal basal ganglia regions may be a worthwhile future clinical strategy.

GPi AND SNr

While there is evidence of dopamine loss in the GPi of humans with PD (Hornykiewicz, 1998) and of MPTP-treated monkeys (Pifl et al., 1990), the behavioral consequences of this loss are not fully understood. As mentioned above, PET studies in humans have suggested that dopamine loss in the GPi may be involved in some of the early compensatory changes in PD (Whone et al., 2003).

An involvement of dopamine loss in the substantia nigra is supported by experiments exploring the effects of nigral infusions of dopamine receptor antagonists or reductions of nigral dopamine release in rats (see section on SNr above). These studies have suggested that actions of dopamine in the SNr may be involved in the control of normal movement and in the early compensation for striatal dopamine loss (Andersson et al., 2006). This is also suggested by behavioral experiments described earlier in this review wherein dopamine receptor activation can facilitate movement in rodents. It is unclear whether such motor effects would also occur in primates, as the primate SNr is more strongly involved in non-motor rather than motor behaviors (Parent and Hazrati, 1994; Haber and Fudge, 1997; Middleton and Strick, 2002). However, intraventricular and intranigral infusions of glial derived nerve-growth factor (GDNF) were shown to reduce parkinsonian motor deficits in MPTP-treated monkeys, associated with increased dopamine levels in the SN and the GP, but not in the striatum (Gash et al., 1996). GDNF has been clinically tested, but the therapeutic value of the explored GDNF treatment strategies, specifically the chosen delivery method and targeting, remains controversial (Gill et al., 2003; Nutt et al., 2003; Slevin et al., 2005; Lang et al., 2006). It is perhaps worth noting that the available human studies have not specifically examined (in isolation) the use of GDNF in extrastriatal tissues.

Several groups have shown that intranigral grafts of embryonic mesencephalic tissue attenuate rotational behavior and other behavioral abnormalities in 6-OHDA-treated rats (Nikkhah et al., 1995a,b; Olsson et al., 1995; Yurek, 1997; Johnston and Becker, 1999; Mukhida et al., 2001; Palmer et al., 2001) and in MPTP-treated monkeys (Starr et al., 1999; Collier et al., 2002). Furthermore, dual intrastriatal and intranigral grafts of fetal dopaminergic tissue in humans helped to improve parkinsonism in PD patients, although not with greater benefit than intrastriatal grafts (Mendez et al., 2002).

EFFECTS OF CLINICALLY USED DRUG TREATMENTS AT EXTRASTRIATAL SITES

One of the factors that determines whether clinically used dopaminergic antiparkinsonian drugs act at basal ganglia sites outside of the striatum is the availability and functional integrity of dopamine receptors at these sites in the parkinsonian state. There are, in fact, some reports of changes in the density of extrastriatal dopamine receptors in parkinsonian animals and in patients with PD. For instance, altered D1LR- and D2LR binding has been demonstrated in the STN (Flores et al., 1999; Murer et al., 1999; Mehta et al., 2000) and SN (Narang and Wamsley, 1995). Furthermore, the fraction of membrane-bound D1LRs in SNr and GPi appears to increase in

dopamine-depleted animals (Kliem et al., 2010). In human studies, D1LR radioligand binding was decreased while the mRNA levels remained unchanged in the GPe (Hurley et al., 2001). Another study did not detect any changes in D1LR- or D2LR binding at these sites (Cortes et al., 1989).

There is little evidence that the function of D1LRs or D2LRs in the extrastriatal basal ganglia changes from the normal to the dopamine-depleted state. In our recent comparison of changes in neuronal firing rates and patterns in response to local administration of agonists at D1LRs or D2LRs in GPe, STN, GPi, and SNr, no response differences were detected between normal and parkinsonian animals (Kliem et al., 2010; unpublished observations). Taken together the extrastriatal basal ganglia could be targets for clinically used dopaminergic agonists, such as the commonly used agonists pramipexole and ropinirole. These D2LR-preferring agents may not only act in the striatum, but also at the level of the GPe or its afferents, and perhaps at glutamatergic synapses in GPi and SNr (see above). Activation of extrastriatal D2LRs may act to reduce the irregularity of neuronal firing (through actions in the STN) and the overall activity at the level of the basal ganglia output nuclei (through actions in SNr and GPi).

A more detailed understanding of the effects of extrastriatal dopamine activation could also lead to a better understanding of the mechanisms involved in the frequent non-motor side effects of D2LR agonist therapies, such as disturbances in the control of impulsivity (Isaias et al., 2008), fatigue or hallucinations (Stowe et al., 2008; Truong et al., 2008). Such effects are most likely due to striatal actions of these drugs; however, extrastriatal actions may also play a role. Thus, recent studies have suggested that the STN and probably other basal ganglia areas may be part of the circuitry regulating impulsivity (Uslaner and Robinson, 2006) and reward related behaviors (Baunez et al., 2005; Joshua et al., 2009; Rouaud et al., 2010).

CONCLUDING REMARKS

It is now clear that not only the striatum, but also all of the extrastriatal basal ganglia nuclei receive dopaminergic projections. While biochemical studies have shown measurable dopamine levels in all of these nuclei, our pharmacological studies in monkeys found evidence for an endogenous tone only in GPe and GPi.

The signals carried by the dopaminergic fibers to the extrastriatal basal ganglia may overlap with those carried to the striatum, but are probably not identical with them. For instance, because the STN receives collaterals of the nigrostriatal projection, it can be expected that the dopaminergic inputs to this nucleus carry some of the same information that is also transmitted from the SNc to the striatum. In contrast, the GPi and to a much lesser extent, the GPe receives a dopaminergic projection that is separate from that terminating in the striatum so that the signals it receives may differ from those that reach the striatum. There may also be substantial heterogeneity within the nuclei themselves. Thus, in monkeys, histological studies have demonstrated dopaminergic inputs to the dorsal regions of GPe and STN, while more ventral portions of these nuclei may receive fewer (or no) dopaminergic inputs. The actual "reach" of dopamine, and the timing and strength of its effects within each of these nuclei will, of course, not only be determined by the anatomical innervation, but also by the range of diffusion. The factors influencing diffusion, in these nuclei such as the distribution and density of dopamine receptors, and the expression pattern and concentration of DAT are not sufficiently known at this time.

The large body of literature that is reviewed in this article demonstrates that virtually all of the dopamine receptor subtypes are expressed in each of the extrastriatal basal ganglia, albeit with different patterns of pre- or postsynaptic expression. With some exceptions, it appears that activation of D1LRs and D2LRs within the individual nuclei generates similar responses. For instance, our primate studies have demonstrated that activation of D1LRs in GPi and SNr leads to an inhibition of firing, most likely explained through increased GABA release in these nuclei. We also found that D2LR activation reduces neuronal activity in these nuclei, perhaps through reductions of glutamate release from STN inputs. Another example for the overall similarity of D1LR and D2LR activation would be the actions of dopamine in the STN. D1LR activation may increase the activity of STN neurons via postsynaptic effects, while D2LR activation could achieve the same effect through presynaptic inhibition of GABA release. The view that D1LR and D2LR effects are in some sense similar is obviously simplistic, but it may result in the recognition of overall response patterns of neurons in these nuclei to endogenous dopamine: the activity of GPe and STN neurons appears to be increased, while the activity of the basal ganglia output nuclei, GPi and SNr, appears to be reduced.

As mentioned above, there is clear evidence that dopamine is lost at extrastriatal sites in PD, and it is possible that the loss of dopamine at these sites contributes to the development of some aspects of parkinsonism. While the behavioral effects of activation or blockade of dopamine receptors at extrastriatal sites still needs to be clarified, it is clear that dopamine receptor activation in all of the nuclei discussed have strong effects on neuronal activities, even in the parkinsonian state. It seems therefore likely that these receptors mediate some of the beneficial and adverse effects of commonly used antiparkinsonian dopamine receptor agonist regimens.

In practical terms, the knowledge regarding dopaminergic effects at extrastriatal sites could be used for site-specific dopaminergic therapies in PD patients. By targeting some of the known key steps in the pathophysiology of PD, some of the well-known side effects of existing dopaminergic treatments could potentially be avoided. For instance replacement of dopamine in STN or GPe may help us to reduce neuronal bursting activities, while replacement of dopamine in GPi or SNr could reduce overall basal ganglia output. Given the ubiquitous presence of dopamine receptor subtypes in the striatum and extrastriatal basal ganglia, it will be challenging to devise systemic pharmacological treatments to achieve dopaminergic effects at specific basal ganglia locations. However, such specificity could be achieved by surgical procedures to re-establish dopaminergic stimulation in specific basal ganglia nuclei, through grafting, stem cell therapies, viral transfection methods, or even some of the newly developed optogenetic approaches targeting G-protein coupled receptors (Airan et al., 2009). Thus, understanding the functions of extrastriatal dopamine could not only provide a more comprehensive view of the role of dopamine in the basal ganglia, but also may prove therapeutically fruitful in the long-term.

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Centrality of striatal cholinergic transmission in basal ganglia function

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Work over the past two decades revealed a previously unexpected role for striatal cholinergic interneurons in the context of basal ganglia function. The recognition that these interneurons are essential in synaptic plasticity and motor learning represents a significant step ahead in deciphering how the striatum processes cortical inputs, and why pathological circumstances cause motor dysfunction. Loss of the reciprocal modulation between dopaminergic inputs and the intrinsic cholinergic innervation within the striatum appears to be the trigger for pathophysiological changes occurring in basal ganglia disorders. Accordingly, there is now compelling evidence showing profound changes in cholinergic markers in these disorders, in particular Parkinson's disease and dystonia. Based on converging experimental and clinical evidence, we provide an overview of the role of striatal cholinergic transmission in physiological and pathological conditions, in the context of the pathogenesis of movement disorders.

Keywords: acetylcholine, striatum, interneuron, Parkinson's disease, dystonia, movement disorders

INTRODUCTION

The basal ganglia include different interconnected subcortical nuclei that are involved in serving critical motivation, motor planning, and procedural learning function (Graybiel et al., 1994; Yin and Knowlton, 2006; Nicola, 2007; Kreitzer and Malenka, 2008). The striatum represents the main input nucleus of the basal ganglia. It receives excitatory afferents from the cortex and thalamus, and is densely innervated by midbrain dopamine neurons (Bolam et al., 2000; Kreitzer and Malenka, 2008).

The large majority of striatal neurons are GABAergic. Most of these GABAergic neurons are represented by medium spiny projection neurons (MSNs; Izzo et al., 1987). At least three types of GABAergic interneurons have been identified, according to their electrophysiological and neurochemical properties. GABAergic interneurons may colocalize with the calcium-binding proteins parvalbumin or calretinin, or neuropeptide Y, somatostatin, and NADPH diaphorase (Kawaguchi, 1993; Tepper and Bolam, 2004). Accordingly, they have been classified, respectively, as fast-spiking (FS) neurons, persistent and low-threshold spike (PLTS) neurons, or low-threshold spike (LTS) neurons (Kawaguchi et al., 1989; Tepper and Bolam, 2004). A recent study has characterized an additional group of GABAergic interneurons, expressing tyrosine hydroxylase (TH^+), which have been electrophysiologically classified into four distinct types (Tepper et al., 2010). Indeed, the existence of TH^+ neurons in the striatum of rodents and primates had been reported since the late 1980s (for review, see Ibáñez-Sandoval et al., 2010).

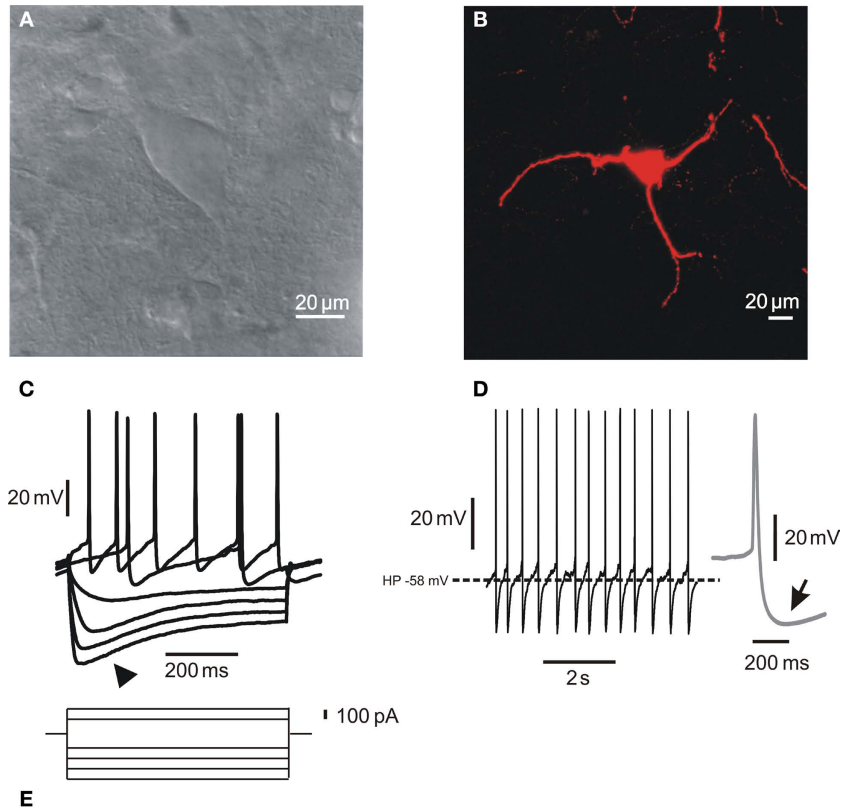
In addition to the numerically prevailing population of GABAergic neurons, the striatum also contains a small percentage of interneurons which provide this area with one of the highest acetylcholine (ACh) levels in the brain (Graybiel, 1990; Mesulam et al., 1992; Contant et al., 1996). These are the large aspiny cholinergic interneurons (ChIs) characterized by dense local axonal arborizations, and by tonic firing activity (Bolam et al., 1984; Wilson et al., 1990; Kawaguchi, 1993; Aosaki et al., 1995; Bennett and Wilson, 1998; Bennett et al., 2000; Zhou et al., 2002).

It has long been known that striatal ChIs play a central role in the basal ganglia circuitry both in the control of voluntary movements and in the pathophysiology of movement disorders, such as Parkinson's disease (PD), and dystonia (Pisani et al., 2003a, 2007; Aosaki et al., 2010). Indeed, anticholinergic drugs have long been a first choice therapy for PD and dystonia (Duvoisin, 1967; Jankovic, 2006). Here, in light of the most recent findings, we will review the role of ChIs in striatal function and in the pathogenesis of basal ganglia disorders.

MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF CHOLINERGIC INTERNEURONS

Large aspiny ChIs represent less than 2% of the entire striatal neuronal population. Their neurochemical identification is due to the expression of ChAT, the biosynthetic enzyme for ACh. Morphologically (**Figures 1A,B**), they are characterized by a large polygonal soma (Ø 20–50 μm), widespread dendritic and axonal fields (Bolam et al., 1984; Smith and Bolam, 1990; Wilson et al., 1990), and a preferential distribution in the matrix area flanking the patches border (van Vulpen and van der Kooy, 1998). These features suggest that ChIs may integrate synaptic inputs over relatively large regions, and act as an associative interneuron in the striatum (Kawaguchi et al., 1995; Miura et al., 2007).

In vitro electrophysiological recordings have described the peculiar membrane properties of ChIs, that distinguish these neurons from all other striatal neuronal subtypes (**Figures 1C–E**). These include a relatively depolarized resting membrane potential, long-lasting action potential, high input resistance, prominent afterhyperpolarization (AHP) current, and hyperpolarization-activated



E

Resting membrane potential (mV)	Action potential amplitude (mV)	AHP duration (ms)	Input resistance (MΩ)
- 62 ± 2.7	64.5 ± 3.9	340 ± 50	143 ± 41

FIGURE 1 | Morphological and electrophysiological properties of striatal cholinergic interneurons. (A) Infrared differential interference contrast image of a cholinergic interneuron in a striatal slice showing the peculiar polygonal shape and large somatic size of this neuronal subtype. **(B)** Confocal microscope image of a biocytin-loaded cholinergic interneuron. The cell was loaded with 2% biocytin by means of the recording electrode during an electrophysiological experiment. Note the absence of spines along the dendrites. **(C)** Representative current-clamp recording of the I–V relationship. The arrowhead indicates the prominent I_h evoked by hyperpolarizing current injection. **(D)** Spontaneous firing activity of a cholinergic interneuron. The inset on the right (gray) shows a single action potential, followed by a prominent AHP (arrow). **(E)** Table summarizing the main electrophysiological properties characterizing striatal cholinergic interneurons. Data are presented as mean ± SEM; $n = 5$.

cation current (I_h; Bolam et al., 1984; Wilson et al., 1990; Kawaguchi, 1993; Aosaki et al., 1995; Bennett and Wilson, 1998; Bennett et al., 2000; Zhou et al., 2002).

These cells are autonomously active, showing a range of spontaneous tonic firing patterns, from irregular single spiking to rhythmic bursting, even in the absence of synaptic input, suggesting that they are intrinsic in origin (Bennett and Wilson, 1999; Bennett et al., 2000; Goldberg and Wilson, 2005; Wilson, 2005; Wilson and Goldberg, 2006; Goldberg et al., 2009). The prevalence of a spiking pattern in any single neuron was shown to be dependent on the underlying Ca^{2+}-activated K^+ conductances. In particular, single spiking depends on a medium-duration AHP (mAHP) current generated by rapid SK currents, which are associated with high-voltage-activated (HVA) $Ca_V2.2$ Ca^{2+} channels. On the other hand, periodic bursting is driven by a delayed and slowly decaying AHP (sAHP) current, associated with Ca_V1 Ca^{2+} channels (Bennett et al., 2000; Goldberg and Wilson, 2005; Wilson and Goldberg, 2006). The specific association between HVA Ca^{2+} channel subtypes and the K^+ currents underlying the mAHP and sAHP currents is generated by the dynamics of Ca^{2+} redistribution among cytoplasmic binding sites with different binding kinetics (Goldberg et al., 2009).

Striatal ChIs are recipients of a prominent glutamatergic drive from both the cortex and the centromedian and parafascicular (Cm–Pf) thalamic nuclei (Lapper and Bolam, 1992; Sidibe and Smith, 1999; Thomas et al., 2000), as well as of an extensive dopaminergic innervation from the substantia nigra pars compacta (Olson et al., 1972; Lavoie et al., 1989; Dimova et al., 1993; Smith and Villalba, 2008).

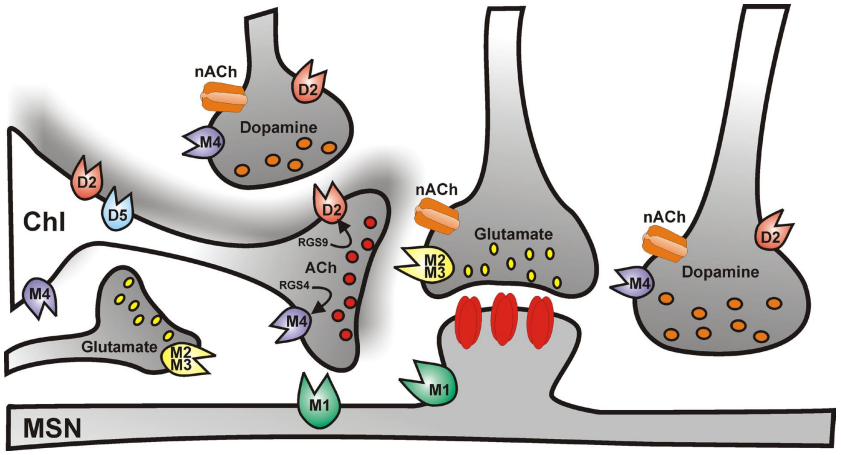


FIGURE 2 | Cholinergic control of striatal medium spiny neuron activity. Simplified cartoon of the striatal circuitry reporting the distribution of muscarinic and nicotinic receptors. Cholinergic receptors regulate the activity of medium spiny neurons both at the postsynaptic level, and presynaptically, by modulating glutamate, dopamine, and acetylcholine neurotransmission.

The predominant effect of dopamine on ChIs is mediated by activation of D2-like D_2 receptors (**Figure 2**), which inhibit striatal ACh efflux (DeBoer et al., 1996), by reducing both autonomous action potential firing and synaptic inputs to ChIs. The former effect is achieved by enhancing the slow inactivation of voltage-dependent Na^+ channels (Maurice et al., 2004) and by modulating I_h current (Deng et al., 2007). The reduction of synaptic inputs is achieved through inhibition of HVA Ca^{2+} channel (Yan and Surmeier, 1996; Pisani et al., 2000).

In addition, striatal ChIs express D1-like D_5 subtype receptors (**Figure 2**; Bergson et al., 1995; Yan and Surmeier, 1997), which are mainly somatodendritic and depolarize the cell by promoting the non-selective opening of cation channels and the closure of K^+ channels, thus, in turn, enhancing ACh release (Damsma et al., 1990; Imperato et al., 1993; DeBoer and Abercrombie, 1996; Aosaki et al., 1998; Pisani et al., 2000).

An additional level of control of striatal ACh release is represented by M2/M4 muscarinic autoreceptors (**Figure 2**). Autoreceptor activation reduces ACh release by closing Ca_V2 Ca^{2+} channels which mediate exocytosis, and by increasing opening of Kir3 potassium channels, which hyperpolarize terminals and further reduce Ca^{2+} channel opening (Yan and Surmeier, 1996; Calabresi et al., 1998b).

Furthermore, ChIs receive extrinsic excitatory serotonergic (Lavoie et al., 1989; Bonsi et al., 2007) and noradrenergic afferents (Pazos et al., 1985; Pisani et al., 2003b), and an intrinsic inhibitory GABAergic innervation from both MSNs and FS interneurons (Bolam et al., 1986; Martone et al., 1992; Aosaki et al., 2010).

Postsynaptic potentials evoked by electrical stimulation of fibers innervating ChIs are mediated by activation of ionotropic NMDA, AMPA, and $GABA_A$ receptors. Upon complete inhibition of both the glutamatergic and GABAergic synaptic components, a slow inhibitory synaptic potential is unmasked, which is mediated by a K^+ conductance activated by M2-like receptors (Calabresi et al., 1998b).

The activity of striatal ChIs is therefore highly regulated, through a complex interaction between intrinsic properties and the neuromodulatory control exerted by several transmitters.

ORIGIN OF THE PAUSE RESPONSE IN ChI TONIC FIRING ACTIVITY

Striatal ChIs exhibit a variety of spontaneous firing patterns also during *in vivo* recordings (Wilson et al., 1990; Reynolds et al., 2004). Indeed, these neurons correspond to the tonically active neurons (TANs) recorded *in vivo* from the primate striatum, which respond with a pause in their ongoing firing activity to reward-related stimuli (Apicella et al., 1991, 1998; Aosaki et al., 1994;). Several mechanisms are likely to contribute to this pause response, through the modulation of both intrinsic and synaptic properties of ChIs. It has been suggested that these pauses in firing may be due to AHP currents intrinsically generated via I_h transient deactivation following cortical excitatory synaptic inputs (Reynolds et al., 2004; Oswald et al., 2009). Of interest, I_h is regulated by dopamine (Deng et al., 2007). In fact, it is known that synaptic inputs arising both from the dopaminergic nigrostriatal system and from thalamic nuclei involved in sensorimotor integration modulate the responsiveness of these neurons to reward-related stimuli (Aosaki et al., 1994; Matsumoto et al., 2001). High-frequency stimulation (HFS) of the substantia nigra during *in vivo* recordings increases the AHP (Reynolds et al., 2004). Similarly, in neurons exhibiting regular firing *in vitro* exogenous application of dopamine causes a prolongation of a depolarization-induced pause and an increase in the duration of sAHP (Deng et al., 2007). Recent *in vitro* experimental evidence shed further light on the origin of the pause response in striatal ChIs (Ding et al., 2010). This report showed that high-frequency thalamic stimulation elicits an initial burst followed by a pause in the firing activity of ChIs. Both D_2 dopamine and nicotinic ACh (nACh) receptors were shown to be involved in this response. These data suggest that the biphasic response to thalamic stimulation might be driven by the initial excitation of ChIs, which induces ACh release and activation of presynaptic nACh receptors located on dopaminergic

terminals (**Figure 2**); hence the stimulation of dopamine release and D_2 receptor activation, which prolongs the AHP by inhibiting I_h and Na^+ channel currents (Aosaki et al., 2010).

Striatal ChIs have been shown, both *in vivo* and *in vitro,* to undergo long-term plastic changes of synaptic efficacy, which might lastingly influence the pattern of firing activity (Suzuki et al., 2001b; Bonsi et al., 2004; Reynolds et al., 2004; Fino et al., 2008). In slice preparations HFS of glutamatergic afferent fibers induces a long-term potentiation (LTP) of both the AMPA-mediated excitatory and GABAergic inhibitory postsynaptic potentials, which is dependent on D_5 receptor activation, and on a critical level of intracellular Ca^{2+} rise through Ca_V1 channels (Suzuki et al., 2001b; Bonsi et al., 2004). Interestingly, intracellular recordings of ChIs from striatal slices of rats that have learned a rewarded, externally cued sensorimotor task show an increase in spontaneous $GABA_A$-mediated synaptic activity with respect to untrained animals (Bonsi et al., 2003), further suggesting a role for GABAergic transmission in the generation of the pause response. More recently, spike-timing-dependent plasticity (STDP) protocols were shown to induce bidirectional long-term plasticity in ChIs (Fino et al., 2008). STDP–LTP was mainly presynaptic and involved NMDA-receptor activation, while long-term depression (STDP–LTD) had a postsynaptic origin and involved metabotropic glutamate receptors.

Thus, it is plausible that long-term changes of both glutamatergic and GABAergic synaptic potential amplitude are also involved in the generation of the firing activity pattern (Aosaki et al., 2010).

The pattern of spiking and pauses of ChIs is able to filter the striatal output, by directly and indirectly influencing MSN activity (Phelps et al., 1985; Izzo and Bolam, 1988; Chang and Kita, 1992; Wang et al., 2006; Pakhotin and Bracci, 2007; Bonsi et al., 2008). There is experimental evidence indicating that the pauses in ChIs activity might powerfully enhance the salience of dopamine signaling (Threlfell et al., 2010) and transform the reward signal arising from dopaminergic neurons into a gating signal for LTD induction at MSNs (Wang et al., 2006). Further, the thalamic-induced burst-pause response of ChIs might provide a neural substrate for attentional shift and cessation of ongoing motor activity (Ding et al., 2010). Indeed, the patterned activity of ChIs has been suggested to differentially gate the cortical drive to striatopallidal and striatonigral MSNs. Upon thalamic stimulation, the initial burst response of ChIs triggers the transient suppression of cortical inputs to MSNs, through presynaptic muscarinic M2-class receptor activation, but also initiate a slower, muscarinic M1 receptor-dependent postsynaptic facilitation of striatopallidal MSNs. This facilitation extends during the pause response, when the cortical drive resumes, thus creating a late temporal window when the corticostriatal input can selectively drive activity in the striatopallidal network thought to control action suppression (Ding et al., 2010).

MUSCARINIC AND NICOTINIC MODULATION OF MSN ACTIVITY

A very dense cholinergic innervation of the striatum arises from intrinsic ChIs. By tonically firing action potentials at about 5 Hz, these interneurons provide an ongoing ACh signal, that is rapidly terminated by acetylcholinesterase (AChE). ACh may act both at synaptic sites, predominantly onto distal dendrites and spine necks (Bolam et al., 1984; Phelps et al., 1985), and via volume transmission (Descarries et al., 1997; Koos and Tepper, 2002).

In the striatum, different subtypes of nACh receptors have been identified, containing a combination of the α4, α6, α7, and β2, β3 subunits (Wada et al., 1989; Seguela et al., 1993; for review, see Quik et al., 2007). In addition, both M1-like and M2-like muscarinic ACh (mACh) receptors, predominantly the M1 and M4 subtypes, are expressed at high density (**Figure 2**).

In MSNs, M1 receptor activation enhances NMDA-receptor-mediated currents, promoting cell depolarization and corticostriatal LTP (Calabresi et al., 2000), and increases the synchrony in the NMDA-induced network dynamics, via enhancement of persistent Na^+ current (Carrillo-Reid et al., 2009). In addition, M1 receptors modulate HVA Ca^{2+} currents (Howe and Surmeier, 1995; Galarraga et al., 1999; Olson et al., 2005; Perez-Rosello et al., 2005; Perez-Burgos et al., 2008, 2010). Recently, M1 receptor activation has been suggested to have cell-specific effects on striatopallidal vs. striatonigral MSNs, due to specific characteristics of the downstream effectors (Chen et al., 2006; Shen et al., 2007; Day et al., 2008).

In addition to direct postsynaptic effects on MSNs, presynaptic ACh receptors regulate both glutamate and GABA release from striatal afferents. While mACh receptors inhibit neurotransmitter release, presynaptic nACh receptors exert the opposite effect (Calabresi et al., 1998a; Koos and Tepper, 2002; Zhou et al., 2002; Grilli et al., 2009; McClure-Begley et al., 2009).

The autonomous activity of ChIs ensures a sufficient level of endogenous ACh to tonically activate mACh and nACh receptors, thereby constantly influencing striatal activity. Through mACh receptor activation, ACh provides a presynaptic inhibitory tone on the excitatory glutamatergic drive onto MSNs (Pakhotin and Bracci, 2007). Indeed, a single spike in a ChI is able to induce a significant mACh receptor-mediated depression of glutamatergic synaptic currents in a MSN. However, a mechanism to limit this powerful inhibitory control of ChIs over the glutamatergic input to MSNs has been recently proposed to reside in the nicotinic excitation of striatal GABAergic interneurons (Sullivan et al., 2008).

Overall, nACh and mACh receptors would act to translate the pattern of the ongoing cholinergic activity into a strong influence over striatal output (Koos and Tepper, 2002): nACh receptor activation would rapidly affect the activity of MSNs, while the muscarinic impact might become more evident on a slower time scale, and in particular when additional extrasynaptic volume transmission extends the duration of the ACh signal, such as during periods of more intense cholinergic activity (Singer et al., 2002).

STRIATAL ACETYLCHOLINE AND SYNAPTIC PLASTICITY

Enduring changes in synaptic efficacy at corticostriatal synapses are viewed as the cellular basis underlying motor learning and associative memory processes. HFS of corticostriatal afferents may induce either LTD or LTP at MSN synapses, depending on a variety of cell-specific mechanisms (Calabresi et al., 1992; Lovinger et al., 1993; Surmeier et al., 2009). Induction of LTD and LTP requires an intact nigrostriatal projection, and depends upon both dopamine and ionotropic glutamate receptor subtypes involved (Lovinger, 2010). Complex biochemical processes follow the activation of glutamatergic and dopaminergic receptors and their mutual interplay (Calabresi et al., 1994; Gerdeman et al., 2002).

M1 mACh receptors are abundantly expressed on dendrites and spines of MSNs (**Figure 2**), and are therefore likely to exert a relevant influence on synaptic plasticity (Hersch et al., 1994; Yan et al., 2001). In fact, activation of postsynaptic M1 muscarinic receptors increases MSN excitability, by reducing dendritic K^+ currents (Galarraga et al., 1999; Shen et al., 2005). As a consequence, M1 receptor activation promotes MSN depolarization and plays a permissive role in corticostriatal LTP (Calabresi et al., 1999). Accordingly, the M1 receptor antagonist pirenzepine prevents LTP, whilst methoctramine, an M2-like receptor blocker, enhances the magnitude of this form of synaptic plasticity (Calabresi et al., 2000). In addition, M1 receptor activation reduces the opening of Ca_V1 channels, in response to depolarization, that is necessary for LTD induction (Calabresi et al., 1994; Choi and Lovinger, 1997; Kreitzer and Malenka, 2005). Indeed, LTD induction requires D_2 receptor activation in order to pause ChI firing activity and reduce M1 receptor tone (Wang et al., 2006).

In summary, manipulation of ACh tone is expected to affect the direction of corticostriatal synaptic plasticity. In fact, loss of autoreceptor function in M2/M4 receptor knockout mice increases striatal ACh tone and impairs selectively LTD induction at MSN synapses. Accordingly, in these mice LTD can be restored by reducing ACh levels with hemicholinium-3, which depletes endogenous ACh (Bonsi et al., 2008).

CHOLINERGIC SIGNALING IN DISEASE STATES

PARKINSON'S DISEASE

In the early 1960s anticholinergic drugs were introduced in the pharmacological treatment of PD, according to the evidence of an imbalance between dopaminergic and cholinergic transmission within the striatum (Barbeau, 1962; Duvoisin, 1967; Hornykiewicz and Kish, 1987).

Although the increased striatal ACh level has long been attributed to the removal of tonic inhibitory control by D_2 receptors on ChIs (Maurice et al., 2004), recent experimental work has investigated in more detail ChI function in acute dopamine depletion models of PD (Fino et al., 2007; Salin et al., 2009). As expected, in dopamine-depleted animals ChIs displayed an increased excitability *in vitro* (Fino et al., 2007), and became highly synchronized in firing rhythmic bursts *in vivo* (Raz et al., 1996, 2001). This altered pattern of activity might result in periodic outbreaks of ACh release into the striatum which might not be readily hydrolyzed by AChE. Such alterations in ACh input are likely to underlie the loss of synaptic plasticity (Pisani et al., 2005) and to contribute to the pruning of spines (Shen et al., 2007) reported in MSNs from dopamine-depleted animals, contributing to imbalanced striatal outflow in the parkinsonian state.

Interestingly, recent experimental evidence revealed a novel mechanism by which mACh receptor signaling would disrupt striatal activity (Ding et al., 2006). "Regulators of G protein signaling" (RGS) proteins are GTPase accelerating proteins (GAPs), which terminate G protein coupling between receptors and effectors. Alterations in dopamine content have been shown to rapidly modify the expression of several RGS proteins. These authors report that dopamine depletion does not alter D_2 dopamine receptor signaling in ChIs, but leads to a decreased mACh M4 receptor coupling to Ca^{2+} channels, thereby modifying ChIs excitability. Moreover, they show that this impaired coupling is caused by the selective upregulation of RGS4 expression (Ding et al., 2006).

A very recent paper by Ding et al. (2011) has suggested unexpected roles for ChIs also in the adverse motor effects, dyskinesias, induced by prolonged treatment of PD patients with the dopamine replacing agent 3,4-L-dihydroxphenylalanine methyl ester (L-DOPA). These authors have shown in PD rodent models that repeated L-DOPA exposure causes activation of extracellular signal-regulated kinase 1/2 (ERK) and, in turn, an increased basal firing rate and dopamine-dependent excitation in striatal ChIs. These specific responses of ChIs to chronic L-DOPA treatment correlated with the expression of dyskinesia. Accordingly, muscarinic receptor antagonism reduced L-DOPA-induced dyskinesia.

DYSTONIA

As in PD, anticholinergic drugs targeting mACh receptors are also effective in the treatment of another movement disorder, dystonia. DYT1 dystonia is a severe form of inherited dystonia, characterized by involuntary twisting movements and abnormal postures. Although the pathogenesis of this disabling disorder remains to be fully elucidated, an altered coupling of dopaminergic and cholinergic signaling has been recently demonstrated in the striatum of mice over-expressing the human protein torsinA with the mutation responsible for DYT1 dystonia (Pisani et al., 2006). In these mice, D_2 receptor activation induces an excitatory, rather than inhibitory, effect in ChIs. This paradoxical effect was associated to an increase in the functional representation of Ca_V2 Ca^{2+} channels, that regulate Ca^{2+} entry and the physiological pacemaking activity of these interneurons, likely enhancing ACh release. Indeed, the activity of endogenous AChE was increased in the striatum of DYT1 mice, suggesting a compensatory mechanism to reduce an increased cholinergic tone. In accordance to the proposed role of ACh levels in determining the direction of corticostriatal synaptic plasticity (Bonsi et al., 2008), the elevation in cholinergic tone in DYT1 mice was correlated to the loss of LTD and synaptic depotentiation, and the enhancement of LTP (Martella et al., 2009). This notion was supported by the observation that these alterations were normalized by lowering ACh tone with hemicholinium-3, a depletor of endogenous ACh. Moreover, the clinical drug trihexyphenidyl as well as pirenzepine, both mACh M1 receptor antagonists, were effective in restoring normal synaptic plasticity. These observations might explain the efficacy of anticholinergic drugs in the treatment of dystonia.

OTHER MOVEMENT DISORDERS

Functional imaging and post-mortem studies have revealed a significant loss of striatal cholinergic markers in different basal ganglia disorders (Suzuki et al., 2002; Warren et al., 2005; Smith et al., 2006; Kataoka et al., 2010). Huntington's disease (HD) is an autosomal dominant neurodegenerative disease, caused by a mutation in the gene encoding Huntingtin, characterized by involuntary choreiform movements, behavioral and cognitive impairment. Though striatal ChIs have been reported to be spared during striatal degeneration in HD (Graveland et al., 1985), recent studies suggest that they might be functionally altered. Indeed, the levels of both the vesicular ACh transporter (VAChT) and choline acetyltransferase

(ChAT) are markedly decreased in the striatum of HD transgenic mice, as well as in post-mortem striatal tissue from HD patients (Spokes, 1980; Suzuki et al., 2001a; Vetter et al., 2003; Smith et al., 2006). Moreover, in two experimental models of HD, 3-nitropropionic acid-treated rats and R6/2 transgenic mice, striatal ChIs did not express LTP (Picconi et al., 2006). Progressive supranuclear palsy (PSP) is a progressive neurodegenerative disease characterized by accumulation of tau protein and akinetic-rigid features, falls, supranuclear gaze palsy, and subcortical dementia. In this disorder, cholinergic dysfunction is indicated by reduced levels of ChAT and VAChT in post-mortem samples, as well as by loss of striatal ChIs (Suzuki et al., 2002; Warren et al., 2005).

Chronic clinical use of dopaminergic drugs is often associated with the development of different types of motor complications, such as dystonia, parkinsonism, hyperkinesia, and stereotyped behavior. These reactions to dopaminergic agents points to an imbalance between striatal ACh and dopamine levels. Accordingly, the treatment of choice for these complications is represented by cholinergic drugs (Sethi and Morgan, 2007; Cubo et al., 2008). In the case of motor stereotypy, as well as of Tourette's syndrome (TS), a childhood-onset neuropsychiatric disease characterized by motor and vocal tics (Graybiel and Canales, 2001) and by a reduced striatal volume (Peterson et al., 2003), a cholinesterase inhibitor has been reported to be effective in the clinical practice (Cubo et al., 2008). Notably, a recent stereological analysis of post-mortem brains showed a significant reduction in the number of ChIs in the sensorimotor regions of the striatum in TS patients (Kataoka et al., 2010). Accordingly, a key role of striatal ChIs has been demonstrated in the arrest of cocaine-induced motor stereotypy in a rat model, where pharmacological treatments restoring ACh release rapidly blocked movement dysfunction (Aliane et al., 2010). Interestingly, this study showed that, while the striatal dopamine/ACh balance is effective during the period of strong motor stereotypy (dopamine increases and ACh decreases), it becomes dissociated during the phase of motor recovery. Indeed, in this period dopamine level still remains high, while ACh returns to its basal level mirroring the decreasing intensity of stereotypy, suggesting an important role of cholinergic transmission in the arrest of motor stereotypy. In accordance with this hypothesis, pharmacological blockade of muscarinic receptors as well as lesion of ChIs significantly prolonged motor stereotypy.

Although simplistic, the striatal ACh/dopamine balance view finds support in clinical pharmacological evidence. To date, two major categories of drugs are successfully utilized in the management of most movement disorders: drugs interfering with either dopaminergic or cholinergic function, suggesting that the interplay between these transmitters is relevant to the maintenance of a correct motor control. In our view, a perspective based upon cholinergic dysfunction may prove useful to orientate clinical pharmacological reasoning.

CONCLUDING REMARKS

Clues from neurobiology, functional imaging studies and post-mortem data converge to suggest that both pathogenic features and clinical phenomenology of distinct movement disorders are closely related to dysfunction of striatal cholinergic signaling.

To date, therapeutic intervention to most of these disorders is unsatisfactory. In those conditions where an increased cholinergic activity is documented, targeting muscarinic receptors with more selective drugs is warranted. Indeed, both in PD and dystonia, enhancing M2/M4-mediated autoreceptor function, either by developing selective agonists, or by modulating Ca_V2 Ca^{2+} channels appears a promising strategy.

The development of new animal models, including transgenic mice, as well as muscarinic and nicotinic receptor knockout mice, is moving research to a level in which the physiology of the receptor subtypes could be addressed *in vivo*, offering new perspectives for their pharmacological manipulation.

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Heterogeneity and diversity of striatal GABAergic interneurons

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The canonical view of striatal GABAergic interneurons has evolved over several decades of neuroanatomical/neurochemical and electrophysiological studies. From the anatomical studies, three distinct GABAergic interneuronal subtypes are generally recognized. The best-studied subtype expresses the calcium-binding protein, parvalbumin. The second best known interneuron type expresses a number of neuropeptides and enzymes, including neuropeptide Y, somatostatin, and nitric oxide synthase. The last GABAergic interneuron subtype expresses the calcium binding protein, calretinin. There is no overlap or co-localization of these three different sets of markers. The parvalbumin-immunoreactive GABAergic interneurons have been recorded *in vitro* and shown to exhibit a fast-spiking phenotype characterized by short duration action potentials with large and rapid spike AHPs. They often fire in a stuttering pattern of high frequency firing interrupted by periods of silence. They are capable of sustained firing rates of over 200 Hz. The NPY/SOM/NOS interneurons have been identified as PLTS cells, exhibiting very high input resistances, **l**ow **t**hreshold **s**pike and **p**rolonged plateau potentials in response to intracellular depolarization or excitatory synaptic stimulation. Thus far, no recordings from identified CR interneurons have been obtained. Recent advances in technological approaches, most notably the generation of several BAC transgenic mouse strains which express a fluorescent marker, enhanced green fluorescent protein, specifically and selectively only in neurons of a certain genetic makeup (e.g., parvalbumin-, neuropeptide Y-, or tyrosine hydroxylase-expressing neurons etc.) have led to the ability of electrophysiologists to visualize and patch specific neuron types in brain slices with epifluorescence illumination. This has led to a rapid expansion of the number of neurochemically and/or electrophysiologically identified interneuronal cell types in the striatum and elsewhere. This article will review the anatomy, neurochemistry, electrophysiology, synaptic connections, and function of the three "classic" striatal GABAergic interneurons as well as more recent data derived from *in vitro* recordings from BAC transgenic mice as well as recent *in vivo* data.

Keywords: neostriatum, interneuron, GABAergic, tyrosine hydroxylase, EGFP, NPY, SOM, NOS

INTRODUCTION

It was recognized from the earliest neurocytological studies that the neostriatum comprised a large number of small to medium sized neurons (less than 20 μm in diameter) of varying morphology and a small number of large neurons (Mehler, 1981). Estimates of the ratio of small-medium to large cells varied widely in these reports, from 20:1 up to 270:1 due to the lack of application of rigorous quantitative techniques (Parent, 1986). Although the large neurons were first identified by Kölliker (1896) as giant interneurons, Ramon y Cajal (1911), in a rare error, identified them as striatal projection neurons (SPNs), a claim reiterated by Vogt and Vogt (1920), thus presenting a picture of the striatum as a nucleus comprised primarily of interneurons with a very small number of projection neurons (Zhou et al., 2002). This resulted in decades of controversy and confusion about the functional identities of the large and the small striatal cells, that was not resolved conclusively until retrograde labeling from substantia nigra and globus pallidus unambiguously identified the medium sized spiny neurons as the SPN and the giant aspiny neurons as interneurons (Grofova, 1979) that were later shown to be uniformly cholinergic (Kimura et al., 1980).

Striatal projection neurons are now recognized to make up approximately 95% of all the neurons in the rodent striatum (Gerfen and Wilson, 1996; the proportion is significantly lower in higher vertebrates, especially in primates, Graveland and DiFiglia, 1985), and the cholinergic interneurons make up only 0.5–1% of the neurons. The remaining neurons, thus comprising approximately 3–4% of the total number of neurons in the rodent striatum, are made up of several different subtypes of aspiny GABAergic interneurons.

Striatal GABAergic interneurons were first identified as such by their avid uptake of ^{3}H-GABA combined with Golgi staining (Bolam et al., 1983). Medium-sized aspiny striatal neurons with varicose dendrites and indented nuclear envelopes accumulated ^{3}H-GABA at a rate almost one order of magnitude greater that of the spiny projection neurons. Subsequent studies revealed that a population of aspiny interneurons with similar or identical characteristics also

exhibited significantly stronger glutamate decarboxylase (GAD) activity than spiny projection neurons (Bolam et al., 1985; Cowan et al., 1990; Kita, 1993; Kubota et al., 1993).

By the mid 1990s, there were reliable reports of three distinct subtypes of medium sized striatal GABAergic interneurons that could be distinguished in striatum of mammalian species on the basis of their expression of the calcium binding proteins parvalbumin (PV) or calretinin (CR), or their expression of the neuropeptides somatostatin (SOM), or neuropeptide Y (NPY) or nitric oxide synthase (NOS) (Vincent and Johansson, 1983; Vincent et al., 1983; Chesselet and Graybiel, 1986; Cowan et al., 1990; Kita et al., 1990; Bennett and Bolam, 1993; Kubota and Kawaguchi, 1993, 1994, 1995; Kubota et al., 1993). Each of these exists in very low abundance compared to the SPNs. In the case of PV^+ interneurons, 0.7%, CR^+ interneurons, 0.5% and SOM/NOS/NPY^+ interneurons 0.6%, as determined by unbiased stereological cell counts of immunostained rat striatum (Luk and Sadikot, 2001; Rymar et al., 2004). Of course, these numbers should be regarded as *minimum* estimates of the numbers of GABAergic interneurons, since immunostaining is of relatively low and variable sensitivity compared to other techniques for neurochemical phenotyping, so what this really means is that *at least* 1.8% of striatal neurons consist of GABAergic interneurons, and the proportion is almost certainly higher than that.

These reports were followed by groundbreaking *in vitro* electrophysiological studies by Yasuo Kawaguchi and colleagues that showed that the PV-immunoreactive (PV^+) and SOM–NPY–NOS^+ neurons also exhibited distinct somatodendritic and axonal morphologies, as well as readily distinguishable electrophysiological properties (Kawaguchi, 1993, 1997; Kubota et al., 1993; Kawaguchi et al., 1995). The first part of this article will concentrate on these earlier studies, reviewing the original core data on the "classic" striatal GABAergic interneurons, as well as updating them with the most recent findings on synaptic connectivity and pharmacology.

More recently, the widespread availability of several strains of transgenic mice engineered to express enhanced green fluorescent protein (EGFP) under the control of endogenous transcriptional regulatory sequences (Gong et al., 2003) has provided researchers with additional powerful tools with which to target specific neuronal subtypes for visually guided recording, thereby simplifying the electrophysiological characterization and biocytin labeling of different striatal GABAergic interneurons. These in turn have resulted in an expansion of the number of distinct subtypes of striatal GABAergic interneurons that have been electrophysiologically and morphologically characterized. These data will be reviewed in the next part of this article.

We will conclude with a discussion of the changing concepts of striatal organization and the functional diversity and potential roles of striatal GABAergic interneurons.

PV^+ INTERNEURONS

NEUROCYTOLOGY

Parvalbumin-immunoreactive striatal neurons were first reported by Gerfen et al. (1985) who showed the existence of medium-sized, aspiny interneurons that were distinct from previously described cholinergic and SOM^+ neurons, and which were more abundant laterally than medially. Subsequent studies revealed that these neurons were strongly immunoreactive for GAD (Kita et al., 1990), particularly the GAD_{67} isoform (Lenz et al., 1994) and were present in both patch and matrix compartments with dendrites that freely crossed patch–matrix boundaries (Cowan et al., 1990) and thus were almost certainly the striatal GABAergic interneurons that were originally identified on the basis of intense ^{3}H-GABA uptake (Bolam et al., 1983).

Most PV^+ striatal interneurons are categorized as medium-sized (Kita et al., 1990; Kawaguchi, 1993; Koós and Tepper, 1999), although a rare variant has been described that can be as large as the giant cholinergic interneuron (Bennett and Bolam, 1994a). In a sample of intracellularly labeled PV^+ interneurons Kawaguchi (1993) described two distinct morphologies, one characterized by a medium size soma and more compact axonal and dendritic fields, and another exhibiting larger somatic diameters as well as axonal and dendritic fields. These observations suggest that PV^+ interneurons may not represent a single homogenous cell type. PV^+ interneurons are not distributed homogeneously throughout striatum; rather they obey a ventral to dorsal, medial to lateral, and caudal to rostral gradient of increasing density (Gerfen et al., 1985; Kita et al., 1990; Mura et al., 2000; Wu and Parent, 2000). PV^+ interneurons emit five to eight aspiny dendrites that range from almost smooth to extremely varicose (Cowan et al., 1990; Kita et al., 1990; Kawaguchi, 1993; Kita, 1993). Most dendrites branch within a few 10 s of μm from the soma, and then only sparingly, with dendrites greater than third or fourth order rare. The entire dendritic field is compact and roughly spherical, extending 200–300 μm in diameter centered around the soma. A relatively small fraction of PV^+ neurons display more extended dendritic fields (Kawaguchi, 1993).

The axon is highly branched, extends through and beyond the dendritic tree of the parent cell and is one of the densest axonal arborizations of any striatal neuron. The axonal field is also roughly spherical or ovoid and extends about 1.5–2 times the diameter of the dendritic field with its maximal density roughly coextensive with the dendritic arborization (Kawaguchi, 1993; Koós and Tepper, 1999; Tepper and Bolam, 2004).

In EM studies the nuclear envelope of PV^+ neurons is deeply invaginated (Kita et al., 1990), consistent with that of the aspiny GABAergic interneurons described previously (Bolam et al., 1983; Takagi et al., 1984a). In addition, striatal PV^+ dendrites were observed to form dendro-dendritic gap junctions with other PV^+ dendrites (Kita et al., 1990; Kita, 1993). PV^+ interneurons are the only striatal neuron of any type where gap junctions have been directly observed morphologically.

INTRINSIC ELECTROPHYSIOLOGICAL PROPERTIES

Striatal PV^+ interneurons exhibit a distinctive electrophysiological profile that enables them to be identified strictly on that basis of intracellular or whole cell recordings *in vitro* or *in vivo*. The electrophysiological characteristics of striatal PV^+ interneurons were first identified in slices from juvenile rats by Kawaguchi and colleagues (Kawaguchi, 1993; Kawaguchi et al., 1995), and these observations have subsequently been replicated in slices from mature rats and mice (Koós and Tepper, 1999, 2002; Bracci et al., 2002, 2003; Centonze et al., 2003; Plotkin et al., 2005; Taverna et al., 2007; Ibáñez-Sandoval et al., 2010).

All striatal PV+ interneurons are classified electrophysiologically as fast-spiking interneurons (FSI) and in some instances can fire at frequencies over 400 Hz (**Figure 1C**) in response to strong depolarizing current injections with little spike frequency adaptation (**Figures 1A,C**). PV+ FSI are strongly hyperpolarized *in vitro* and do not exhibit spontaneous activity. They exhibit a low input resistance similar to that of SPNs (50–150 MΩ), but significantly less inward rectification. Action potentials evoked by depolarizing current injection are of short duration (<0.5 ms) and exhibit deep, rapid onset and short duration spike after hyperpolarizations. Similar characteristics are exhibited by FSI in cortex and hippocampus that are also PV+ (Freund and Buzsáki, 1996; Galarreta and Hestrin, 1999, 2001, 2002) and are likely due, at least in part to the expression of Kv 3.1, a high threshold, rapidly activating and slowly inactivating voltage-gated K+ channel that is expressed selectively in PV+ FS interneurons (Lenz et al., 1994; Rudy and McBain, 2001).

Characteristically, unlike most neurons, the intrinsic ionic mechanisms of striatal PV+ interneurons cannot sustain repetitive firing at arbitrarily low frequencies. As shown in **Figure 1**, increasing the amplitude of current injection steps finally depolarizes the neuron to threshold (about −50 mV), eliciting a single, delayed spike. However, further small increments of injected current do not elicit gradually increasing rates of firing, instead, a tiny increment in stimulus strength of 10 pA above threshold results in firing at the neuron's minimal sustainable frequency typically exceeding 20 Hz (**Figure 1**).

Among neostriatal interneurons defined as FSIs based on these electrophysiological criteria two subtypes may be distinguished based on the temporal structure of action potential trains elicited with current injection. One of these is characterized by continuous firing that is maintained for the duration of the current injection except for very high current amplitudes where the action potential train is sometimes terminated earlier, presumably due to Na+-channel inactivation. The other type of FSIs exhibit a "stuttering" response consisting of brief trains of action potentials separated by silent periods of variable duration during which subthreshold membrane potential oscillations are observed (Kawaguchi, 1993; Koós and Tepper, 1999; Bracci et al., 2003). It remains unclear if these two firing patterns reflect different states of the same neurons or are indicators of genuinely distinct neuronal phenotypes. In one study using BAC–PV–EGFP transgenic mice that enabled immunocytochemically verifiable targeting of PV+ interneurons Freiman et al. (2006) observed only the continuous firing pattern in EGFP+ FSIs. In contrast, FSIs exhibiting stuttering responses have been shown to stain for PV using immunocytochemistry in the rat neostriatum (Kawaguchi, 1993) and nucleus accumbens (Taverna et al., 2007). In addition, both firing types have been reported in both rats (Bracci et al., 2002, 2003; Plotkin et al., 2005; Taverna et al., 2007) and mice (Centonze et al., 2003; Gittis et al., 2010) among electrophysiologically defined FSIs, although the stuttering phenotype appears to be less frequent in mice than in rats (Tecuapetla et al., unpublished). Since developmental effects cannot account for these findings (Plotkin et al., 2005) the data together suggest

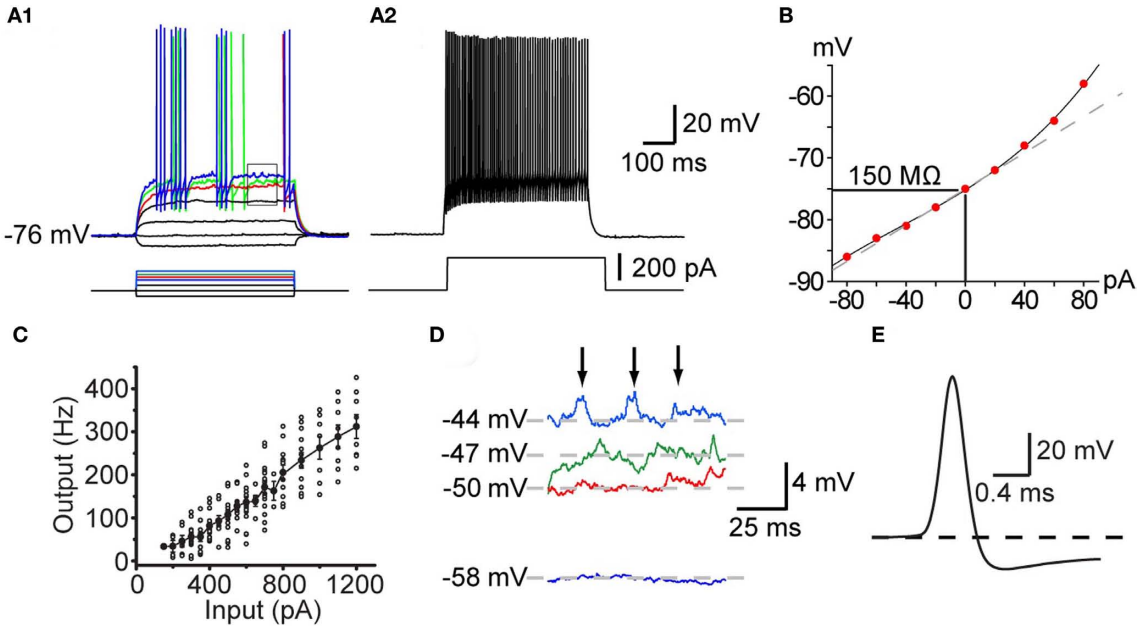

FIGURE 1 | Intrinsic electrophysiological properties of striatal FSIs recorded in whole cell current clamp *in vitro*. **(A1)** Responses to current pulses reveal the non-linear current–frequency relation around threshold. Minimal effective stimulation results in a single action potential a long delay (red trace). Increasing the stimulus by in 20 pA steps results in the appearance of the characteristic stuttering firing pattern (green and blue traces). **(A2)** At 220 pA the neuron fires continuously at high frequency with little spike frequency adaptation. **(B)** IV curve of the neuron illustrated in **(A)**. **(C)** IF curve plotted for a population of FSIs shows that they are capable of sustained firing in excess of 350 Hz. **(D)** Expanded view of the rectangle shown in **(A1)** illustrating voltage dependent subthreshold membrane oscillations that give rise to episodes of spiking in the chattering mode. **(E)** Expanded view of the singe spike shown in the red trace in **(A1)** illustrating the brief duration of the FSI action potential and the rapid onset of the deep spike AHP.

either a species *and* cell type specific difference in the expression of PV, so that in mice PV would be present only in continuous firing but not in stuttering FSIs; while in the rat, PV would be expressed in FSIs exhibiting either characteristics, or alternatively, stuttering neurons may be PV^+ in the mouse but much less frequent of much less frequently encountered in slice experiments than in rats. These interpretations should be considered tentative however, until the findings of Freiman et al. (2006) are confirmed with a more systematic characterization of a larger sample of neurons.

Another characteristic feature of these neurons is the presence of large amplitude (2–3 mV) voltage dependent membrane oscillations at depolarized subthreshold membrane potentials (Koós and Tepper, 1999; Bracci et al., 2003), which is particularly prominent in stuttering neurons during the periods between action potentials bursts. In stuttering neurons the oscillations appear to trigger the firing of bursts by bringing the membrane potential to threshold. The oscillations and the intermittent firing pattern are Ca^{2+} or SK channel independent, but are completely eliminated by TTX, suggesting that they are due to an interaction between voltage-gated K^+ conductances and a persistent or possibly the inactivating sodium conductance responsible for spike generation (Bracci et al., 2003, Koós and Tepper, unpublished).

An additional characteristic of striatal PV^+ interneurons is that they are interconnected by electrotonic synapses (Koós and Tepper, 1999), as are PV^+ FSI in cortex and hippocampus (Galarreta and Hestrin, 2001). Although the coupling ratio is usually not strong enough to evoke spiking, it is powerful enough to allow evoked spikes in electrotonically coupled FSI to fire near synchronously (Tepper, 2010). Although it has been proposed that this would allow syncytial activation of groups of FSI firing in near synchrony (e.g., Kita et al., 1990; Koós and Tepper, 1999), recent *in vivo* recordings from presumed FSI showed that striatal FSI activity is largely uncoordinated, at least during the cue and reward phases of a conditioned maze task (Berke, 2008).

AFFERENT CONNECTIVITY

Both symmetric and asymmetric synapses are seen to contact PV^+ dendrites and somata. Somatic inputs of both types are relatively sparse, but the dendrites are heavily innervated. Nearly two-thirds of the afferents form asymmetric synapses (Kita et al., 1990), originating mostly from cortex, with rather little thalamic innervation (Kita, 1993). Single cortical axons make multiple contacts with PV^+ interneurons (Ramanathan et al., 2002), which may account, in part, for the greater responsivity of PV^+ interneurons to cortical stimulation (Parthasarathy and Graybiel, 1997) compared to SPNs (Mallet et al., 2005). The symmetric synapses arise from both extrinsic and intrinsic GABAergic and dopaminergic inputs (Kubota et al., 1987; Bevan et al., 1998).

Choline acetyltransferase immunoreactive boutons have been seen in contact with PV^+ somata and dendrites in striatum (Chang and Kita, 1992), and striatal FSI are powerfully excited by stimulation of non-desensitizing nicotinic receptors *in vitro* (Koós and Tepper, 2002). However, at present, there have been no demonstrations of EPSP/Cs^+ originating from stimulation of cholinergic interneurons.

Dopaminergic inputs to presumed PV^+ interneurons have also been observed ultrastructurally (Kubota et al., 1987), as have GABAergic inputs originating from the GP (Bevan et al., 1998). The pallidostriatal inputs are largely selective for FSIs (Bevan et al., 1998) and *in vivo*, increased firing of FSI during choice selection in a simple discrimination task coincide with a decrease in firing of GP neurons (Gage et al., 2010).

EFFERENT CONNECTIVITY

Action potentials in striatal FSI evoke large amplitude IPSPs in SPNs in organotypic cell culture (Plenz and Kitai, 1998) and in acute striatal slices (Koós and Tepper, 1999, 2002). Synapses between striatal FSIs and SPNs are largely proximal (Kita et al., 1990; Kita, 1993; Bennett and Bolam, 1994b) and paired recordings show that they exhibit extremely low failure rates (<1%) and effective temporal summation, and are powerful enough to delay or completely block spiking in postsynaptic SPNs (Koós and Tepper, 1999). This is in contrast to the axon collateral synapses between SPNs (Tunstall et al., 2002), which typically evoke significantly smaller IPSPs/IPSCs than FSI-evoked synaptic responses when recorded somatically (Koós et al., 2004; Tepper et al., 2004, 2008; Gustafson et al., 2006) due to a combination of predominantly distal synaptic locations (88%; Wilson and Groves, 1980) and relatively few synaptic (2–3) connections made by each SPN on each postsynaptic SPN (Koós et al., 2004). Note, however, that a recent report described sixfold larger collateral synaptic currents in a small fraction of the SPN–SPN pairs recorded (Tecuapetla et al., 2009). Powerful inhibition of SPNs by FSIs *in vivo* has also been demonstrated during periods of increased cortical activity (Mallet et al., 2005).

The probability of a synaptic connection between a FSI and a SPN within the radius of the FSIs axonal field is extremely high, ranging from 25% in the first studies in rat slices (Koós and Tepper, 1999) to between 48 and 75% in more recent experiments in mouse slices (Gittis et al., 2010; Planert et al., 2010). These numbers are significantly greater than the connection probability between pairs of SPNs, which is consistently reported to be between 10 and 20% (Czubayko and Plenz, 2002; Tunstall et al., 2002; Koós et al., 2004; Taverna et al., 2004).

Striatal FSIs make synapses onto both direct and indirect pathway SPN. The biophysical properties of the synaptic contacts do not differ and exhibit short-term depression. Further, single FSI often make synapses with both types of SPN (Gittis et al., 2010; Planert et al., 2010; see **Figure 2**). In addition to synapses with SPNs, FSIs have been shown to make functional synaptic connections with other FSIs, but not with cholinergic or PLTS interneurons. Unlike SPN axon collateral interactions, which are almost exclusively unidirectional (see Tepper et al., 2008 for review), the probability of reciprocal synaptic connections between pairs of FSIs is high (Gittis et al., 2010).

It is now generally accepted that striatal PV^+ FSIs are the major components of a powerful, feedforward inhibition that regulate spike timing in SPNs, thereby regulating striatal output.

PHARMACOLOGY

Bath application of DA or selective D_1/D_5 dopamine agonists induces depolarization and an increase in input resistance in striatal FSI in brain slices, both blocked by SCH23390, a selective D_1/D_5 dopamine antagonist (Bracci et al., 2002). A subsequent study with D_1 knockout mice revealed that this effect was due to activation of postsynaptic D_5 receptors located on the FSI (Centonze et al., 2003). The

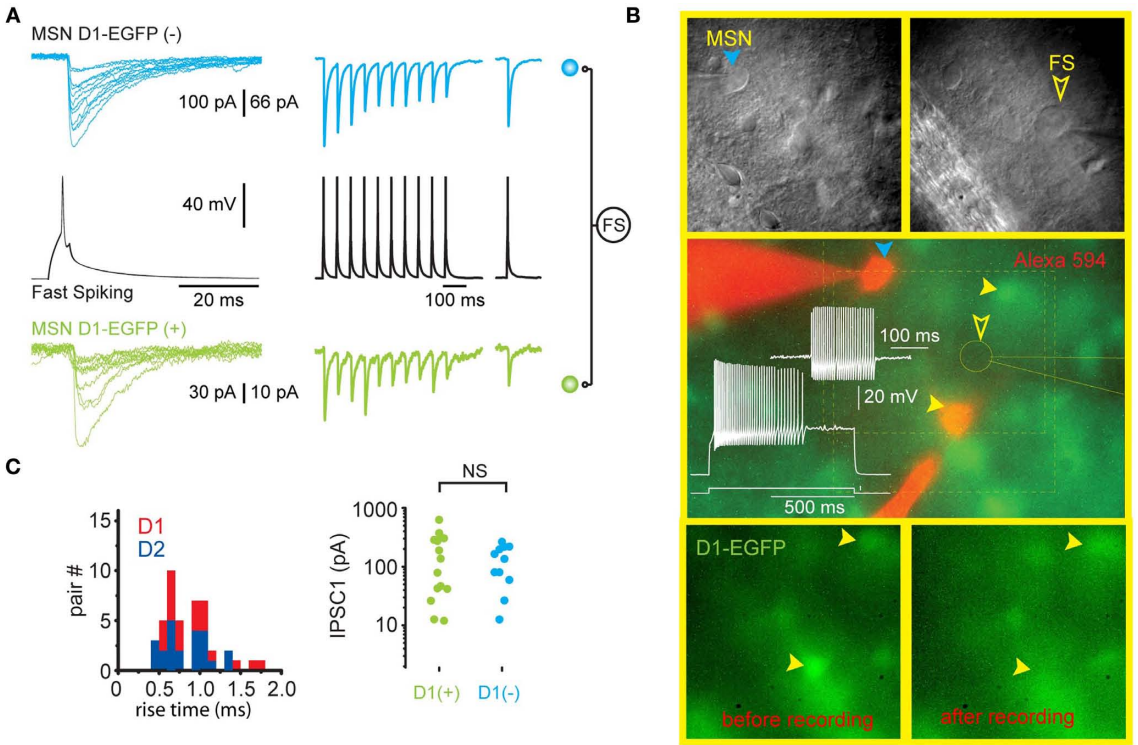

FIGURE 2 | Single FS interneurons evoke large IPSCs in both direct and indirect pathway SPNs. (A) Representative recording from two SPNs that are postsynaptic to the same FSI (black traces) in a striatal slice from a BAC transgenic D_1-EGFP mouse. The green traces are from the EGFP-D_1-expressing striatonigral SPN shown in **(B)**. The blue traces are from another SPN that does not express EGFP and is therefore a D2-expressing striatopallidal neuron. Note similar characteristics (IPSC amplitude, extremely reliable [zero failures] synaptic transmission, short term depression) of each synaptic connection. **(B)** Upper panels show the striatopallidal SPN and FSI and patch pipettes visualized with DIC. Middle panel shows electrophysiological identification of the FSI. The striatopallidal neuron is recorded and stained with a patch pipette containing Alexa 594 (orange). Bottom panels show the disappearance of the EGFP from the striatonigral neuron as a result of dialysis during recording. **(C)** Summary data from all triple recordings consisting of one FSI presynaptic to a striatonigral ($n = 14$) and striatopallidal ($n = 11$) neuron reveals no difference in IPSC rise time or amplitude in striatonigral and striatopallidal neurons.

ionic mechanism of the depolarization remains unknown. *In vivo*, systemic application of amphetamine leads to a dose-dependent increase in firing rate of most FSIs whereas the selective DA D_2 receptor antagonist, eticlopride, produced a consistent increase in FSI firing rate (Wiltschko et al., 2010). As there are apparently no postsynaptic D2-class receptors on striatal FSIs, these effects are likely due to presynaptic modulation of GABAergic inputs, possibly originating from the GP (Wiltschko et al., 2010), although this remains to be demonstrated directly.

ACh has a dual action on striatal FSIs. *In vitro*, these interneurons are strongly depolarized and induced to fire by bath or local application of carbachol, a response that is sustained for the duration of application. The response is completely blocked by mecamylamine but not by methyllycaconitine, indicating the involvement of a non-desensitizing, rapidly acting nicotinic receptor distinct from Type-1 receptors.

In addition to the nicotinic excitation, the FSI–SPN synapse is subject to powerful presynaptic inhibition by a pirenzapine-sensitive muscarinic receptor. It has been suggested (Koós and Tepper, 2002) that this presynaptic effect predominates during periods of cortical arousal when ACh levels are high (Abercrombie and DeBoer, 1997) and FSIs are firing rapidly (Mallet et al., 2005).

SOM/NOS/NPY$^+$ INTERNEURONS

Originally, the GABAergic nature of these neurons was a matter of some debate, with early studies making a clear distinction between SOM$^+$ interneurons and GABAergic interneurons (e.g., Lenz et al., 1994) because these neurons did not appear to express either GAD mRNA (Chesselet and Robbins, 1989) or GAD_{67} or GABA immunoreactivity (Kubota et al., 1993). A subsequent experiment in which rats were pretreated with colchicine to block axonal transport and increase somatic levels of proteins synthesized in the soma revealed that striatal NOS$^+$ neurons were also immunoreactive for GAD_{67}. A later EM study showed that the synaptic terminals of these neurons were also immunoreactive for GABA (Kubota and Kawaguchi, 2000), ending the controversy once and for all.

Several early immunocytochemical experiments indicated that striatal SOM$^+$ interneurons were also immunoreactive for NPY (formerly referred to as avian pancreatic polypeptide; Vincent and Johansson, 1983; Vincent et al., 1983) and the enzyme NADPH-diaphorase, which is equivalent to NOS (Hope et al., 1991), but not for PV or ChAT, thus forming a distinct subtype of striatal GABAergic interneuron (Fujiyama and Masuko, 1996; Gerfen and Wilson, 1996). For the remainder of this review, this neurochemically defined striatal interneuron subtype will be referred to as NPY interneurons.

NEUROCYTOLOGY

SOM/NPY/NOS$^+$ interneurons are medium sized, with round, polygonal, or fusiform somata with diameters ranging from 9 to 25 μm, making them on average the second-largest striatal neuron after the large aspiny cholinergic interneuron. Typical NPY neurons emit from 3 to 5 thick, aspiny mostly non-varicose proximal dendrites that branch within 30–50 μm of the cell body and taper rapidly, becoming more varicose in the distal regions. The entire arborization is relatively simple, branching sparsely and extends to a diameter of about 600 μm (DiFiglia and Aronin, 1982; Vincent and Johansson, 1983; Aoki and Pickel, 1988; Kawaguchi, 1993).

At the electron microscopic level, SOM/NPY/NOS$^+$ interneurons are characterized by a deeply indented nuclear membrane and a rich cytoplasm, like the PV$^+$ GABAergic interneurons but in marked contrast to SPN (DiFiglia and Aronin, 1982; Aoki and Pickel, 1988) which exhibit smooth, non-indented nuclear envelopes (Wilson and Groves, 1980).

The axonal arborization of SOM/NPY/NOS$^+$ is unique among striatal interneurons. It is the least dense axonal arborization of all striatal neurons (although there are exceptions – see **Figure 1** in Kubota and Kawaguchi, 2000) and also the largest in overall extent, and consists largely of long, sparsely branching axons extending in straight lines for up to 1 mm (Kawaguchi, 1993; Kubota and Kawaguchi, 2000). At least some of the axons are myelinated (DiFiglia and Aronin, 1982).

Kawaguchi (1993) reported that a PLTS neuron intracellularly labeled with biocytin appeared to possess two axons, as previously reported for a medium-sized aspiny striatal interneuron in a Golgi preparation (Takagi et al., 1984a). We have encountered the same phenomenon in a mouse PLTS interneuron as shown in **Figure 3**.

INTRINSIC ELECTROPHYSIOLOGICAL PROPERTIES

Kawaguchi (1993) obtained whole cell recordings from neurons in slices from juvenile rats that exhibited electrophysiological characteristics that were readily distinguishable from those of SPNs and FSIs. The most characteristic attributes of these neurons were the presence of a low threshold Ca^{2+} spike (LTS), a very high input resistance (>600 MΩ), a depolarized resting membrane potential

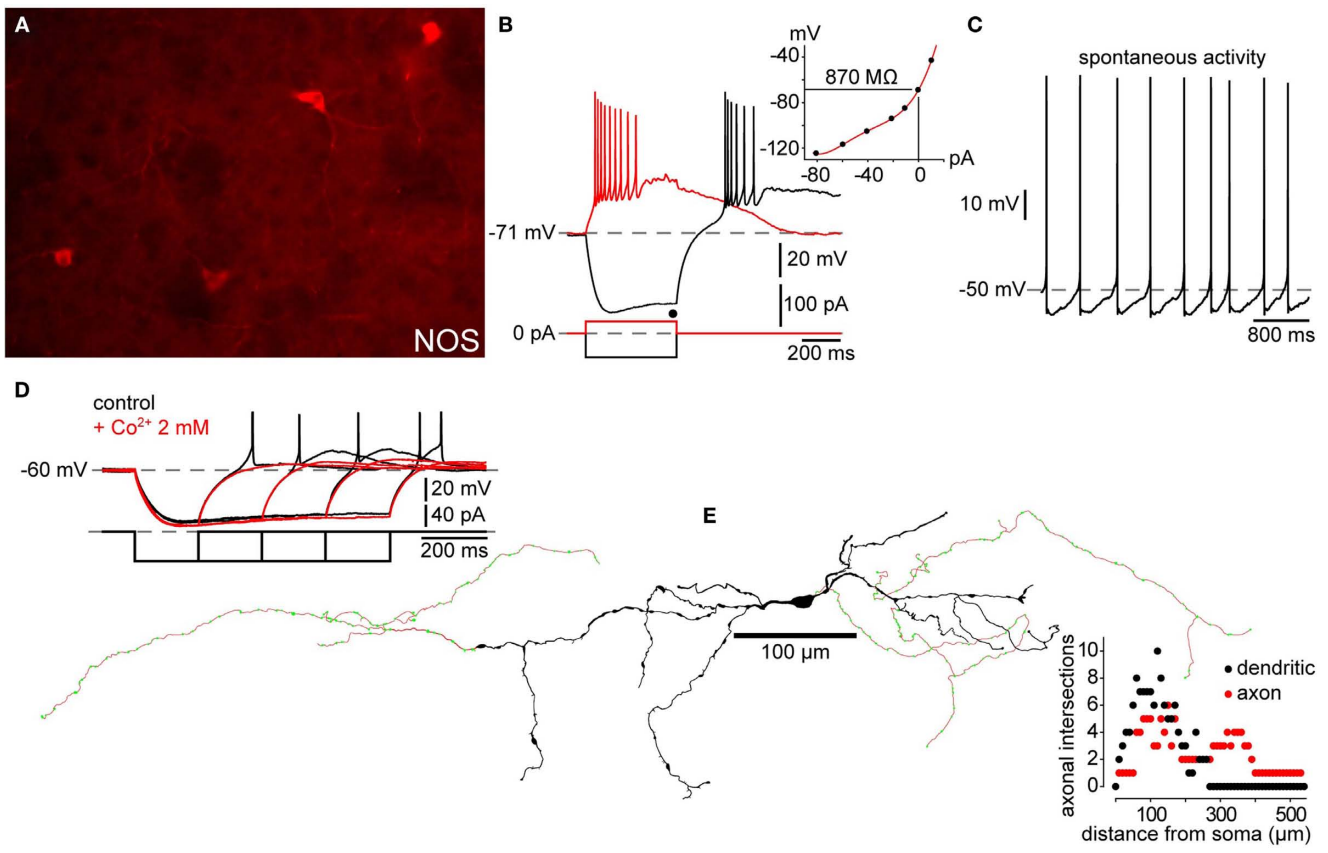

FIGURE 3 | Electrophysiology and morphology of striatal SOM/NPY/NOS interneurons. (A) Immunofluorescence photomicrograph of NOS$^+$ mouse striatal interneurons. **(B)** Passive electrophysiological properties of a NPY$^+$ interneuron from a striatal slice from a BAC transgenic EGFP–NPY$^+$ mouse shows the characteristic relatively depolarized resting membrane potential, LTS following cessation of a hyperpolarizing current injection (black trace), I_h and, prolonged depolarizing plateau potentials elicited from rest by depolarizing current injection (red trace) and following the rebound LTS after a hyperpolarizing current injection. Inset: IV curve from data in **(B)** shows typical high input resistance of striatal PLTS interneurons, even at maximum of I_h activation (black dot). **(C)** Spontaneous activity typical of many SOM/NPY/NOS interneurons. **(D)** Rebound depolarizations display the time dependent de-inactivation, all-or none nature and blockade by Co^{2+} characteristic of a LTS. **(E)** Reconstruction of a biocytin filled PLTS interneuron shows typical varicose dendritic arborization with a few sparsely scattered spines. Note the few arborizations in both, axon and dendrites (Sholl plot). This particular example was selected because it had two distinct axons emerging from opposite poles of the soma.

(approximately −56 mV) and the expression of long-lasting plateau potentials following depolarization from rest, in rebound from strong hyperpolarizing current injections or in response to strong excitatory synaptic inputs. These neurons exhibited long duration action potentials (1 ms at half amplitude). Due to the **LTS** and the **p**ersistent depolarizing **p**lateau potentials, these neurons were termed PLTS interneurons (Kawaguchi, 1993) and were subsequently shown to be the SOM$^+$ neurons described in the immunocytochemical studies reported above. Subsequent observations in adult rats and mice replicated these findings (Kawaguchi et al., 1995; Kubota and Kawaguchi, 2000; Centonze et al., 2002, 2003; Ibáñez-Sandoval et al., 2010). Although not mentioned in the original reports, a significant proportion (30 out of 44 cells) of the PLTS neurons in our mouse slices exhibited tonic spontaneous activity (Ibáñez-Sandoval et al., unpublished). The typical physiological characteristics of striatal PLTS interneurons are illustrated in **Figure 3**.

Although it was originally assumed that the SOM/NOS/NPY interneurons represented a single population of neurons, more recent stereological cell counting has revealed slightly different numbers for SOM$^+$ neurons (21,300/striatum = 0.8% of the total) and NPY$^+$ neurons (0.57%; Rymar et al., 2004). Furthermore, a multiple immunocytochemical labeling study concluded that nearly 25% of the striatal interneurons that expressed various combinations of SOM, NOS, or NADPH diaphorase were *not* immunoreactive for NPY (Figueredo-Cardenas et al., 1996). Therefore, there appear to be at least three distinct subpopulations of striatal SOM/NPY/NOS$^+$ interneurons, each expressing different combinations of these three markers. Whether these all subpopulations express the same "classical" electrophysiological phenotype of PLTS interneurons described above is unknown at present.

AFFERENT CONNECTIVITY

Previous anatomical work showed that PLTS interneurons receive numerous synaptic contacts on their proximal dendrites from both cholinergic and dopaminergic axons, as well as onto their distal dendrites, which receive asymmetric synaptic inputs from the cortex (Kubota et al., 1988; Vuillet et al., 1989a,b, 1992). GABAergic synaptic inputs originating from the globus pallidus were also demonstrated ultrastructurally using juxtacellular labeling and NOS immunocytochemistry (Bevan et al., 1998). The synaptic inputs to NPY neurons were also examined electrophysiologically by Partridge et al. (2009) using a BAC–NPY–GFP transgenic mouse strain and by Gittis et al. (2010) using BAC–Lhx6–GFP transgenic mice. These experiments demonstrated AMPA and NMDA receptor mediated cortical glutamatergic inputs that were relatively weak compared to the inputs of SPNs and $GABA_A$ receptor mediated inhibitory inputs comparable to those of SPNs (Partridge et al., 2009; Gittis et al., 2010).

EFFERENT CONNECTIVITY

Not surprisingly, the major efferent target of PLTS interneurons is the SPN. Axon terminals form symmetric synapses, mostly on the distal regions of the dendrites and on spines, largely avoiding the soma (DiFiglia and Aronin, 1982; Aoki and Pickel, 1988; Vuillet et al., 1989a,b; Kubota and Kawaguchi, 2000). NPY$^+$ boutons have also been observed to make symmetric contact with cholinergic interneurons, but synapses between NPY$^+$ neurons have not been reported (Vuillet et al., 1989a,b, 1992).

Despite the anatomical evidence cited above, in a recent *in vitro* paired recording study, in contrast to PV$^+$ interneurons, PLTS interneurons were found to evoke only sparse (2/60) and relatively weak GABAergic IPSCs in SPNs (Gittis et al., 2010). In this study, PLTS interneurons were first visually identified in slices from BAC transgenic mice engineered to express EGFP in neurons expressing the homeobox protein Lhx6, a marker for interneurons arising from the ganglionic eminence including PV$^+$, CR$^+$, and SOM/NPY/NOS$^+$ interneurons (Marin et al., 2000). No IPSCs were observed in postsynaptic PLTS, FSI, or cholinergic interneurons. Whereas a sparser efferent connectivity than the PV$^+$ interneurons is consistent with the much less dense and elaborate axonal arborization, the almost complete absence of postsynaptic responses is not.

One possible explanation for the lack of synaptic responses is that the principal neuroactive substance released from PLTS interneurons may not be GABA. As reviewed above, in contrast to the PV$^+$ interneurons, PLTS interneurons express far lower levels of GABA and GAD. Perhaps the principal function of these neurons is to release SOM, NOS, and/or NPY, all of which could exert slower neuromodulatory effects on their postsynaptic targets rather than fast synaptic effects. For example, SOM has been shown to exert a potent presynaptic inhibition on GABA release at SPN–SPN synapses (Lopez-Huerta et al., 2008).

PHARMACOLOGY

Like the PV$^+$ FSIs, the PLTS interneurons are excited by D1-class agonists through D1 family dopamine receptors eliciting depolarization and action potential firing *in vitro* (Centonze et al., 2002). Interestingly, as is the case with FSIs, the excitatory effect of dopamine on PLTS neurons was also absent in D1 receptor knock out mice indicating the involvement of D5 receptors. In addition, indirect cholinergic effects through M_2 muscarinic acetylcholine receptors have also been reported (Bernard et al., 1998).

CR$^+$ INTERNEURONS

Of the three classically recognized striatal GABAergic interneurons, by far the least is known about the CR interneuron. Although they make up 0.5% of striatal neurons based on stereological cell counts of immunostained material in rat, just slightly less than the number of PV$^+$ neurons (Rymar et al., 2004), our knowledge of these interneurons is limited to what can be seen in immunostained material, since they have never been recorded and intracellularly labeled. In primates including humans, the proportion of CR$^+$ neurons is much greater than in rodents and CR$^+$ interneurons outnumber PV$^+$ and SOM/NPY interneurons by 3 or 4 to 1 (Wu and Parent, 2000). No EGFP–CR$^+$ transgenic mice are currently available, and so there have been no successful attempts thus far to correlate a set of physiological properties with the CR$^+$ phenotype as has been done for other striatal interneurons. The lack of intracellular labeling has also resulted in only a very limited description of the axonal arborization. The lack of data on CR interneurons underscores the power and utility of transgenic mice that selectively express EGFP under the control of a single, specific promotor.

Early studies in rats described CR-expressing interneurons as medium sized aspiny neurons, 12–20 μm in diameter that issued a small number of smooth, aspiny dendrites that branch sparingly and taper into thin, varicose processes (Bennett and Bolam, 1993; see **Figure 4**). However, subsequent studies in rats and primates consistently describe at least three or four morphologically distinct types of striatal CR$^+$ neurons, ranging from small to large in somatic size (Prensa et al., 1998; Schlosser et al., 1999; Wu and Parent, 2000; Rymar et al., 2004). Indeed, our immunocytochemical studies have revealed the existence of at least three morphologically distinct types of striatal CR interneurons in mouse striatum as shown in **Figure 4**.

Neonatal hypoxia results in the neurogenesis of CR$^+$ striatal interneurons in rats that persists for at least 5 months after induction. Interestingly the neurogenesis appears limited to the CR$^+$ interneurons since there is no neurogenesis of striatal neurons that express markers for any of the other striatal interneurons or projection neurons (Yan et al., 2008).

TH$^+$ INTERNEURONS

Dubach et al. (1987) first described striatal neurons immunoreactive for TH (TH$^+$) in the caudate nucleus of three normal monkeys. The vast majority of these neurons were actually outside the borders of the caudate and putamen, and were located in the white matter ventral to the striatal neuropil. Of the neurons actually located within the striatum, most were restricted to a narrow band in the dorso-medial periphery of the caudate nucleus. These monkey TH$^+$ neurons were reported to be bipolar and 8–12 μm in diameter. Although no rigorous attempt to count the number of striatal TH$^+$ neurons was made,

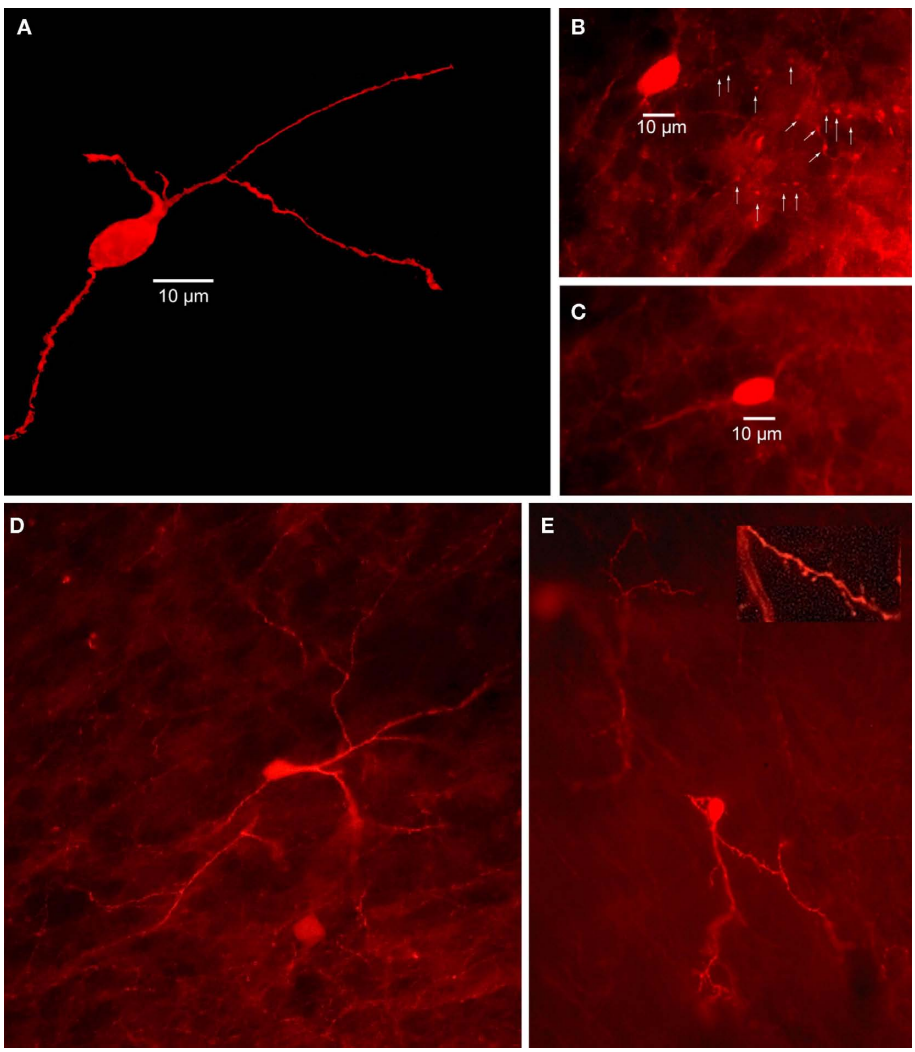

FIGURE 4 | Striatal calretinin immunopositive interneurons. **(A)** Two-dimensional projection of 40 deconvolved 1 μm optical sections through mouse striatum immunostained for a *Type I* CR interneuron shows a single medium sized, aspiny CR$^+$ interneuron. **(B)** Immunofluorescence photomicrograph of a single section containing an aspiny medium-sized mouse immunostained striatal CR$^+$ interneuron. Arrowheads point to axonal varicosities. **(C)** Another immunostained aspiny *Type I* CR$^+$ interneuron. **(D)** Immunostained brightly fluorescent aspiny *Type II* CR$^+$ interneuron just above a less brightly fluorescent *Type I* CR$^+$ interneuron. Note smaller soma of *Type II* neuron and more high branched dendritic arborization. **(E)** Least common type of CR$^+$ interneuron is the *Type III*, intensely fluorescent and spiny.

the authors estimated that they numbered in the "tens of thousands." Identical immunostaining protocols applied to mouse and rat striatum in the same study failed to find any striatal or peristriatal TH$^+$ neurons. Interestingly, when the material from these monkeys was subjected to fluorescence histochemistry, only 5–10 fluorescent neurons were observed in the caudate and putamen (Dubach et al., 1987).

In contrast, a subsequent immunostaining experiment in rat striatum did reveal the existence of a very small number TH$^+$ neurons (7–19 per striatal hemisphere) in control animals. These neurons were 10–20 μm in diameter, multipolar, and exhibited sparse spines on some dendrites. Interestingly, the number of TH$^+$ neurons increased by a factor of 2–4 times following dopaminergic denervation (Tashiro et al., 1989b), suggesting that the expression of TH in these neurons may be under the control of ambient DA levels.

Subsequently, many studies from different laboratories have confirmed the existence of neurons that could be immunostained with different monoclonal or polyclonal antibodies directed against TH in mouse, rat, monkey, and man. However, there remained considerable controversy regarding the number of striatal TH$^+$ neurons, their identification as interneurons or projection neurons, their morphology, species dependence and other factors (Betarbet et al., 1997; Meredith et al., 1999; Mao et al., 2001; Palfi et al., 2002; Jollivet et al., 2004; Cossette et al., 2005; Mazloom and Smith, 2006; Porrit et al., 2006; Tande et al., 2006; Huot et al., 2007; Darmopil et al., 2008).

Recently we have been able to resolve many of these controversies by using genetically modified mice that express EGFP under the control of the endogenous TH regulatory sequences (Tg (Th-EGFP) 1Gsat/Mmnc; Gong et al., 2003). These have allowed us to visualize striatal TH$^+$ neurons in brain slices and target them for whole cell recording and biocytin labeling which allowed us to study the electrophysiology and anatomical properties of striatal EGFP–TH$^+$ neurons (Ibáñez-Sandoval et al., 2010). In that article we reported the existence of four electrophysiological distinct types of striatal EGFP–TH$^+$ neurons, which were named: *Type I*, *Type II*, *Type III*, and *Type IV*. After electrophysiological characterization, biocytin-stained EGFP–TH$^+$ neurons were reconstructed and described neuroanatomically.

NEUROCYTOLOGY

Striatal EGFP–TH$^+$ of all four subtypes neurons exhibited medium sized somata (width = 15.2 ± 0.6 μm and height = 10.8 ± 0.4 μm), which were most frequently round or ovoid for *Types II–IV*, but often polygonal for *Type I*. These neurons emitted at least two to four aspiny and varicose primary dendrites (88%). Estimates from unbiased stereology showed the number of EGFP–TH$^+$ interneurons per striatum was 2684 + 1216 ($n = 6$), a number much greater than that in most previous, non-quantitative estimates of striatal TH$^+$ neurons labeled by immunocytochemistry in rodents (Tashiro et al., 1989b; Mao et al., 2001; Busceti et al., 2008).

Occasionally (in 12% of stained neurons) the dendrites from *Type I* cells exhibited moderately dense, thick stick-like appendages that appeared to lack distinct spine heads (see **Figure 5**). Nevertheless, these neurons could be readily distinguished from SPNs on morphology alone, and as described below, their very distinct electrophysiological properties. These data are consistent with some of the previous reports based on TH$^+$ immunocytochemistry

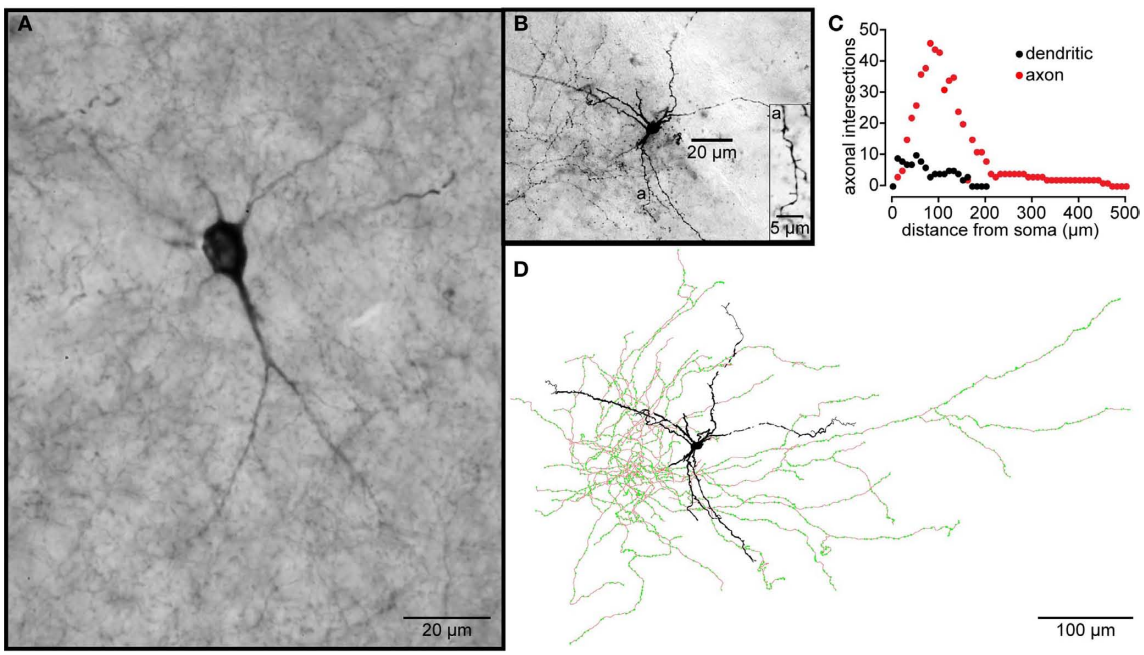

FIGURE 5 | Striatal tyrosine hydroxylase immunopositive interneurons. **(A)** TH-Immunoreactive aspiny striatal neuron from a normal rhesus macaque monkey. **(B)** A *Type I* striatal EGFP–TH$^+$ neuron stained with biocytin during whole cell recording from a striatal slice from an EGFP–TH$^+$ mouse. The dendrites branch infrequently and exhibit sparse, spine-like appendages (**a**). **(C)** Shows a Sholl plot of the reconstructed neuron revealing the extents and close overlap of the dendritic and axonal arborizations. **(D)** Drawing tube reconstruction of the neuron shown in **(B)**. Note the dense axonal arborization studded with varicosities (green dots), presumably axonal boutons.

(Dubach et al., 1987; Betarbet et al., 1997; Cossette et al., 2004, 2005; Mazloom and Smith, 2006; Huot and Parent, 2007) but are in sharp contrast to others that claimed the striatal TH$^+$ neurons to be a subpopulation of SPNs (e.g., Tashiro et al., 1989a,b; Darmopil et al., 2008). *Types II, III*, and *IV* could not be distinguished on morphological grounds.

The biocytin labeling of EGFP–TH$^+$ neurons allowed the first descriptions of their axonal arborizations (Ibáñez-Sandoval et al., 2010). These data revealed that for all four cell types, the axon emerged from the soma or proximal dendrite and branched almost immediately forming a dense local axon collateral plexus that occupied a volume coextensive with and sometimes extending beyond the dendritic tree of the issuing neuron (**Figure 5**). The collaterals were highly branched and studded with large numbers of prominent varicosities. None of the filled cells exhibited a single axonal branch that was larger than the rest or that could be clearly identified as the main axon. These data are consistent with our retrograde labeling data that failed to show any EGFP–TH$^+$ neurons retrogradely labeled from large Fluorogold injections in substantia nigra and GP.

Immunocytochemical studies using rat, monkey, and human material have demonstrated the presence of the GABAergic markers, GAD_{65} and/or GAD_{67} (Betarbet et al., 1997; Cossette et al., 2005; Mazloom and Smith, 2006; Tande et al., 2006; San Sebastián et al., 2007), dopaminergic markers including the dopamine transporter (DAT, Betarbet et al., 1997; Palfi et al., 2002; Cossette et al., 2004; Tande et al., 2006), and the synthetic enzyme a-aromatic amino acid decarboxylase (AACD, Mura et al., 1995, 2000; Meredith et al., 1999; Lopez-Real et al., 2003), and less frequently CR (Mura et al., 2000; Cossette et al., 2004, 2005; Tande et al., 2006) in TH$^+$ interneurons. Moreover, a small number of single cell RT-PCR experiments demonstrated in *Type I* and *IV* TH$^+$ interneurons (the only two types examined with sc-RT-PCR) the expression of an isoform of the obligatory marker of monoamine release, the vesicular monoamine transporter (VMAT), VMAT-1 and the apparent absence of the more common isoform, VMAT-2, expressed by mesencephalic DA neurons (Ibáñez-Sandoval et al., 2010). Importantly, TH$^+$ striatal interneurons were directly demonstrated to be distinct from PV, NOS, or CR expressing neurons in BAC–TH–EGFP mice (Ibáñez-Sandoval et al., 2010). In addition, these interneurons are present in both the matrix and patch compartments of the striatum, but appear to be more frequent in the matrix in primates (Huot et al., 2007).

The presence of TH immunoreactive neurons in the striatum was a matter of some debate in normal animals (Dubach et al., 1987; Tashiro et al., 1989a,b; Betarbet et al., 1997; Palfi et al., 2002; Cossette et al., 2004, 2005; Tande et al., 2006; Huot et al., 2007). In some studies, striatal TH$^+$ neurons were seen only after dopamine denervation (Mura et al., 1995; Meredith et al., 1999; Lopez-Real et al., 2003; Darmopil et al., 2008). A subsequent experiment in which mice were pretreated with colchicine to block axonal transport in an attempt to facilitate somatic accumulation of TH revealed TH immunoreactive neurons in the striatum. It still remains to be clarified if these neurons are able to synthesize and release dopamine.

INTRINSIC ELECTROPHYSIOLOGICAL PROPERTIES

Electrophysiological recordings of striatal EGFP–TH$^+$ cells revealed significant heterogeneity among these neurons with respect to their resting membrane potential, membrane potential responses to current injection, input resistance, spontaneous activity, action potential waveform, and maximum firing rate. Based on a highly reproducible pattern of correlations between a number of electrophysiological characteristics four distinct types of TH$^+$ interneurons could be distinguished and were named *Type I–IV TH interneurons*. The most frequently encountered subtype is *Type I* (60%) followed by *Type IV* (21%), *Type II* (13%), and finally the *Type III* (6%; **Figure 6**). The electrophysiological properties that most distinctly identify the *Type I* interneurons is their extremely high input resistance (346–1500 MΩ), their inability to maintain continuous firing during depolarizing current injections and a depolarization induced long-lasting plateau potential that is generated in a self-sustaining manner by a nimodipine sensitive L-type Ca^{2+} conductance and a flufenamic acid sensitive non-selective cationic conductance (I_{CAN}, **Figure 6C**; Ibáñez-Sandoval et al., 2010). In addition, *Type I* neurons had action potentials exhibiting highly variable durations (0.4–1.9 ms half amplitude) and fast adaptation. In some cases *Type I* neurons also exhibited spontaneous fluctuations in membrane potential of ~10 mV in amplitude, that resembled up and down states in spiny neurons (Wilson and Kawaguchi, 1996). Hyperpolarizing current pulses in the majority of *Type I* cells elicited a membrane potential deflection that could be blocked by ZD7288 (100 μM), indicating that it was attributable to activation of I_h.

In contrast, the *Type II* cells had somewhat lower input resistances (234–758 MΩ; **Figure 6**), and were further distinct in their ability to fire action potentials throughout a depolarizing current injections and by exhibiting higher maximal firing rates (137–265 Hz), and little adaptation (**Figure 6C**). *Type II* neurons also had short duration action potentials (0.3–0.53 ms), and large amplitude after hyperpolarization (16–25 mV). While hyperpolarizing current pulses in almost all of the *Type II* cells, elicited a HCN channel mediated sag response similar to those of *Type I* neurons, *Type II* cells did not exhibit the characteristic plateau potentials of *Type I* neurons.

The *Type III* interneurons could be distinguished by the most negative resting membrane potentials (−89 mV) and the lowest input resistance among TH$^+$ neurons (150–205 MΩ), which was due to a strong inward rectification present at membrane potentials more negative to −80 mV (**Figure 6**). Like *Type I* cells, the *Type III* interneurons were incapable of sustained firing in response to large amplitude current pulses but at much lower intensities DC current injection elicited continuous firing (**Figure 6C**). These neurons also exhibited a nimodipine sensitive plateau potential similar to those of *Type I* neurons.

Finally, like *Type I* and *Type II* cells, *Type IV* interneurons exhibited high input resistances (235–821 MΩ) and a HCN channel mediated sag response to hyperpolarizing current injections similar to those in *Type I* and *Type II* neurons, but were clearly distinguished from these cell types by exhibiting a **l**ow-**t**hreshold **s**pike (LTS), that could be elicited at the resting membrane potential by depolarization or when rebounding from hyperpolarizing current injections (**Figure 6C**). The LTS was accompanied by a short burst of fast action potentials exhibiting intra-burst frequencies in excess of 300 Hz. *Type IV* neurons had short-duration action potentials (0.4–0.85 ms) as well.

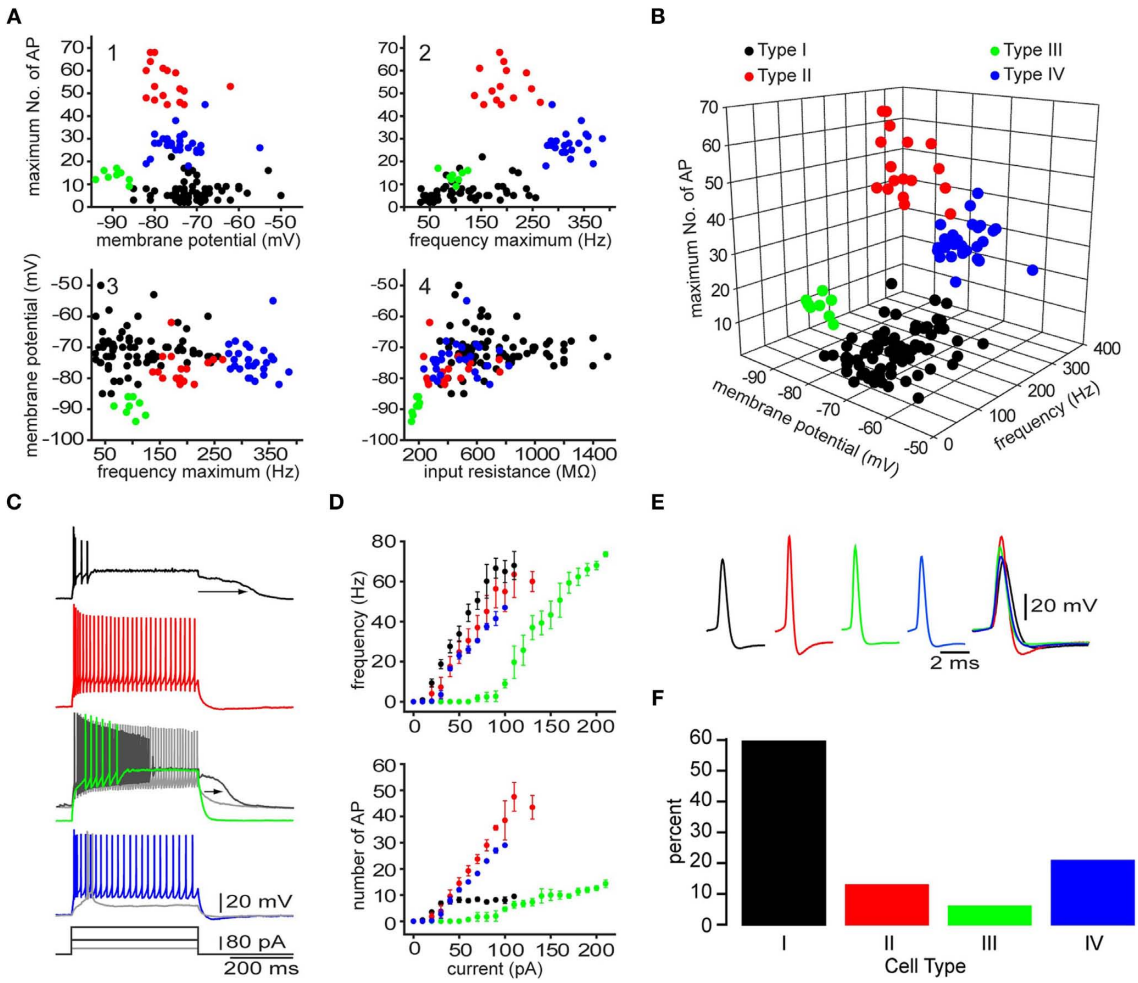

FIGURE 6 | Four different types of striatal EGFP–TH⁺ neurons in mice. **(A)** Selected two-dimensional scatter plots of various electrophysiological parameters reveal the separation of striatal EGFP–TH⁺ neurons into four distinct groups, termed *Types I–IV*. **(B)** Clustering of four distinct cell types in one representative three-dimensional scatter plot. **(C, D)** Voltage response to depolarizing current injection (100 pA), for four striatal TH⁺ neuron types, showing the relationship between injected current and maximum number of spikes evoked or maximum firing rate show clear differences between the four striatal TH⁺ types neurons. Note the plateau potential that was evoked in the *Type I* (arrow) at rest and in a slightly depolarized *Type III* neuron (arrow), using a stronger depolarizing current injection (180 pA). The *Type III* fires throughout the depolarizing current (gray line). In addition, the *Type IV* interneuron exhibits a clear LTS component in response to a small depolarizing current injection (40 pA), at its resting membrane potential. **(E)** Averaged action potentials from cell *Types I-IV* clearly show differences in multiple spike waveform parameters. **(F)** Histogram showing the distribution of the four EGFP–TH⁺ cell types.

Unlike *Type I*, *Type II*, and *Type III* neurons, that exhibited electrophysiological properties unlike those of any previously identified cell type in the neostriatum, the *Type IV* cell closely resembled the LTS interneuron described by Koós and Tepper (1999). However, *Type IV* TH⁺ neurons as well as the previously described LTS neurons both differed from the NPY/NOS/SOM⁺ PLTS neuron described by Kawaguchi and colleagues (Kawaguchi, 1993; Kawaguchi et al., 1995) due to the absence of plateau potentials, lower input resistances and a shorter duration action potentials.

AFFERENT CONNECTIVITY

Previous anatomical work showed sparse but clearly defined asymmetrical and symmetrical axodendritic synaptic contacts on TH⁺ striatal neurons (Mazloom and Smith, 2006), suggesting that TH⁺ neurons are integrated into the striatal circuit. In this regard, recent studies shown that at least *Type I* neurons respond to cortical stimulation (Ibáñez-Sandoval et al., 2010), evoking an EPSP that was blocked by 10 μM of DNQX, showing the involvement of AMPA/kainate type glutamate receptors. In addition, *intrastriatal* stimulation evoked a compound response that consisted of $GABA_A$ receptor mediated inhibitory and AMPA receptor mediated glutamatergic excitatory components. The cellular origin of the excitatory responses has not been determined.

Interestingly, paired recordings showed that both EGFP–TH *Type I* and *Type II* interneurons receive GABAergic inhibitory inputs from SPNs (Ibáñez-Sandoval et al., 2010). Single action potentials elicited in SPN evoked a IPSPs in postsynaptic neurons depolarized with current injection that was sufficient in amplitude to delay action potential firing (**Figure 7B**). The IPSP reversed near to the chloride equilibrium potential (**Figure 7A**), and could be blocked by bicuculline (10 μM), indicating that the IPSPs were mediated by

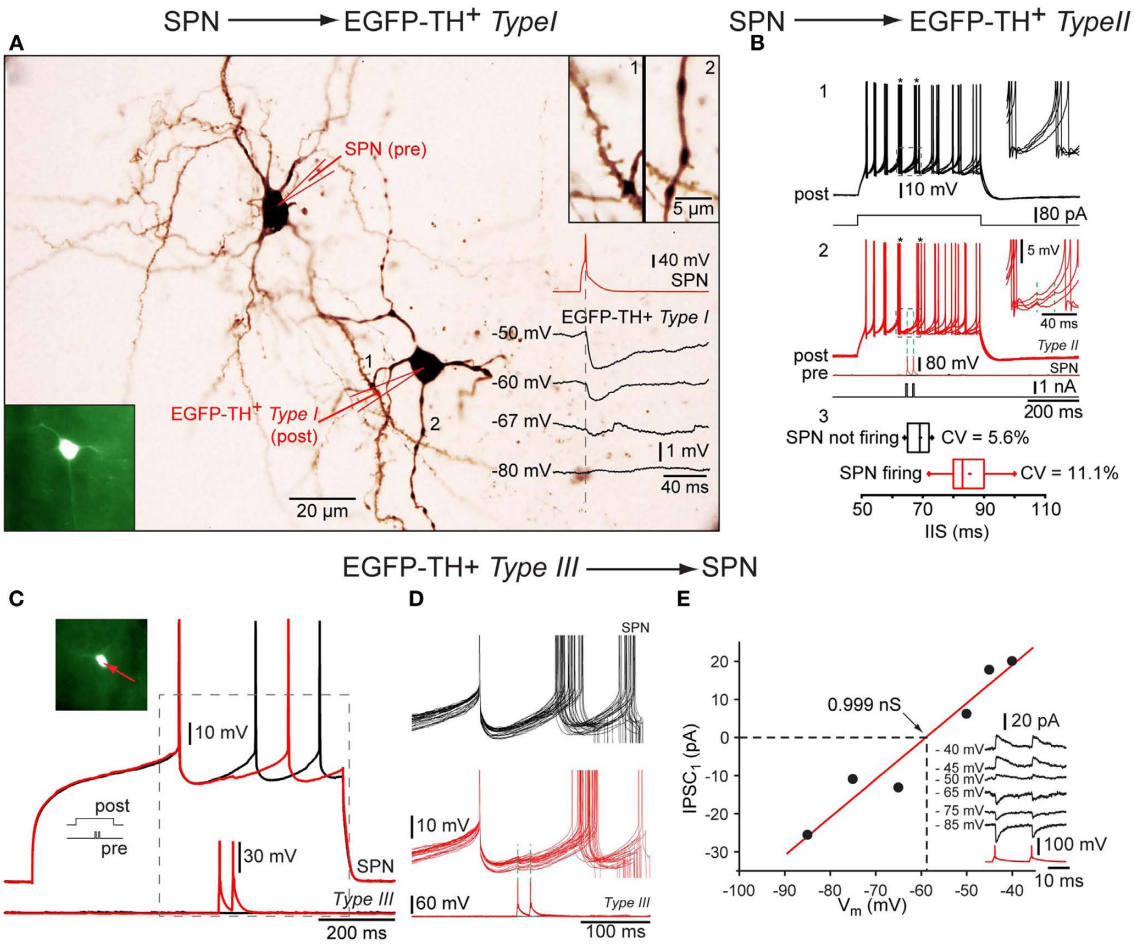

FIGURE 7 | Synaptic interactions of TH interneurons. Whole cell recordings from three connected pairs in which the first pair consisted of a presynaptic SPN and a postsynaptic *Type I* neuron, the second pair a presynaptic SPN and a postsynaptic *Type II* interneuron and the third pair of a presynaptic *Type III* neuron and a postsynaptic SPN. **(A)** Photomicrograph showing pair 1 intracellularly labeled with biocytin. Bottom inset: Fluorescence photomicrograph of the postsynaptic *Type I* EGFP–TH interneuron (bottom left), Top inset: spine laden dendritic segment of the SPN (1) and a varicose dendrite from *Type I* neuron (2) at higher magnification. Traces show a single action potential in the SPN (red), eliciting IPSPs at different postsynaptic membrane potentials (in black) showing reversal near –67 mV. **(B)** Depolarization evoked repetitive firing of a *Type II* neuron (1, black traces) is interrupted at the time indicated by the asterisks by single spikes of evoked in the presynaptic SPN (2, red traces) Inset shows the IPSP enlarged. Panel 3 shows the cumulative data for the inter-spike intervals with (black) and with presynaptic activity (red). **(C)** An analogous experiment shows delay in the depolarization induced spiking in an SPN (top red and black traces) by two spikes in the presynaptic *Type III* interneuron. **(D)** Higher magnification of the IPSP. Note the reliability of transmission. **(E)** *I–V* plot for the synaptic response in **(C)** and **(D)** showing a reversal potential as expected for Cl^- and providing an estimate of the synaptic conductance (~1 nS).

$GABA_A$ receptors (see Ibáñez-Sandoval et al., 2010). These synaptic connections are of particular interest because they represent the only demonstrated fast GABAergic inhibitory input to any striatal interneuron type from SPNs. Furthermore the failure of comparable previous paired recording experiments (Koós and Tepper, 1999) to detect an inhibitory input from SPNs to FSIs suggest the intriguing possibility the TH^+ interneurons and FSIs play fundamentally different roles in the striatal circuitry.

EFFERENT CONNECTIVITY

Using paired recordings, Ibáñez-Sandoval et al. (2010) have demonstrated that *Type I*, *II*, and *III* TH interneurons all innervate SPNs and that single presynaptic action potentials often elicit large amplitude IPSPs in their postsynaptic targets. All three neurons were shown to elicit IPSPs that are mediated by $GABA_A$ receptors confirming the previous identification of these neurons as GABAergic (Betarbet et al., 1997; Cossette et al., 2005; Mazloom and Smith, 2006; Tande et al., 2006; San Sebastián et al., 2007). The biophysical properties of the connections of the three types of interneurons appear to be heterogeneous. While synapses of *Type I* and *II* neurons elicited IPSP/Cs characterized by relatively large amplitudes and undetectably low failure rates, *Type III* neurons elicited responses in control condition that were of similar average amplitudes and were not associated with failure rates (**Figures 7C–E**). All of these inputs were, however, sufficiently strong to delay action potential firing in SPNs depolarized with intracellular current injection (Ibáñez-Sandoval et al., 2010).

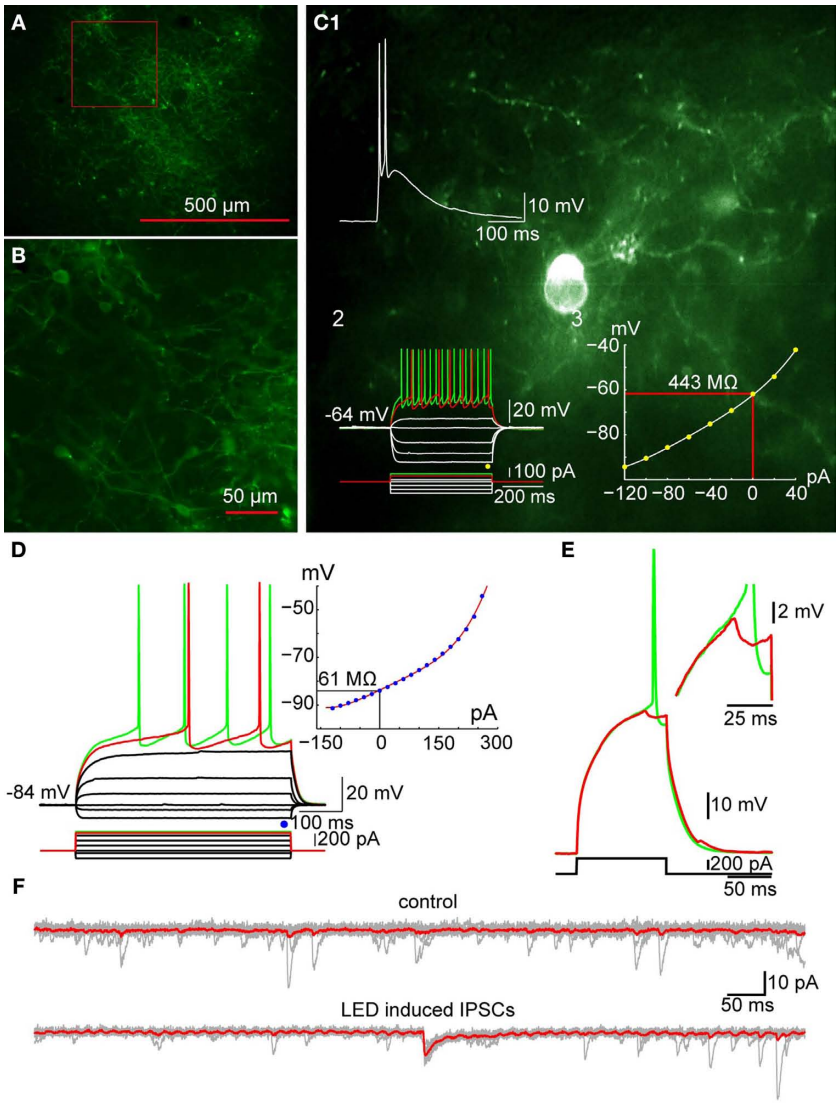

FIGURE 8 | Optogenetic activation of TH⁺ interneurons inhibits SPN firing. **(A)** Photomicrograph of a parasagittal slice obtained from a TH-Cre mouse injected with AAV-2:EF1:DOI:CHR2-EGFP. Note the large area of infection (~1 mm, in diameter). **(B)** Higher magnification of the area indicated by the red rectangle in A showing numerous AAV-infected TH⁺ interneurons as well as axonal and dendritic processes expressing ChR2-YFP (green). **(C)** Photomicrograph of a single ChR2 expressing striatal TH interneuron recorded in current clamp. C1: A 2-ms pulse of blue light elicits action potentials in the TH⁺ interneuron. C2: Responses to injected current identify the trasfected neuron as a *Type II* TH⁺ interneuron. C3: *I–V* plot from data in C2 is typical of *Type II* interneurons. **(D)** SPN recorded in another ChR2-expressing striatal slice. Inset shows typical *I–V* characteristics. **(E)** Action potential firing elicited in the SPN in D with current injection can be blocked by brief (2 ms) optogenetic stimulation of TH⁺ interneurons and axons. Inset shows the IPSP elicited in the SPN at higher magnification. **(F)** Current traces obtained without (top traces) and with optical stimulation (bottom traces). V_h = –80 mV. Note the IPSC elicited in the SPN (bottom).

To asses the role of the relative small population of TH interneurons in the inhibitory control of the neostriatum it is important to determine what fraction of SPNs receive input from these source and the strength of inhibition that may be exerted by a concerted activity of this interneuron population. We have conducted preliminary experiments to address this issue using Channelrhodopsin-2 (ChR2) mediated optogenetic activation of genetically targeted TH interneurons.

Fluorescent-tagged ChR2 (ChR2-YFP or ChR2-dTomato) was expressed in TH⁺ interneurons with viral mediated transfer of a Cre/lox controlled transgenes (Tsai et al., 2009) using serotype-2 or 5 adeno-associated virus in BAC transgenic TH-Cre [Tg(Th-cre)12Gsat mice (**Figures 8A,B**). Using this method postsynaptic responses to brief optical stimulation of TH interneurons could be detected in the majority of SPNs (see **Figure 8**) and these response were able to prevent action potential firing of the postsynaptic

neuron induced by current injection (**Figure 8E**). Together the currently available data supports the conclusion that despite their relatively small population TH interneurons may contribute significantly to the GABAergic inhibitory control of SPNs.

ARE THERE OTHER STRIATAL GABAERGIC INTERNEURONS?

CCK$^+$ AND VIP$^+$ NEURONS

Although a small number of cholecystokinin (CCK) and vasoactive intestinal polypeptide (VIP) aspiny neurons have been described in the striatum of the rat (Takagi et al., 1984b; Theirault and Lamdis, 1987; Hökfelt et al., 1988), as well as CCK neurons in the cat (Adams and Fisher, 1990), nothing is known about their physiological properties or synaptic actions. Both CCK and VIP neurons were reported as medium size (12–17 μm; VIP neurons and 10–20 μm; CCK neurons), with few primary dendrites (3–5), which branched close to the soma and whose dendrites became varicose and aspiny appearing. Moreover, in contrast to the sparsely distributed VIP neuron population observed in all areas of the striatum (Theirault and Lamdis, 1987), the observation of a very small population of CCK neurons in the cat (Adams and Fisher, 1990) could not be replicated in rat (Gilles et al., 1983) or human material (Schiffmann et al., 1989). In rats, the failure to detect CCK in the striatum persisted even after colchicine treatment (Gilles et al., 1983; Záborszky et al., 1985). Nevertheless, the possibility remains that detection of CCK and VIP interneurons is limited or prevented by low levels of neuropeptide expression or high sensitivity to colchicine. Preliminary single cell RT-PCR analysis of a small number of TH interneurons suggest that some if these neurons may express CCK and/or VIP implying a possible overlap among these types of interneurons. Genetic reporting and/or targeting methods as well gene expression assays will be essential to clarify the existence and possible roles these neurons in the striatum.

Finally, indirect electrophysiological evidence has been presented recently demonstrating the existence of a GABAergic population of neurons in the neostriatum which are activated by cholinergic interneurons and provide strong feedback inhibition of the same cholinergic interneurons (Sullivan et al., 2008). Since SPNs do not express nicotinic receptors or display nicotinic EPSPs the feedback neurons must be an interneuron. Recordings from eight pairs of FSIs and cholinergic interneurons failed to demonstrate a synaptic innervation in either direction (Tecuapetla et al., unpublished observation, Koós and Tepper, unpublished observations) suggesting that despite of the presence of soma-dendritic nicotinic receptors on FSIs these neurons may not be responsible for the feedback inhibition of SPNs. Further experiments will be required to identify these interneurons.

FUNCTIONAL IMPLICATIONS OF THE DIVERSITY OF STRIATAL INTERNEURONS

The data reviewed here shows that the neostriatal circuit incorporates an unexpectedly large variety of inhibitory interneurons that exhibit highly specialized intrinsic properties and connectivity. While the existence of such an intricate organization provides in itself a compelling argument for a fundamental role of the local circuitry in the behavioral functions of the striatum, the precise function(s) of interneuronal inhibition in the striatum remain largely unknown. Moreover, it is puzzling why such a large variety of interneurons exists in the striatum and what distinct functions these individually small and in some cases minute neuron populations may serve.

Understanding the information encoded by GABAergic striatal interneurons or other contingencies of their activity is in its infancy. Recently, multiunit recording experiments have provided some information about fast-spiking units (FSUs), which exhibit action potential waveforms and firing properties resembling FSIs. Interestingly, in behaving rats navigating in a baited maze, the activity of these neurons does not systematically co-vary with the animal's position relative to rewarded or otherwise significant locations (Berke, 2008). Instead, in a choice paradigm these neurons fire during choice execution exhibiting specificity to the direction of the movement chosen by the animal. Interestingly, the directional selectivity of FSUs and SPNs were the opposite, suggesting that inhibition from interneurons may contribute to the direction selectivity of the SPN responses. A perhaps related spatial pattern of activation of FSIs was described earlier based on immediate early gene expression used as a reporter of PV$^+$ interneuron activity in response to cortical activation (Parthasarathy and Graybiel, 1997).

A highly intriguing possibility is that a major function of at least some interneuron types is the coordination or control of gamma frequency oscillations observed primarily in the ventral parts of the neostriatum and the nucleus accumbens. Gamma oscillations are generated by synchronous periodic activity of GABAergic interneurons which act as a pacemaker for the firing of principal neurons throughout diverse neuronal systems including the neocortex, hippocampus, and thalamus (Freund and Buzsáki, 1996; Traub et al., 1999; Bartos et al., 2007) as well as the insect olfactory system (Laurent, 2002). Although it remains uncertain if gamma oscillations in the ventral striatum are of local origin, spiking activity of FSUs (Berke, 2008; van der Meer and Redish, 2009) and at least under some conditions the activity of presumed SPNs (Popescu et al., 2009; Kalenscher et al., 2010), phase lock to this activity, and the striatal gamma becomes synchronized with a non-zero phase lag to oscillations in the amygdala after conditioning (Popescu et al., 2009), suggesting a significant contribution from local mechanisms. Moreover, in the hippocampus the synchronous oscillatory activity of FSIs is generated by interplay of mutual inhibition and intrinsic resonant properties of interneurons (Traub et al., 1999) and in part through electrotonic coupling (Pais et al., 2003). Remarkably, the same features of dense synaptic interconnections, electrotonic coupling and unique intrinsic properties, including resonance at a gamma frequency range are displayed by FSIs in the striatum (Koós and Tepper, 1999; Bracci et al., 2003; Tepper, 2010), and there is also an overlap of the location of high voltage spindles and FSI units in the dorsal striatum (Berke et al., 2004). Based on these considerations it is possible that a resonant network of connected FSIs in the neostriatum can selectively phase lock to the oscillatory component(s) of excitatory inputs and further, that the activity of these interneurons is partially responsible for the observed gamma activity in the LFP and the moderate phase locking of a subset of SPNs. In addition to PV$^+$ FSIs, CR$^+$ and TH$^+$ interneuron networks may also contribute, possibly with the three cell types playing distinct roles in the low (~50 Hz) and high (~80 Hz) frequency gamma oscillations observed in the striatum. The differential sensitivity of the two types of oscillations to psychostimulants (Berke, 2008, 2009) may also reflect the involvement of different GABAergic circuits.

With regard to the function of the diversity of GABAergic interneurons a comparison with the functional organization of interneurons in hippocampus and the neocortex may be instructive. This comparison

is well motivated considering the shared developmental origin and in some respects striking similarity of interneurons in the striatum and in cortical structures (i.e., the neocortex and the hippocampus), including the pattern of expression of a similar complement of calcium binding proteins and neuropeptides as well as the similarity of the physiological properties and connectivity of striatal FSIs and basket cells in cortical areas mentioned above (Kawaguchi and Kubota, 1993). It is equally important to note however, that the 20 or more GABAergic interneurons identified in the CA1 field of the hippocampus greatly outnumber those currently recognized in the striatum and in most cases do not correspond to specific types of striatal interneurons. While it is likely that further investigation will reveal significantly more diversity among striatal interneurons than known today the homology of interneurons between the striatum and cortical structures will most likely be manifested as a common logic of cell type determination (and hence rules of classification) and not as a detailed correspondence of the majority of individual cell types.

These complexities not withstanding we believe that useful insights may be gained from certain emerging principles of the organization of hippocampal GABAergic interneurons and circuits. For a comprehensive discussion of these principles the reader is referred to the excellent recent review of Klausberger and Somogyi (2008), only the most relevant issues will be mentioned here. First, GABAergic inhibition of pyramidal neurons is highly organized, so that functionally distinct subcellular domains, including the axon initial segment, the soma, proximal and distal dendrites and even dendritic spines receive innervation from a unique but partially overlapping complement of GABAergic interneuron types. Conversely, individual types of interneurons display precise selectivity in innervating different postsynaptic subcellular domains. In the neostriatum, the observation of perisynaptic versus mostly dendritic localization of PV^+ and NPY^+ terminals respectively on SPNs (Kubota and Kawaguchi, 1993) suggests a similar specialization of interneurons.

Second several classes of interneurons have been discovered in the hippocampus that innervate primarily or exclusively other GABAergic interneurons. In principle the effect of these "higher order" neurons on the overall network activity may be significantly amplified through their control of powerful inhibitory circuits potentially resulting in a degree of influence that may not be readily predicted from their population size, the density of their axonal arborization, or the strength of their unitary synaptic connections. Therefore it will be interesting to examine if infrequent types of striatal interneurons, especially TH^+ interneurons provide significant inhibition of other interneurons. This possibility is supported by our preliminary optogenetic experiments showing synaptic inhibition of FSIs by TH interneurons (English et al., unpublished).

Finally, investigation of the firing activity of several identified types of interneurons in the hippocampus in relation to the three main oscillatory patterns of the hippocampus (theta rhythm, gamma oscillations, and high frequency ripples) revealed that there is no unique correspondence between individual cell types and inhibitory network functions. Instead, specific inhibitory functions (such as inhibition of distinct subcellular compartments, or inhibition associated with different oscillations) are provided cooperatively by multiple interneuron types and conversely, each interneuron type contributes to more then one (but not all) distinct inhibitory functions (Klausberger and Somogyi, 2008). This organization probably allows the fine tuning of each inhibitory function through complementing the unique properties, such as intrinsic firing properties, neuromodulation or use dependent plasticity of synaptic output, offered by individual types neurons. By analogy, one might expect a similar functional overlap between various neostriatal interneuron types with significant implications for future *in vivo* and *in vitro* investigation of the functioning of interneurons.

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Interactions between the midbrain superior colliculus and the basal ganglia

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An important component of the architecture of cortico-basal ganglia connections is the parallel, re-entrant looped projections that originate and return to specific regions of the cerebral cortex. However, such loops are unlikely to have been the first evolutionary example of a closed-loop architecture involving the basal ganglia. A phylogenetically older, series of subcortical loops can be shown to link the basal ganglia with many brainstem sensorimotor structures. While the characteristics of individual components of potential subcortical re-entrant loops have been documented, the full extent to which they represent functionally segregated parallel projecting channels remains to be determined. However, for one midbrain structure, the superior colliculus (SC), anatomical evidence for closed-loop connectivity with the basal ganglia is robust, and can serve as an example against which the loop hypothesis can be evaluated for other subcortical structures. Examination of ascending projections from the SC to the thalamus suggests there may be multiple functionally segregated systems. The SC also provides afferent signals to the other principal input nuclei of the basal ganglia, the dopaminergic neurones in substantia nigra and to the subthalamic nucleus. Recent electrophysiological investigations show that the afferent signals originating in the SC carry important information concerning the onset of biologically significant events to each of the basal ganglia input nuclei. Such signals are widely regarded as crucial for the proposed functions of selection and reinforcement learning with which the basal ganglia have so often been associated.

Keywords: superior colliculus, thalamus, subthalamus, striatum, substantia nigra, dopamine, selection, reinforcement learning

The basal ganglia are one of the fundamental processing units of the vertebrate brain. As such they have evolved multiple connections with most regions of the cerebral cortex, limbic system, thalamus, and numerous structures in the hindbrain. An important, although not exclusive, component of the basal ganglia connectional architecture are the parallel looped projections that originate in and return to external structures. The most prominent examples of this configuration are the looped projections connecting the basal ganglia with the cerebral cortex (Alexander et al., 1986). However, prior to the evolutionary expansion of the cerebral cortex, it was probably the co-evolution of the basal ganglia with subcortical sensorimotor structures that established basic looped circuitry onto which the cortex was later grafted (Reiner, 2010). The purpose of the present article is to detail the functional anatomy of connections between one of the evolutionary primitive sensorimotor structures of the brainstem, the superior colliculus (SC), and the basal ganglia. The SC was chosen as a template structure because its anatomy (Grantyn and Moschovakis, 2004; May, 2006), electrophysiology (Boehnke and Munoz, 2008), and especially its role in the re-direction of gaze (Sparks, 1986; Dean et al., 1989; Stein and Meredith, 1993; Grantyn and Moschovakis, 2004), are comparatively well understood. The connections between the SC and basal ganglia are also well characterized (Hikosaka et al., 2000; McHaffie et al., 2005). Our appreciation of the structure and function of the SC can therefore help provide insights, first, into how the basal ganglia might contribute to shifting the direction of gaze (which may serve as a general model), and second, how the SC might contribute to general functions performed by the basal ganglia.

TECTO-BASAL GANGLIA CONNECTIONAL ARCHITECTURE

PARALLEL LOOPS

Alexander et al. (1986) were the first to appreciate the parallel-loop configuration of the connections between the cerebral cortex and the basal ganglia. These parallel, partially segregated loops, pass sequentially through the basal ganglia nuclei and return to cortical regions of origin via a relay in the thalamus (Joel and Weiner, 1994; Groenewegen et al., 1999; Haber, 2003). Although the loops originate from functionally diverse regions of cerebral cortex, the internal micro-circuits of the basal ganglia with which they make contact are qualitatively similar in terms of cell-type, neurochemistry, and intrinsic connectivity (Voorn et al., 2004). Much experimental evidence now supports the concept that cortico-basal ganglia-thalamo-cortical channels have an important anatomical and functional significance (Alexander et al., 1986; Parent and Hazrati, 1995; Middleton and Strick, 2000). Consequently, they have been incorporated into many contemporary conceptual and computational

models of the basal ganglia (Mink, 1996; Redgrave et al., 1999a; Gurney et al., 2001a,d; Frank and Claus, 2006; Humphries et al., 2006; Frank et al., 2007). However, it is likely that the cortico-basal ganglia loops were pre-dated by looped projections connecting sub-cortical structures with the basal ganglia (**Figure 1**).

This idea was originally proposed with specific reference to the SC by May and Hall (1984) and later expanded upon by McHaffie et al. (2005). Thus, in addition to descending projections to the pons and the medulla (Redgrave et al., 1987), both the superficial and deep layers of the SC have ascending connections with targets in the thalamus, including the lateral posterior nucleus (Lin et al., 1984; Abramson and Chalupa, 1988; Berson and Graybiel, 1991; Harting et al., 2001b) and the midline/intralaminar nuclear complex (Chevalier and Deniau, 1984; Krout et al., 2001). Significantly, the ascending projections of the SC specifically target regions of the thalamus that provide major afferents to the striatum and STN (Takada et al., 1985; Feger et al., 1994; Van der Werf et al., 2002) (**Figure 2**). This arrangement suggests the SC is an important afferent source of sensory and motor information, as well as being a principal recipient of basal ganglia output (McHaffie et al., 2005).

A detailed examination of tecto-thalamic projections suggests there are at least two functionally segregated systems, one originating from the superficial layers and the other from the deep layers. Output from the exclusively visual superficial layers is directed to the extrageniculate visual thalamus (lateral posterior/pulvinar complex) (Lin et al., 1984; Abramson and Chalupa, 1988; Berson and Graybiel, 1991; Harting et al., 2001b). In addition to its efferent connections with extrastriate visual cortex (Updyke, 1981; Raczkowski and Rosenquist, 1983), this lateral posterior region of the thalamus also projects extensively to localized regions of the striatum, including lateral aspects of the body and tail of the caudate and dorsal putamen (Lin et al., 1984; Takada et al., 1985; Harting et al., 2001a,b; Cheatwood et al., 2003). This tecto-thalamic projection therefore provides a fairly direct route by which early visual input can be made available to the striatum (Lin et al., 1984; Takada et al., 1985; Harting et al., 2001a,b). The next link of this loop is the "direct" striatonigral projection which relays information from the visual thalamus to the ventrolateral aspects of substantia nigra, pars reticulata (Deniau et al., 1996; Gerfen and Wilson, 1996; Deniau et al., 2007). It is within these nigral regions that signals related to visual orienting are most frequently encountered (Hikosaka and Wurtz, 1983) and from which the final nigrotectal link of the visual loop returns to the SC (May and Hall, 1984; Harting et al., 1988; Redgrave et al., 1992a).

The ascending projections from the SC deep layers are to the thalamic intralaminar nuclei; the caudal intralaminar complex (centromedian and parafasicular nuclei) and the rostral intralaminar thalamic group (central lateral, paracentral, and central medial nuclei) (Chevalier and Deniau, 1984; Krout et al., 2001). Since both the caudal and rostral intralaminar thalamic nuclei provide topographically ordered projections to all functional territories within the striatum (Mengual et al., 1999; Van der Werf et al., 2002; Smith et al., 2004), the colliculo-thalamo-basal ganglia-collicular projections involving these sub-regions of the intralaminar thalamus may themselves represent sub-components of functionally independent parallel loops. The "direct" and "indirect" components of the tecto-thalamo-basal ganglia-tectal loops that project between the intrinsic nuclei of the basal ganglia are well known and have been reviewed extensively elsewhere (Tulloch et al., 1978; Deniau et al., 1996; Gerfen and Wilson, 1996; Smith et al., 1998). Similarly, the projections from both basal ganglia output nuclei (substantia nigra pars reticulata and the internal globus pallidus) back to the SC have also been described in detail (Graybiel, 1978; Harting et al., 1988; Deniau and Chevalier, 1992; Redgrave et al., 1992a,b; Takada et al., 1994).

Together these observations provide strong evidence for a primitive pattern of looped connections which originate in different parts of the SC, project in parallel via the thalamus through the basal ganglia and return to the same regions in the SC. Before leaving this

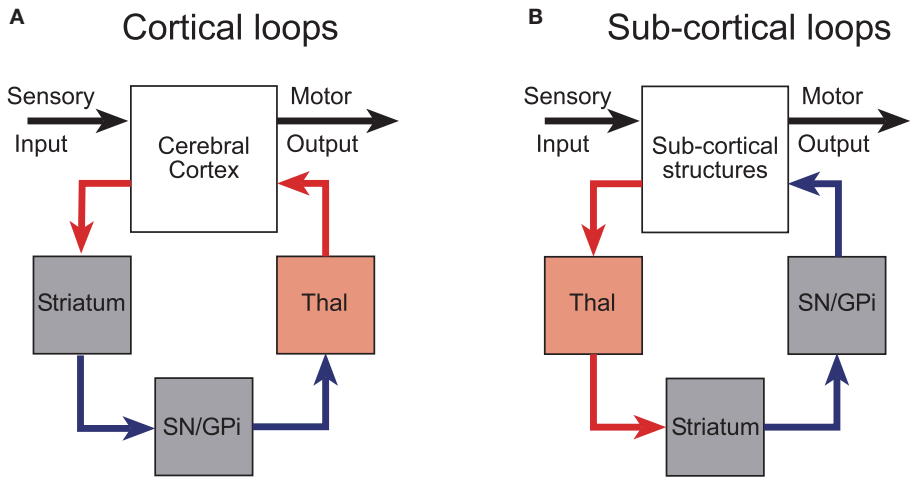

FIGURE 1 | Cortical and subcortical sensorimotor loops through the basal ganglia (modified with permission from McHaffie et al., 2005). **(A)** The position of the thalamic relay is on the return arm of cortical loops, while for subcortical loops, the thalamic relay is on the input side. **(B)** Predominantly excitatory regions and connections are in red; inhibitory regions and connections are in blue. GPi, internal globus pallidus; SN, substantia nigra; Thal, thalamus.

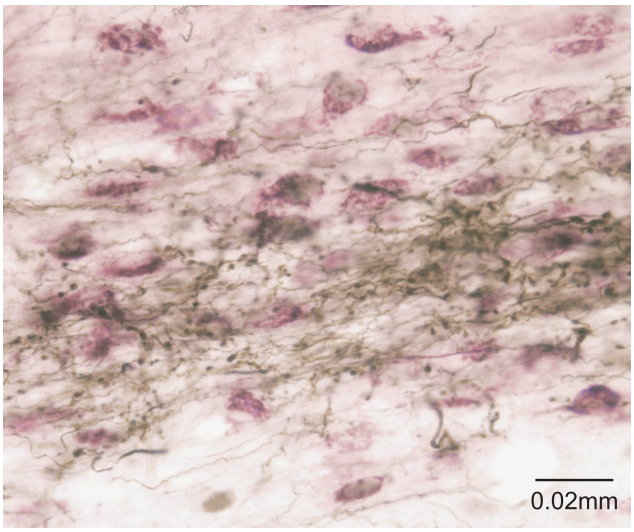

FIGURE 2 |The tecto-thalamo-striatal projection. Thalamo-striatal neurones in the central medial nucleus of the thalamus labeled with CTb (purple) retrogradely transported from the striatum, surrounded by terminal boutons labeled with biotinylated dextran (brown) anterogradely transported from the deep layers of the superior colliculus.

topic, it is worth noting that to view the sub-cortical basal ganglia loops as segregated closed channels of communication is undoubtedly simplistic (cf. Joel and Weiner, 1994; Haber, 2003) for similar comments about the cortico-basal ganglia loops). The synaptic relays between the different parts of each loop (in the structures of origin, the thalamus, and the various basal ganglia nuclei), represent nodal points whereby signals originating from outside the loop can modulate activity circulating within the loop.

A SUB-CORTICAL "HYPER-DIRECT" PROJECTION

In recent years, evidence has accumulated that the STN should be considered as an important entry point to the basal ganglia (Nambu et al., 2002), in addition to being an intrinsic relay in the classical "indirect-projection" (Albin et al., 1989). Thus, cortical afferents arising mainly from prefrontal and motor areas make direct contact with the STN (Afsharpour, 1985; Nambu et al., 2002). However, since the basal ganglia, including the STN, were present prior to the evolutionary expansion of cerebral cortex (Reiner, 2010), we might expect the STN also to receive inputs from ancient brainstem structures.

Evidence consistent with this suggestion for the SC was initially provided by Tokuno et al. (1994) and further emphasized by Coizet et al. (2009). In the latter study, tracer injections into the lateral deep SC layers produced dense anterogradely labeled terminals in the STN (**Figure 3**). Ultrastructural examination of the tecto-subthalamic projection revealed a high proportion of asymmetrical synaptic contacts suggesting that the projection is predominantly excitatory. In contrast, the STN was virtually devoid of terminal labeling when injections were directed to the medial SC. Injections of retrograde tracers into the STN confirmed small- to medium-sized multi-polar neurones concentrated in the lateral deep layers of the SC were the source of the tecto-subthalamic projection. Together these results suggest that phylogenetically older structures such as the SC established direct access to the basal ganglia output nuclei via a subcortical "hyperdirect" projection.

A DIRECT TECTO-NIGRAL PROJECTION

A further important input to the basal ganglia occurs via afferents to DA cell groups in the ventral midbrain (substantia nigra pars compacta, SNc and the ventral tegmental area, VTA) (Lindvall and Bjorklund, 1974). Inputs to DA containing regions of the ventral midbrain from several brainstem structures, including the pedunculopontine tegmental nucleus (Mena-Segovia et al., 2004; Winn, 2006), the lateral dorsal tegmental nucleus (Omelchenko and Sesack, 2005), the dorsal raphe (Benarroch, 2009), the rostromedial tegmental nucleus (Jhou et al., 2009), the periacqueductal gray (Omelchenko and Sesack, 2010) and the parabrachial nucleus (Coizet et al., 2010), have been described. However, given the sensitivity of DA neurones to unexpected biologically salient events (Schultz, 1998; Redgrave et al., 1999b; Horvitz, 2000; Redgrave and Gurney, 2006; Redgrave et al., 2008) it is the direct connections with sensory structures such as the SC that has attracted much recent interest.

A direct tectonigral pathway (**Figure 3**) was first described in rodents by Comoli et al. (2003) and later confirmed in cat (McHaffie et al., 2006), and monkey (May et al., 2009). Using anterograde and retrograde tracing techniques supported by ultrastructural analysis in the rat, Comoli et al. (2003) showed that the tectonigral pathway comprises several functionally distinguishable components. Anterogradely labeled tectonigral boutons formed both asymmetric (presumed excitatory) and symmetric (presumed inhibitory) synapses with both tyrosine hydroxylase-positive and -negative elements in substantia nigra pars compacta. The projection is also broadly topographical with neurones in lateral SC projecting strongly to lateral SNc while more medial regions of SNc and VTA receive predominantly from the medial intermediate layers (Comoli et al., 2003) and periaqueductal gray (Omelchenko and Sesack, 2010).

A particularly important feature of SC projections to SNc is that many tectonigral cells-of-origin also appear to send an ascending axon collateral to the thalamus (**Figure 4**). In a double retrograde tracing study (Coizet et al., 2007), significant double-labeling was reported after injections involving tectonigral fibers and ascending tectothalamic projections. Separate injections into the rostral-intralaminar, caudal-intralaminar, and ventromental thalamic nuclei each double-labeled between 15 and 30% of tectonigral neurones in the lateral deep SC. However, whether the double labeling associated with different targets in the thalamus are part of the same or separate projection systems is unresolved. If they are separate, the proportions of double labeled cells added together could potentially give a total of ~70%.

This brief anatomical overview confirms that the SC, one of the primitive sensorimotor structures in the brainstem, is not only an important recipient of basal ganglia processed information but is also a critical source of input. Direct afferent connections target both the STN and DA cell groups in the ventral midbrain while indirect input to the striatum occurs via relays in the thalamus. The functional implications of such this sub-cortical architecture will now be considered, first, in terms of how the basal ganglia

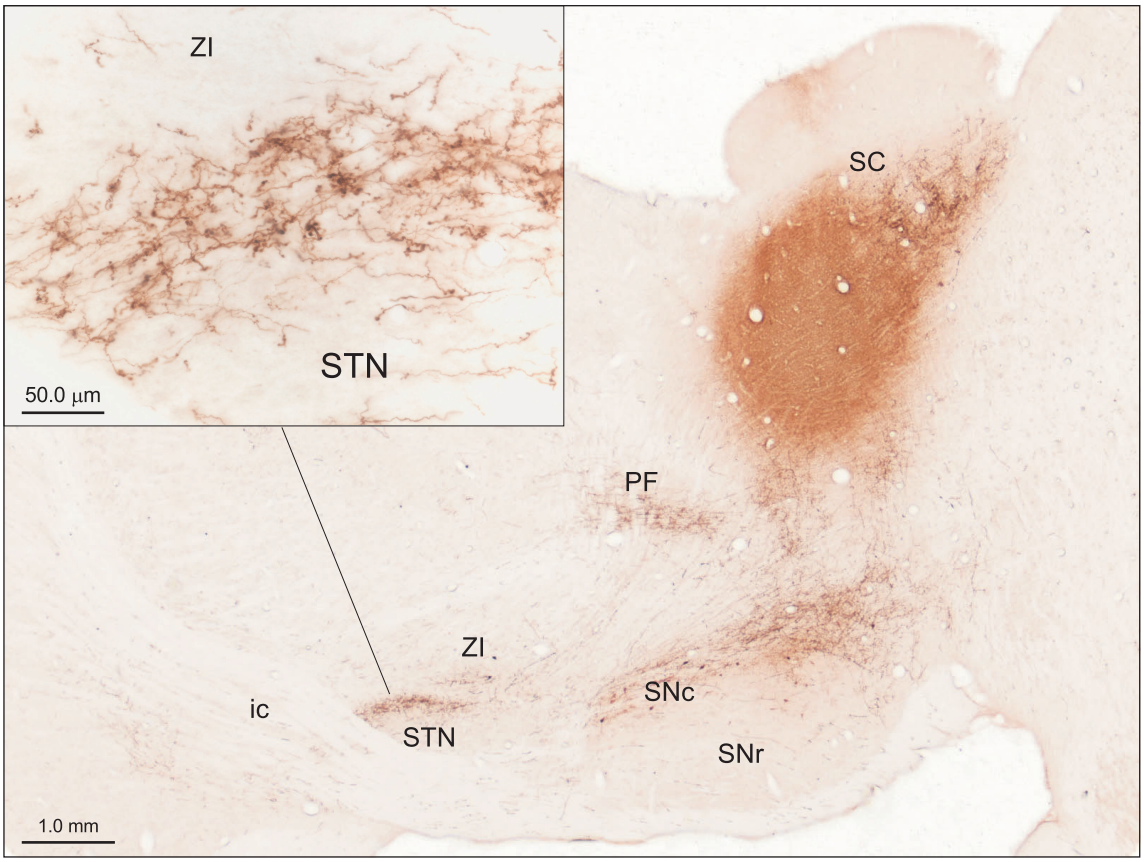

FIGURE 3 | The tecto-subthalamic and tecto-nigral projections in rat (modified with permission from Coizet et al., 2009). A large injection of the anterograde tracer PHA-L into the deep layers of the lateral superior colliculus (SC) produced dense fiber and terminal labeling in substantia nigra pars compacta (SNc) and subthalamic nucleus (STN). Note the dense clusters of terminal boutons, presumably surrounding neuronal cell bodies in STN (see inset). The tecto-thalamic projection was also confirmed in this case with terminal labeling evident in the parafasicular thalamic nucleus (PF). ic, Internal capsule; SNr, substantia nigra pars reticulate; ZI, zona incerta.

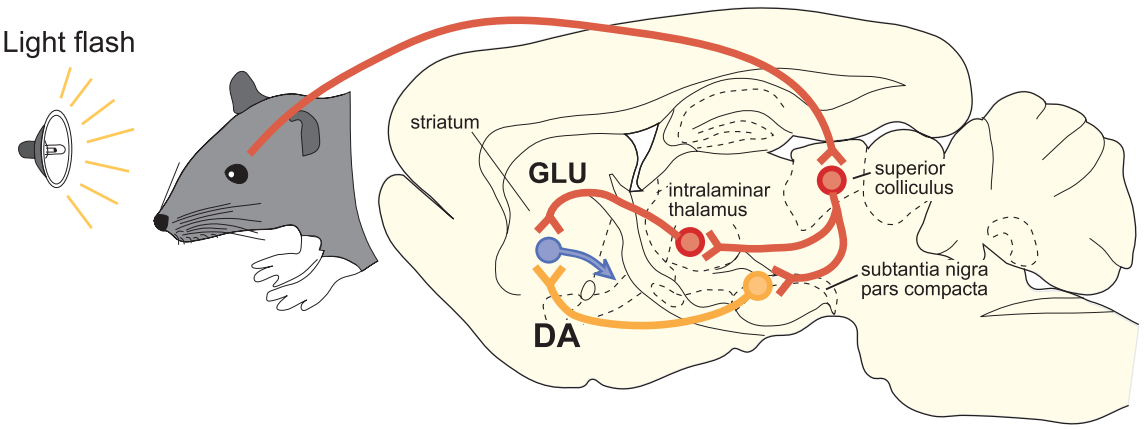

FIGURE 4 | A schematic illustration of the proposed convergence of short-latency phasic inputs to the striatum elicited by an unpredicted visual event. Direct retinal input to the superior colliculus could be re-directed, via branched projections to the intralaminar thalamic nuclei and to the substantia nigra pars compacta. At present, the identities of the neurotransmitters used in branched connections from the superior colliculus are unknown. Consequent, potentially converging phasic inputs to the striatum from intralaminar nuclei (GLU, glutamate) and substantia nigra (DA, dopamine) are likely to play a critical role in reinforcement learning.

modulates SC-mediated gaze shifts, and second, how aspects of basal ganglia function might benefit from short-latency sensory input from the SC.

FUNCTIONAL IMPLICATIONS

Despite suggestions implicating the basal ganglia in a wide range of functions, accumulating evidence points to a generic role in selection (Mink, 1996; Redgrave et al., 1999a; Hikosaka et al., 2000) and reinforcement learning (Schultz, 1998; Wise, 2004; Berridge, 2007). Selection would be an emergent property of the parallel looped-architecture that connects the basal ganglia with most external structures (Alexander et al., 1986; McHaffie et al., 2005). Output signals from the basal ganglia are tonically active and inhibitory (Chevalier and Deniau, 1990). Selective suppression of inhibitory output in some loops while maintaining or increasing it in others (selective disinhibition Chevalier and Deniau, 1990) would select the targets of disinhibited loops (Redgrave et al., 1999a). Selection as a property of basal ganglia macro-architecture has been confirmed computationally in biologically constrained simulations (Gurney et al., 2001b,c; Humphries et al., 2006) and the control of robot action selection (Prescott et al., 2006). In addition, the basal ganglia have also been associated with reinforcement learning, in particular, instrumental conditioning (Schultz, 1998; Wise, 2004; Berridge, 2007). Insofar as reinforcement acts to bias future selections, by increasing or decreasing the probability that reinforced selections will be re-selected (Thorndike, 1911), reinforcement learning is likely to be closely associated with the mechanism(s) of selection (Wickens et al., 2007; Redgrave et al., 2008). We will now consider how connections between the SC and the basal ganglia could contribute to these two important functions.

SELECTION

Faced with competing motivations and multiple sensory inputs, early vertebrates, like their modern relatives, required a means to select the most pressing stimuli and adaptive responses while suppressing less favored options. As the primary structure responsible for re-directing gaze toward or away from unexpected novel events (Dean et al., 1989; Stein and Meredith, 1993), the SC has always been confronted by pressing problems of selection. The situation arises because the retinotectal visual system can simultaneously represent numerous events, each one of which could potentially initiate a change of gaze. A selection architecture that can evaluate which of multiple simultaneously presenting stimuli is the most urgent, is essential.

One possibility would be to solve the problem locally with mutually inhibitory connections between all elements in the SC's sensorimotor maps (Snaith and Holland, 1990). However, on what basis would this reciprocally connected inhibitory network operate? Simple rules (e.g., size/speed/contrast) would run the risk of ignoring the near threshold sensory stimuli (which often foretell the beginning of life-or-death events, e.g., a small movement in the bushes or the snap of a twig), in favor of physically salient but innocuous stimuli, or on other occasions, miss the obvious. An alternative would be to have a system fully appraised of the range current motivational, contextual, and sensory variables perform selections according to the most pressing needs of the organism (Mink, 1996; Redgrave et al., 1999a). Perhaps it is for this reason the SC requires a contribution from the basal ganglia to perform what seems to be a simple reflex redirection of gaze. Note that the initiation of gaze-shifts to un-predicted sensory events is typically preceded by a pause in inhibitory nigrotectal output activity (Hikosaka et al., 2000). The looped architecture connecting the SC to the basal ganglia via the dorsal thalamus is a candidate mechanism to perform the pre-attentive selections required to determine whether gaze should be shifted, and if so, to which stimulus (McHaffie et al., 2005).

INTERRUPT?

The "hyper-direct" connections from the SC to the STN could provide a mechanism whereby early visual signals can influence basal ganglia output in advance of information circulating in tecto-basal ganglia loops. Because the subthalamo-nigral projection is excitatory (Smith et al., 1998) and the nigrotectal pathway is inhibitory (Chevalier and Deniau, 1990), a burst of activity in the STN transmitted to nigrotectal neurones would deliver a pulse of inhibitory signals to the SC. Two potential, although not necessarily exclusive, functions for such signals have been proposed. First, a vital part of the process by which attention is switched and gaze redirected is to interrupt or close down currently open/selected channels. A short-latency burst of inhibitory nigrotectal activity initiated by the STN could interrupt or break current gaze-fixation (Gillies and Willshaw, 1998; Redgrave et al., 1999a). Alternatively, Mink (1996) has proposed that a widely broadcast excitatory signal from the STN to basal ganglia output nuclei could be part of a mechanism to suppress motor programs that would otherwise interfere with desired movements. However, our finding in the rat that "hyper-direct" input to the STN comes only from the lateral SC (Coizet et al., 2007) raises problems for both suggestions. First, the ecology of the rodent is such that threatening stimuli are most frequently detected in the upper visual field, which according to the retinocentric map, is represented in the medial SC (Dean et al., 1989). In the class of stimuli that required the ongoing behavior of rodents to be interrupted, overhead predators would certainly be included. Similarly, it is not immediately apparent why selection mechanisms required to distinguish between multiple visual events in the upper field might require a radically different, non-STN associated architecture. The question of why typically non-threatening events in the lower visual field of rodents have direct access to the basal ganglia via the STN, whereas defense-related circuitry of the medial colliculus appears to operate through a different architecture remains to be answered.

REINFORCEMENT

Insofar as reinforcement operates to bias future behavioral selections, the association between reinforcement learning and the basal ganglia is to be expected (Wickens, 1993; Schultz, 1998; Wise, 2004; Arbuthnott and Wickens, 2007). The mechanisms proposed for adjusting the sensitivities of the striatum to "reinforced" and "non-reinforced" inputs are long term potentiation (LTP) and long term depression (LTD) (Centonze et al., 2001; Reynolds and Wickens, 2002; Calabresi et al., 2007). Selectivity is achieved by reinforcement acting specifically on recently or concurrently active inputs (Redgrave and Gurney, 2006; Arbuthnott and Wickens, 2007). In this model, reward-related inputs from the cerebral cortex are reinforced by signals from DA neurones in the ventral midbrain

(Schultz, 1998). The pattern of neural activity that has received most attention in this regard is the short-latency, short-duration phasic DA response (Schultz, 1998). The apparent capacity of DA neurones to signal events that are "better" or "worse" than expected (reward prediction errors) has captured the imagination of both the neuroscience (Schultz, 1998; O'Doherty et al., 2003; Ungless, 2004) and computational communities (Montague et al., 1996; Dayan and Balleine, 2002).

However, over the past decade we have argued that for DA neurones to signal estimates of reward prediction errors, the current "reward value" of an unexpected eliciting event must first be evaluated. Presumably, this would rely on sensory systems afferent to the DA neurones being able to determine the value of unpredicted stimuli. In mammals, an unexpected event typically elicits an orienting gaze-shift that brings it onto the fovea for analysis by cortical visual systems (Thorpe and Fabre-Thorpe, 2001). The latency of these gaze-shifts are normally in the range 150–200 ms (Hikosaka and Wurtz, 1983; Jay and Sparks, 1987). Since the latencies of phasic DA responses are normally shorter (~70–100 ms) (Schultz, 1998), they must be initiated on the basis of pre-attentive, pre-gaze-shift sensory processing. In a series of investigations, we established that the SC is the most likely source of short-latency visual input that drives phasic DA responses. First, as described above, a direct tectonigral projection (**Figure 3**) has been observed in rat (Comoli et al., 2003), cat (McHaffie et al., 2006), and monkey (May et al., 2009). Second, SC visual response latencies are always shorter than those recorded in DA neurones (Jay and Sparks, 1987; Schultz, 1998; Comoli et al., 2003). Third, visual-evoked activity in substantia nigra is abolished when the visual (superficial) layers of the SC are removed (Comoli et al., 2003). Fourth, local disinhibition of the SC in anesthetized animals restores visual sensitivity to DA neurones in substantia nigra and the resultant visually evoked DA release into the striatum (Dommett et al., 2005). Consequently, we have proposed the SC as the primary, if not exclusive, source of short-latency visual input to ventral midbrain DA neurones (Comoli et al., 2003; Dommett et al., 2005).

If the tectonigral projection is responsible for the short-latency visual activation of DA neurones, the branching collaterals to the thalamus would assume great importance for the mechanisms of reinforcement in the striatum. Presumably, short-latency sensory signals originating from the SC would be transmitted simultaneously to the striatum, via DA neurones in substantia nigra and via glutamatergic thalamostriatal neurones (**Figure 4**). The likely temporal convergence of widely broadcast thalamostriatal and nigrostriatal inputs evoked by the same sensory event could have important implications for experience-dependent plasticity in the basal ganglia (Centonze et al., 2001; Reynolds and Wickens, 2002; Arbuthnott and Wickens, 2007; Calabresi et al., 2007; Shen et al., 2008). Specifically, under natural conditions, striatal neurones might expect that a sensory-evoked phasic input of DA from SNc would be accompanied by a phasic input of glutamate from the thalamus. The processes most likely to be reinforced by these subcortical signals are those associated with learning the causal structure between behavioral output and biologically salient sensory events (Redgrave and Gurney, 2006; Redgrave et al., 2008). Finally, on a practical note, the possibility of having the same sensory event initiate a coincident afferent convergence of glutamatergic and DA inputs in the striatum could also have important implications for how functional MRI studies investigating basal ganglia responses to biologically salient sensory events, including reward, are interpreted (McClure et al., 2003; O'Doherty et al., 2003; Zink et al., 2004; Knutson and Cooper, 2005). The possibility that signals from the SC to the striatum via the thalamus could act in concert with phasic input from ascending DA neurones to influence striatal hemodynamic responses to salient visual events should now be considered.

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Topographical organization of the pedunculopontine nucleus

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Neurons in the pedunculopontine nucleus (PPN) exhibit a wide heterogeneity in terms of their neurochemical nature, their discharge properties, and their connectivity. Such characteristics are reflected in their functional properties and the behaviors in which they are involved, ranging from motor to cognitive functions, and the regulation of brain states. A clue to understand this functional versatility arises from the internal organization of the PPN. Thus, two main areas of the PPN have been described, the rostral and the caudal, which display remarkable differences in terms of the distribution of neurons with similar phenotype and the projections that originate from them. Here we review these differences with the premise that in order to understand the function of the PPN it is necessary to understand its intricate connectivity. We support the case that the PPN should not be considered as a homogeneous structure and conclude that the differences between rostral and caudal PPN, along with their intrinsic connectivity, may underlie the basis of its complexity.

Keywords: pedunculopontine, brainstem, basal ganglia, reticular activating system, neuronal heterogeneity, synaptic organization, microcircuits, connectivity

INTRODUCTION

The pedunculopontine nucleus (PPN) is located in the upper brainstem and has an irregular shape delimited by the borders of its population of cholinergic neurons. The PPN has been conserved in evolution across species and is present in early mammals and amphibians (Marin et al., 1998; Grillner et al., 2008). Defined in humans in 1982 (Olszewski and Baxter, 1982), it is considered a part of the reticular activating system and, as a reticular structure, it has been argued that PPN has no clear boundaries. One of the main characteristics of neurons of the PPN is their long-range axonal projections, reaching numerous targets across the brain, from distant forebrain structures (Woolf and Butcher, 1986; Hallanger and Wainer, 1988a) to the spinal cord (Rye et al., 1988; Spann and Grofova, 1989). Although initially considered to function as a relay nucleus within ascending activating systems, increasing evidence on the neuronal heterogeneity of the PPN and its local synaptic organization, suggest that this high level of connectivity with functionally distinct neuronal systems underlies an integrative function rather than a role as a simple relay nucleus. An example of this heterogeneous connectivity is the way the PPN is integrated into basal ganglia circuits: distinct functional types of neurons in the PPN innervate basal ganglia and, in turn, basal ganglia projects back to PPN and innervate different neuronal populations. This remarkable interconnectivity has been the subject of previous reviews that have stressed that most structures of the basal ganglia project to, and receive inputs from, the PPN (Pahapill and Lozano, 2000; Mena-Segovia et al., 2004). In the present review we will discuss recent evidence on the heterogeneous distribution of neurochemical subtypes of neurons within the PPN and correlate this with data on their connectivity. We will make use of the large amount of information available that describe the anatomical relationship that the PPN maintains with the basal ganglia and that provide evidence of a topographical organization. We will also correlate such organization with the connectivity of the PPN with other neuronal systems to integrate a theory supporting functional domains in the PPN.

NEUROCHEMICAL DIVERSITY

It has now been widely agreed that the PPN is composed by a mixture of neurons of different sizes, of different neurochemical phenotype and with distinct connectivity. Cholinergic neurons represent a minority of the neurons in the PPN and are intermingled amongst a large number of GABAergic and glutamatergic neurons, which are heterogeneously distributed across its rostrocaudal axis (Mena-Segovia et al., 2009; Wang and Morales, 2009). A parasagittal view of the PPN illustrates the different distributions of the neuronal populations and therefore better represents the anatomical organization of the PPN. Using external landmarks such as the substantia nigra (SN) and the superior cerebellar peduncle, which maintain a constant spatial relationship with the PPN across different medio-lateral levels, it is possible to follow the distribution of the cell types that compose the PPN. GABAergic neurons are more densely concentrated in the rostral PPN, compared to cholinergic and glutamatergic neurons. In the rat they are detectable from the rostral border of the PPN (limiting the caudal part of the SN) and their density decreases dramatically at a level about 1.2–1.5 mm further caudal (Mena-Segovia et al., 2009; **Figure 1**). Such a drop in the density of GABAergic neurons coincides with a change in the cytoarchitecture and organization of cholinergic neurons: bipolar-shaped cholinergic neurons tend to be organized in a layer-like structure close to the SN, where GABAergic neurons are several times more abundant. Following the decline in the number of GABAergic neurons, rounded-shaped cholinergic neurons show a distinct configuration. Instead of lying in the layer-like arrangement, cholinergic neurons show an apparently random distribution and an increased number of processes. The change in density of cholinergic neurons, however, is not as marked as that of the GABAergic neurons or the glutamatergic neurons. In contrast to the rostral PPN, the caudal part of the nucleus has a larger proportion of glutamatergic neurons (Wang and Morales, 2009). Until now, no other neurochemical cell types have been identified

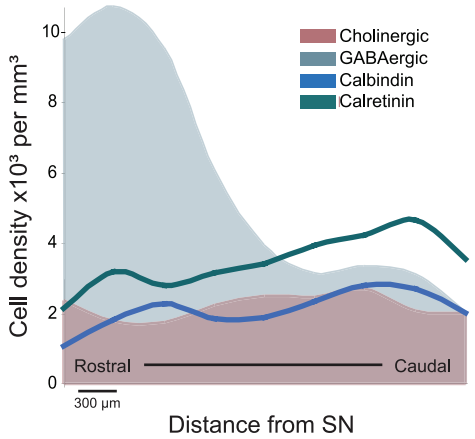

FIGURE 1 | Schematic representation of the distribution of distinct neuronal populations in the PPN. GABAergic neurons are highly concentrated in the rostral PPN, whereas cholinergic, glutamatergic (not shown), calbindin- and calretinin-expressing neurons are more abundant in the caudal PPN. The PPN was divided into 300 µm segments and cell density was evaluated throughout its rostro-caudal extent (Martinez-Gonzalez et al., 2009; Mena-Segovia et al., 2009). The difference in the rostro-caudal distribution of GABAergic neurons correlates with the differences in cytoarchitecture of the cholinergic neurons traditionally used to identify PPN regions (i.e., *pars dissipata* and *pars compacta*). As shown in this figure, the rostral PPN is an area of high neuronal density. SN, substantia nigra.

although differences have been observed within each neuronal type in relation to the co-expression of other neurochemical markers and their firing properties.

In different regions of the brain including hippocampus (Acsady et al., 1993; Somogyi and Klausberger, 2005), cortex (Staiger et al., 2004), and basal ganglia (Parent et al., 1996), neurons that express calcium-binding proteins have been shown to have distinct functional properties despite the fact that they may use the same transmitter. They have thus proved to be useful markers to distinguish sub-populations of neurons. Calcium-binding proteins have also been reported to be expressed by neurons in the PPN in monkeys (Cote and Parent, 1992; Fortin and Parent, 1999) and rats (Dun et al., 1995), and indeed, calbindin and calretinin are expressed by a similar number of neurons to that of cholinergic neurons in the rat PPN (Martinez-Gonzalez et al., 2009). Although they are rarely expressed in cholinergic neurons, they are commonly expressed by GABAergic and glutamatergic neurons suggesting functional subtypes of GABAergic and glutamatergic neurons.

Significant differences have been observed also in terms of the *in vivo* firing properties of PPN neurons. Cholinergic neurons show two types of firing patterns: slow-firing cholinergic neurons that are associated to the cortical upstate during slow oscillations, and fast-firing cholinergic neurons that are correlated to the cortical downstate during slow oscillations (Mena-Segovia et al., 2008). No neurochemical markers of these subtypes have been identified. Neurons that have been identified as non-cholinergic and have been assigned as putative glutamatergic neurons because they give rise to asymmetric synaptic contacts in their targets, also show two main subtypes: fast-firing neurons that are associated with the cortical slow oscillations (Mena-Segovia et al., 2008), and quiescent (or very slow firing) neurons whose firing is independent of the cortical activity (Ros et al., 2010). These putative glutamatergic neurons have a different axonal trajectory and pattern of innervation from those of cholinergic neurons, although some targets are shared by both types (notably, the basal ganglia). Other neurons that have not been characterized in terms of their neurochemical nature include tonic firing neurons and irregular firing neurons; it is likely that at least one of these subtypes are GABAergic (Ros et al., 2010). The correlation between neurochemical subtypes and electrophysiological properties recorded in *in vitro* experiments is more limited. Three types of neurons have been described on the basis of their membrane properties (A-current type, low-threshold spikes type and mixed A-current plus LTS type; Leonard and Llinas, 1994; Takakusaki et al., 1997; Saitoh et al., 2003), although this classification does not seem to be related to their neurochemical nature (and therefore not to their synaptic targets). The different membrane properties, however, are likely to underlie some of the functional differences within each cell subtype.

In summary, each main neuronal type in the PPN is composed of at least two subtypes; the PPN is thus a highly heterogeneous structure at the cellular, molecular, and electrophysiological levels. The different neuronal types are heterogeneously distributed in the PPN, perhaps delineating functional territories (rostral and caudal) determined by a greater density of GABAergic axons arising from the rostral PPN in contrast to a greater density of cholinergic and glutamatergic axons arising from the caudal PPN, thus producing contrasting effects on their target structures.

INTERNAL STRUCTURE AND CONNECTIVITY: THE CASE FOR SUBDIVISIONS OF THE PPN

The notion that the PPN is not homogeneous in terms of its cellular organization is not recent; the PPN was originally divided in *pars dissipata* (rostral) and *pars compacta* (caudal) on the basis of the density of cholinergic neurons (Olszewski and Baxter, 1982), which were believed to be the most representative, if not the only, neuronal type in the PPN (Rye et al., 1987). Other subdivisions included rostral, middle, and caudal thirds, and the inclusion of an area referred to as the midbrain extrapyramidal area (MEA) which receives a dense innervation from the basal ganglia but lies outside the cholinergic borders of the PPN (Steininger et al., 1992). While all of these subdivisions are based on identifiable characteristics in the anatomy of the PPN, it is perhaps the rostral and caudal division that is the most appropriate since it is based on the distribution of all known cell types rather than only the cholinergic neurons. Indeed, GABAergic neurons provide a unique distribution that correlates with the cholinergic *pars dissipata* and *pars compacta* subdivisions (Mena-Segovia et al., 2009). The rostro-caudal division is also the basis for many anatomical studies describing afferents and efferents to and from the PPN, as discussed in the following sections, but essentially does not rely on cell density (as the terms *dissipata* and *compacta* denote).

The idea of two functionally distinct regions of the PPN is supported by the distribution of its cell types defined on the basis of neurochemistry and the connectivity of the PPN with other neuronal systems. Thus, two neurochemically distinct projections arising from rostral and caudal PPN diverge, innervating distinct

structures, but also converge in others. This functional dichotomy seems to be locally regulated, as suggested by the evidence of axonal collaterals of PPN neurons. Thus, a local synaptic network has been identified after tracing the axons of individually labeled neurons (Mena-Segovia et al., 2008; Ros et al., 2010). Both cholinergic and non-cholinergic neurons contribute to this connectivity, although the number of axonal varicosities was found to be greater in cholinergic neurons. Interestingly, a large proportion of PPN projection neurons have axons that travel within the PPN in a rostro-caudal direction, providing local innervation that synaptically links the rostral and the caudal portions. This evidence of a local network of heterogeneous neurochemical nature supports the role of the PPN as an integrator between its input and output systems.

EFFERENT CONNECTIVITY

Neurons of the PPN give rise to long axons that innervate several targets. The longest axons arise from cholinergic neurons and give rise to as many as five or six collaterals that innervate the basal ganglia, thalamus, tectum, and lower brainstem, among other regions (Mena-Segovia et al., 2008). The axons of non-cholinergic neurons are more restricted in terms of length and number of collaterals (typically two; Ros et al., 2010). Single-cell labeling experiments, have demonstrated that both cholinergic and non-cholinergic neurons project preferentially to the basal ganglia and that several divisions of the basal ganglia are innervated. Tracer studies have also produced extensive data on the connections of neurons in the PPN (**Table 1**).

BASAL GANGLIA

Tracer experiments have shown that the STN receives input from the PPN in the cat (Nomura et al., 1980; Edley and Graybiel, 1983), rat (Saper and Loewy, 1982; Hammond et al., 1983), and monkey (Carpenter et al., 1981; Lavoie and Parent, 1994). More recently, tractography studies have confirmed these findings in humans (Muthusamy et al., 2007). In the monkey and rat, the neurons that project to the STN are located in the caudal PPN (Carpenter et al., 1981; Martinez-Gonzalez et al., 2009; Kita and Kita, 2010). Such projections have been identified to include cholinergic, GABAergic, and glutamatergic components (Bevan and Bolam, 1995).

The entopeduncular nucleus (EP, in rat and cat, equivalent to the internal segment of the globus pallidus or GPi in the monkey) receives input from the PPN (Saper and Loewy, 1982; Jackson and Crossman, 1983; Woolf and Butcher, 1986; Shink et al., 1997), and these projections have an excitatory influence on EP neurons in the rat and cat (Gonya-Magee and Anderson, 1983; Scarnati et al., 1988). A species-specific difference seems to exist regarding the density of these projections, since they have been reported to be larger in the monkey than in the cat (Edley and Graybiel, 1983). In the monkey, tracer injections in the GPi give rise to a large number of retrogradely labeled PPN neurons in the rostral PPN, around 40% of which are cholinergic. In contrast, a restricted GPe injection produced a smaller number of labeled neurons in the PPN (Charara and Parent, 1994). This difference was confirmed by anterograde labeling, *Phaseolus vulgaris* leucoagglutinin injections in the PPN give rise to a much higher density of anterogradely labeled fibers in the GPi than in the GPe (Lavoie and Parent, 1994).

The SN *pars compacta* (SNc) and *pars reticulata* (SNr) are interconnected with the PPN (Saper and Loewy, 1982; Woolf and Butcher, 1986). In the rat and monkey, SNc dopaminergic neurons receive direct glutamatergic and cholinergic input from PPN neurons (Sugimoto and Hattori, 1984; Clarke et al., 1987; Scarnati et al., 1987; Gould et al., 1989; Bolam et al., 1991; Futami et al., 1995; Oakman et al., 1995; Charara et al., 1996). These projections emit collaterals that innervate the medial reticular formation in the cat (Nakamura et al., 1989). Furthermore, the activation of the PPN can elicit an excitatory effect on SN neurons in the rat (Scarnati et al., 1984, 1987), evoking monosynaptic glutamatergic and cholinergic excitatory postsynaptic potentials in dopamine neurons (Futami et al., 1995) and non-dopamine neurons (Rohrbacher et al., 2000). In the monkey, the neurons that project to the SN are concentrated in the rostral PPN and about 25% of these are cholinergic. However, very few projecting neurons are located in the caudal PPN (Lavoie and Parent, 1994). In the rat, cholinergic and non-cholinergic neurons that arborize within the SNc are preferentially located in the rostral PPN (Takakusaki et al., 1996). These projections are mostly ipsilateral (Oakman et al., 1995, 1999).

The ventral tegmental area (VTA) also receives substantial cholinergic innervation from the PPN in the rat and monkey (Sugimoto and Hattori, 1984; Oakman et al., 1995; Charara et al., 1996; Geisler and Zahm, 2005). In monkeys, retrograde tracing experiments have shown the existence of glutamatergic and GABAergic afferents from the PPN to the VTA (Charara et al., 1996). In the rat, neurons that send projections to the VTA are concentrated in the caudal PPN, project bilaterally and involve cholinergic (Sugimoto and Hattori, 1984; Oakman et al., 1995), GABAergic and glutamatergic axons (Mena-Segovia et al., 2005). *In vitro*, stimulation of cortical and PPN afferents to the VTA induce glutamatergic synaptic currents in VTA dopaminergic and non-dopaminergic neurons (Bonci and Malenka, 1999). More recently, it has been described that GABAergic and monosynaptic glutamatergic PPN inputs do not converge on the same VTA neurons (Good and Lupica, 2009). Nicotinic acetylcholine receptors (nAChR) can also modulate this excitatory synaptic transmission (Good and Lupica, 2009).

Last but not least, a direct projection from the PPN to the striatum (or caudate/putamen in primates) has been identified in the rat (Saper and Loewy, 1982) and in the monkey (Nakano et al., 1990).

THALAMUS

The thalamus is heavily innervated by the PPN in the rat (Saper and Loewy, 1982; Hallanger and Wainer, 1988a), cat and monkey (Parent et al., 1988), and a large proportion of this output is cholinergic (Sugimoto and Hattori, 1984; Sofroniew et al., 1985; Hallanger et al., 1987; Hallanger and Wainer, 1988a,b; Takakusaki et al., 1996; Oakman et al., 1999; Parent and Descarries, 2008). The projections are widespread, innervating several thalamic nuclei (Smith et al., 1988; Steriade et al., 1988; Kolmac and Mitrofanis, 1998), they are topographically organized in the cat (Steriade et al., 1988) and monkey (Lavoie and Parent, 1994), and arise from neurons that are predominantly located in the caudal PPN. In the cat and monkey, the majority of the thalamic nuclei receive less than 10% of their PPN innervation from the rostral PPN, with the exception of the mediodorsal thalamic nucleus, which receives up to 20% of its innervation from the rostral PPN (Steriade et al., 1988).

Table 1 | Efferent connectivity of the pedunculopontine nucleus.

Target brain area	PPN region	References
BASAL GANGLIA		
STN	Caudal	Nomura et al. (1980), Carpenter et al. (1981), Saper and Loewy (1982), Edley and Graybiel (1983), Hammond et al. (1983), Lavoie and Parent (1994), Bevan and Bolam (1995), Muthusamy et al. (2007), Martinez-Gonzalez et al. (2009), Kita and Kita (2010)
EP/GPi	Rostral	Saper and Loewy (1982), Gonya-Magee and Anderson (1983), Jackson and Crossman (1983), Woolf and Butcher (1986), Scarnati et al. (1988), Charara and Parent (1994), Lavoie and Parent (1994), Shink et al. (1997)
SN	Rostral	Saper and Loewy (1982), Scarnati et al. (1984, 1987), Sugimoto and Hattori (1984), Woolf and Butcher (1986), Clarke et al. (1987), Gould et al. (1989), Nakamura et al. (1989), Bolam et al. (1991), Lavoie and Parent (1994), Futami et al. (1995), Oakman et al. (1995), 1999, Charara et al. (1996), Takakusaki et al. (1996)
VTA	Caudal	Sugimoto and Hattori (1984), Oakman et al. (1995), Charara et al. (1996), Geisler and Zahm (2005), Mena-Segovia et al. (2005), Good and Lupica (2009)
STR		Saper and Loewy (1982), Nakano et al. (1990)
THALAMUS		
MD	Caudal	Steriade et al. (1988), Lavoie and Parent (1994)
VA	Caudal	Smith et al. (1988)
VL	Caudal	Smith et al. (1988)
VM	Caudal	Smith et al. (1988)
Pf	Caudal	Sugimoto and Hattori (1984), Kobayashi and Nakamura (2003), Kobayashi et al. (2007), Parent and Descarries (2008)
DG	Caudal	Parent and Descarries (2008)
Rt	Caudal	Parent and Descarries (2008)
LM-Sg	Caudal	Hoshino et al. (1997, 2000)
CM/CL/PV	Caudal	Steriade et al. (1990), Erro et al. (1999), Krout et al. (2002)
TECTUM		
IC	Caudal	Mena-Segovia et al. (2008), Motts and Schofield (2009)
SC	Caudal	Beninato and Spencer (1986), Hall et al. (1989), Krauthamer et al. (1995), Mena-Segovia et al. (2008)
FOREBRAIN		
MgPA	Rostral	Semba and Fibiger (1992), Losier and Semba (1993)
NbMC	Rostral	Semba and Fibiger (1992)
Hypothalamus		Woolf and Butcher (1986), Hallanger et al. (1987), Ford et al. (1995)
LM-Sg		Hoshino et al. (2004)
RMTg		Jhou et al. (2009)
BRAINSTEM		
PRF	Caudal	Mitani et al. (1988), Semba et al. (1990), Takakusaki et al. (1996)
GiN	Caudal	Mitani et al. (1988), Rye et al. (1988), Grofova and Keane (1991), Martinez-Gonzalez et al. (2009)
MVM	Caudal	Skinner et al. (1990b)
MRF	Caudal	Nakamura et al. (1989), Grofova and Keane (1991)
NPO	Caudal	Garcia-Rill et al. (2001)
Spinal cord	Caudal	Rye et al. (1988), Spann and Grofova (1989), Skinner et al. (1990a)

Abbreviations: STN, subthalamic nucleus; EP, entopeduncular nucleus; GPi, internal segment of the globus pallidus; SN, substantia nigra; VTA, ventral tegmental area; STR, striatum; MD, mediodorsal; VA, ventral anterior; VL, ventro-lateral; VM, ventro-medial; Pf, parafascicular; DG, dorsal geniculate; Rt, retocular thalamic; LM-Sg, lateralis medialis-supragenicuIate; CM, centromedian; CL, centrolateral; PV, paraventricular; IC, inferior colliculus; SC, superior colliculus; MgPA, magnocellular preoptic area; NbMC, nucleus basalis magnocellularis; RMTg, mesopontine rostromedial tegmental nucleus; PRF, pontine reticular formation; GiN, gigantocellular nucleus; MVM, medioventral medulla; MRF, medial reticular formation; NPO, nucleus pontis oralis.

The thalamic input from the PPN is both cholinergic and non-cholinergic (Smith et al., 1988; Steriade et al., 1988). Retrograde labeling from the mediodorsal thalamic nucleus produces prominent labeling of non-cholinergic neurons in both the ipsilateral and contralateral PPN in the cat (Smith et al., 1988). The ventro-anterior and the ventro-lateral thalamic nuclei receive between 50 and 75% of their cholinergic afferents from the central–caudal PPN, whereas the ventro-medial thalamic nucleus receives its cholinergic input mainly from the caudal PPN and the laterodorsal tegmental nucleus (Smith et al., 1988). In the rat, the parafasicular nucleus (Pf) receives inputs from the caudal PPN (Sugimoto and Hattori, 1984). The cholinergic innervation to the Pf has a higher density than the

innervation to the dosolateral geniculate and reticular thalamic nuclei (Parent and Descarries, 2008). Individual Pf neurons receive convergent synaptic inputs from the PPN and the SC (Kobayashi and Nakamura, 2003). In turn, Pf neurons that receive inputs from the PPN project to striatum (Erro et al., 1999), linking monosynaptically PPN axons to thalamostriatal neurons (Kobayashi et al., 2007). Cholinergic neurons that project to the thalamus send collaterals to the basal forebrain (Losier and Semba, 1993), the pontine reticular formation (Semba et al., 1990), the superior and inferior colliculi, and the basal ganglia (Mena-Segovia et al., 2008). In the cat, glutamatergic and GABAergic neurons of the lateralis medialis-suprageniculate nuclear complex (LM-Sg) receive cholinergic input from the PPN (Hoshino et al., 1997, 2000). Other thalamic nuclei receive a more heterogeneous mixture of cholinergic and non-cholinergic PPN afferents, such as the centrolateral, centromedial, and paraventricular thalamic nuclei in the rat (Erro et al., 1999; Krout et al., 2002).

OTHER ASCENDING PROJECTIONS

Individual cholinergic neurons of the PPN project to the superior and inferior colliculi in the rat (Mena-Segovia et al., 2008). The large majority of the cholinergic inputs to the inferior colliculus arise from the ipsilateral PPN and to a less extent, from the LTD. These projections arise from cholinergic neurons that are located in the caudal PPN and include a subpopulation that project to both the ipsilateral and contralateral inferior colliculus in the guinea pig (Motts and Schofield, 2009). The superior colliculus receives cholinergic and non-cholinergic innervation from the PPN in the rat and cat (Beninato and Spencer, 1986; Hall et al., 1989). These afferents arise mainly from the caudal PPN (Beninato and Spencer, 1986). PPN neurons projecting to the superior colliculus, as identified by antidromic stimulation, are segregated into two groups: those that are sensitive and those that are insensitive to physiological sensory stimuli (Krauthamer et al., 1995). A small group of neurons located in the rostral PPN have collaterals that innervate the superior colliculus and the LM-Sg in the cat (Hoshino et al., 2004). In the rat, other ascending targets include the mesopontine rostromedial tegmental nucleus (RMTg; Jhou et al., 2009).

In the forebrain, the magnocellular preoptic area (MgPA) and the nucleus basalis magnocellularis receive afferents from the PPN (Semba et al., 1988; Losier and Semba, 1993). The posterior lateral hypothalamus receives cholinergic input from PPN neurons that are scattered throughout the rostro-caudal axis, and GABAergic innervation from neurons that are concentrated in the rostral PPN; the neurochemical nature of the majority of the projection neurons was not identified (Ford et al., 1995).

BRAINSTEM AND OTHER DESCENDING PROJECTIONS

Neurons in the PPN make a dense innervation on structures in the lower brainstem, pons, medulla, and spinal cord. The descending projections arise from collaterals of ascending axons, as seen in cholinergic (Mena-Segovia et al., 2008) and non-cholinergic neurons (Martinez-Gonzalez et al., 2009), or from non-cholinergic neurons with single descending axons (Ros et al., 2010). Both cholinergic and non-cholinergic neurons innervate the pontine reticular formation in the rat (Semba et al., 1990; Takakusaki et al., 1996) and the cat (Mitani et al., 1988). Thus, PPN has been shown to project to the gigantocellular nucleus (GiN) in rats and cats (Mitani et al., 1988; Rye et al., 1988; Grofova and Keane, 1991; Martinez-Gonzalez et al., 2009), the medioventral medulla (Skinner et al., 1990b), rostral ventro-lateral medulla (Yasui et al., 1990), medial reticular formation, medulla oblongata (Nakamura et al., 1989; Grofova and Keane, 1991), and the spinal cord in rats (Rye et al., 1988; Spann and Grofova, 1989; Skinner et al., 1990a). The majority of the PPN projecting neurons to the spinal cord are non-cholinergic (Skinner et al., 1990a). These descending PPN projections are considered to be directly involved in locomotion since the stimulation of neurons in the caudal PPN leads to a prolonged activation of neurons in the nucleus reticularis pontis oralis and changes in the flexor and extensor nerves in decorticated cats (Garcia-Rill et al., 2001).

In summary, the ascending projections from the rostral PPN preferentially innervate the EP/GPi, SN and the lateral hypothalamus in the rat, cat, and monkey. In contrast, ascending projections from the caudal PPN innervate the thalamus, STN, VTA, SC, and IC.

AFFERENT CONNECTIVITY

Although the information available on the afferent innervation to the PPN is not as abundant and detailed as it is with regards to its efferents, it is clear that the PPN receives a heterogeneous modulation arising from functionally diverse areas of the brain. Thus, neurons in the PPN receive afferents from structures that include the cortex, thalamus, hypothalamus, pons, cerebellum, medulla, spinal cord, and the basal ganglia (Saper and Loewy, 1982; Semba and Fibiger, 1992; **Table 2**).

The PPN receives a direct input from the cerebral cortex arising from distinct frontal lobe areas involved in motor control in the monkey. These convergent inputs seem to target the dorsal and caudal PPN areas (Matsumura et al., 2000). In the rat, these afferents have also been demonstrated, although they seem to be less abundant (Semba and Fibiger, 1992). They arise also from the medial prefrontal cortex (Sesack et al., 1989). In addition, cholinergic PPN neurons receive afferents from the primary auditory cortex in guinea pigs (Schofield and Motts, 2009).

BASAL GANGLIA

The PPN receives a direct input from the STN in the rat (Jackson and Crossman, 1981; Kita and Kitai, 1987), the cat and the monkey (Nauta and Cole, 1978). In addition to the anatomical evidence, electrophysiological experiments in the rat have shown that this input is excitatory and it targets neurons in the PPN (Granata and Kitai, 1989). Furthermore, the STN can modulate PPN activity indirectly through the SNr; this pathway has an inhibitory effect on PPN neurons (Hammond et al., 1983; Florio et al., 2007).

Tracer studies show that the EP sends projections that innervate PPN neurons in the rat (Semba and Fibiger, 1992) and in the monkey (Shink et al., 1997). In the latter, the GPi afferents preferentially target NADPH diaphorase-negative neurons in the rostral PPN, establishing symmetric synapses with proximal dendrites. GP afferents to the PPN arise from the caudal GP, in contrast to the rostral GP that projects to the STN (Moriizumi and Hattori, 1992). GPi axons that innervate PPN neurons arise from type I neurons that are abundant in the center of the GPi (Parent et al., 2001),

Table 2 | Afferent connectivity of the pedunculopontine nucleus.

Origin	Target PPN region	References
CEREBRAL CORTEX		
FL	Caudal	Semba and Fibiger (1992), Matsumura et al. (2000)
PAC		Schofield and Motts (2009)
MPC		Sesack et al. (1989)
CEREBELLUM		
DCN		Hazrati and Parent (1992)
BASAL GANGLIA		
STN		Nauta and Cole (1978), Jackson and Crossman (1981), Hammond et al. (1983), Kita and Kitai (1987), Granata and Kitai (1989), Semba and Fibiger (1992), Florio et al. (2007)
EP/GPi	Rostral	Smith et al. (1990), Moriizumi and Hattori (1992), Semba and Fibiger (1992), Shink et al. (1997), Parent et al. (2001)
SN	Rostral	Noda and Oka (1986), Scarnati et al. (1987), Nakamura et al. (1989), Granata and Kitai (1991), Spann and Grofova (1991), Semba and Fibiger (1992), Saitoh et al. (2003)
VTA		Haber et al. (1990), Semba and Fibiger (1992)
STR		Semba and Fibiger (1992)
TECTUM		
SC		Woolf and Butcher (1986), Redgrave et al. (1987), Semba and Fibiger (1992), Steininger et al. (1992)
BRAINSTEM		
LTDg		Satoh and Fibiger (1986), Cornwall et al. (1990), Semba and Fibiger (1992)
Contralateral PPN		Semba and Fibiger (1992)
FOREBRAIN		
LC		Jones and Yang (1985)
Habenula		Semba and Fibiger (1992)
RMTg		Jhou et al. (2009)
ZI		Satoh and Fibiger (1986), Semba and Fibiger (1992), Kolmac et al. (1998)
DR	Caudal	Vertes (1991), Steininger et al. (1997)

Abbreviations: FL, frontal lobe; PAC, primary auditory cortex; MPC, medial prefrontal cortex; DCN, deep cerebellar nuclei; STN, subthalamic nucleus; EP, entopeduncular nucleus; GPi, internal segment of the globus pallidus; SN, substantia nigra; VTA, ventral tegmental area; STR, striatum; SC, superior colliculi; LTDg, laterodorsal tegmental nucleus; PPN, pedunculopontine nucleus; LC, locus ceruleus; RMTg, mesopontine rostromedial tegmental nucleus; ZI, zona incerta; DR, dorsal raphé.

although some authors differ on this, finding retrogradely labeled neurons in the whole GP after retrograde tracer injection in the rat STN (Smith et al., 1990). This is particularly relevant because the PPN is involved into a circuit that involves the STN and GP, one of the principal outflows of the basal ganglia.

The PPN also receives afferents from the SN in the rat and cat (Nakamura et al., 1989; Spann and Grofova, 1991; Semba and Fibiger, 1992), and this input is inhibitory (Noda and Oka, 1984; Scarnati et al., 1987; Granata and Kitai, 1991) and mediated by GABA (Saitoh et al., 2003). It is not clear yet which PPN neurons are the targets of these afferents, but electron microscopy (EM) studies have shown that nigral afferents to the PPN establish synaptic contacts preferentially with non-cholinergic neurons located in the rostral PPN (Spann and Grofova, 1991; Grofova and Zhou, 1998). Some of these neurons are glutamatergic, and a lower proportion are cholinergic (Grofova and Zhou, 1998).

Other basal ganglia projections to the PPN include the ventral striatum in monkey (Haber et al., 1990) and the VTA in rats (Semba and Fibiger, 1992). Indeed, PPN neurons receive a dopaminergic innervation presumably arising from the mesencephalon in the monkey (Rolland et al., 2009).

OTHER AFFERENT SYSTEMS

The PPN receives afferents from the habenula (Semba and Fibiger, 1992) and the zona incerta (Semba and Fibiger, 1992; Kolmac et al., 1998), the deep cerebellar nuclei (Hazrati and Parent, 1992), the mesopontine RMTg (Jhou et al., 2009) and the superior and inferior colliculi in the rat (Woolf and Butcher, 1986; Redgrave et al., 1987; Semba and Fibiger, 1992; Steininger et al., 1992).

In the brainstem, tracer studies in the rat have shown that the rostral and caudal portions of the dorsal raphé send projections that innervate the PPN (Vertes, 1991), where they preferentially target non-cholinergic neurons in the caudal PPN (Steininger et al., 1997). The locus coeruleus (Jones and Yang, 1985) and the laterodorsal tegmental nucleus also innervate the PPN (Satoh and Fibiger, 1986; Cornwall et al., 1990; Semba and Fibiger, 1992). Furthermore, the PPN receives an input from the contralateral PPN (Semba and Fibiger, 1992).

In summary, the PPN receives afferents from the basal ganglia, cortex, thalamus, cerebellum, forebrain, spinal cord, pons, and the contralateral PPN. The rostral PPN receives inhibitory input from the SN and the EP/GPi. The caudal PPN receives inputs from the dorsal raphé and the motor cortex.

FUNCTIONAL IMPLICATIONS OF TOPOGRAPHY

The data arising from the connectivity studies show that a significant number of structures have a selective relationship with distinct regions within the PPN. This is clearly evident from the analysis of retrograde and anterograde tracing studies showing the distribution of PPN projecting neurons, although less clear with regards to the distribution of the PPN afferents. Nevertheless, in the case of PPN inputs, two important neuronal systems, the basal ganglia and the cortex, seem to contact neurons in distinct regions of the PPN. Thus, the GABAergic output neurons of the basal ganglia, arising in the SNr and EP/GPi, mainly contact neurons located in the rostral PPN. In contrast, neurons in the cortex and the dorsal raphé preferentially innervate neurons in the caudal PPN. In terms of its efferents, the rostral PPN projects to the SNr, SNc, GPi, and the hypothalamus. In contrast, the caudal PPN projects to the STN, the VTA, the thalamus, and the superior and inferior colliculi (**Figure 2**).

The differences in connectivity suggest that there is a functional reciprocity in different areas of the PPN with regards to its inputs and outputs. These differences reveal that, (1) the rostral PPN, which contains a significantly larger number of GABAergic neurons, is interconnected with the structures that provide the GABAergic output from the basal ganglia, therefore suggesting a close functional relationship with basal ganglia operations; and (2) the caudal PPN, which contains a larger number of cholinergic and glutamatergic neurons, receives information from cortex and dorsal raphé, and projects to targets in the thalamus and colliculi, suggesting a close relationship with the modulation of brain states mediated through thalamocortical systems. Moreover, it is also the caudal PPN that projects to the STN and to the brainstem locomotor regions involved in gait and posture and the modulation of the muscular tone across different brain states.

Behavioral studies following restricted lesions or selective manipulations in the rostral and caudal PPN have shown functional differences between these two PPN areas that correlate with the functional domains defined by the neurochemical distribution and connectivity (Inglis et al., 2001; Alderson et al., 2006, 2008; Andero et al., 2007). Such functional differences have relevance for deep brain stimulation (DBS) therapy in the PPN in Parkinson's disease patients (Starr et al., 1998; Pahapill and Lozano, 2000; Nandi et al., 2002; Benabid, 2003; Jenkinson et al., 2005; Stefani et al., 2007). As is evident from the cell types and their efferents, it is most likely that the stimulation of an electrode situated in the rostral PPN will have very different effects to an electrode situated in the caudal PPN.

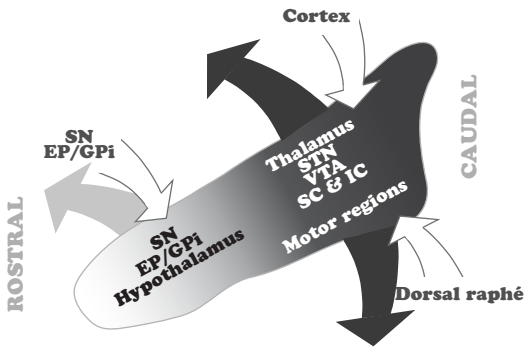

FIGURE 2 | Summary of the topographical distribution of the connectivity in the PPN. The rostral PPN, which is predominantly GABAergic, maintains interconnections with the GABAergic output of the basal ganglia. In contrast, the caudal PPN, where cholinergic and glutamatergic neurons are more abundant, receives input from the cortex and dorsal raphé and projects to the thalamocortical systems, STN and locomotor regions. Only major inputs and outputs, and those structures whose connectivity with the PPN is topographically organized, are depicted in this scheme. EP, entopeduncular nucleus; GPi, internal segment of the globus pallidus; IC, inferior colliculus; SC, superior colliculus; SN, substantia nigra; STN, subthalamic nucleus; VTA, ventral tegmental area.

CONCLUSION

The PPN is subdivided in two functionally distinct regions: the rostral portion, which is predominantly inhibitory and interconnected with the basal ganglia, and the caudal portion, which is predominantly excitatory and closely related to arousal and motor systems. These two functionally distinct areas are locally regulated and synaptically linked by the local axon collaterals of cholinergic and non-cholinergic neurons. The extent of the interaction between these two regions remains to be determined, but will help to elucidate the common mechanism by which PPN neurons seem to participate in a wide range of behavioral functions.

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Somatotopic organization of the primate basal ganglia

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Somatotopic organization is a fundamental and key concept to understand how the cortico-basal ganglia loop works. It is also indispensable knowledge to perform stereotaxic surgery for movement disorders. Here I would like to describe the somatotopic organization of the basal ganglia, which consist of the striatum, subthalamic nucleus, globus pallidus, and substantia nigra. Projections from motor cortical regions representing different body parts terminate in different regions of these nuclei. Basal ganglia neurons respond not only to the stimulation of the corresponding regions of the motor cortices, but also to active and passive movements of the corresponding body parts. On the basis of these anatomical and physiological findings, somatotopic organization can be identified in the motor territories of these nuclei in the basal ganglia. In addition, projections from functionally interrelated cortical areas partially converge through the cortico-basal ganglia loop, but nevertheless the somatotopy is still preserved. Disorganized somatotopy may explain, at least in part, the pathophysiology of movement disorders, such as Parkinson's disease and dystonia.

Keywords: striatum, subthalamic nucleus, globus pallidus, substantia nigra, somatotopy, movement disorders

INTRODUCTION

Somatotopic organization in the cerebral cortex, especially in the primary motor and primary somatosensory cortices, is a well-known and fundamental concept to understand the functions of these areas. Each nucleus of the basal ganglia also shows somatotopy, but it has received little attention. Somatotopy of the basal ganglia is disorganized in movement disorders, suggesting its pathophysiological significance. Knowledge on somatotopy of the human basal ganglia is also indispensable to identify the location of the tip of electrodes during stereotaxic surgery for movement disorders. In this article, I would like to describe the somatotopic organization of the basal ganglia comprehensively and in detail. Although the description is mainly based on monkey studies, it should be applicable to the human basal ganglia because the basal ganglia of non-human primates and humans share a number of common properties, despite their size difference.

BASIC CIRCUITRY OF THE BASAL GANGLIA

The basal ganglia are a group of sub-cortical nuclei, and are composed of the striatum, pallidum, subthalamic nucleus (STN), and substantia nigra (SN). The striatum can be classified into the caudate nucleus, putamen, and ventral striatum. The pallidum can be divided into the external (GPe) and internal (GPi) segments of the globus pallidus and ventral pallidum (VP). The SN is composed of pars reticulata (SNr) and pars compacta (SNc). Among these nuclei, the striatum and STN are input stations of the basal ganglia. The striatum receives inputs from the entire cerebral cortex except the primary visual cortex, and the STN receives inputs mainly from the frontal cortex. On the other hand, the GPi and SNr serve as the output nuclei of the basal ganglia, and project outside the basal ganglia. The GPe connects input stations to the output nuclei. The SNc is composed of dopaminergic neurons, which project widely to the whole basal ganglia, especially to the striatum, and modulate their activity.

Cortical information received in the input stations is transferred to the output nuclei through the following three pathways (**Figure 1**; Alexander and Crutcher, 1990a; Nambu et al., 2002b).

Direct pathway: Striatal neurons expressing substance P receive cortical inputs and project directly to the GPi/SNr.
Indirect pathway: Striatal neurons expressing enkephalin receive cortical inputs and project polysynaptically to the GPi/SNr by way of the GPe and STN.
Hyperdirect pathway: STN neurons receive direct cortical inputs and project to the GPi/SNr. This pathway transfers cortical excitation faster to the GPi/SNr than the direct and indirect pathways.

Information originating from the frontal cortex is processed through these three pathways, and mainly returns to the frontal cortex through the thalamus, thus forming the cortico-basal ganglia loop. Some information is transferred to the brainstem from the output nuclei (Alexander and Crutcher, 1990a).

The primary motor cortex (MI), supplementary motor area (SMA) and premotor cortex (PM) are classically defined motor cortices. In addition, the pre-SMA in the mesial side of the hemisphere anterior to the SMA and the cingulate motor areas (CMA) in the cingulate sulcus have been identified (Picard and Strick, 2001). The PM is not homogeneous and was originally divided into dorsal and ventral parts (PMd and PMv), and is now further subdivided into rostral and caudal parts (PMdr, PMdc, PMvr, and PMvc). The CMA is divided into rostral and caudal parts (CMAr and CMAc). Among them, the most rostral motor cortices, such as pre-SMA, PMvr, PMdr, and CMAr, receive inputs from the frontal association cortex and send outputs to the more caudal motor cortices, such as SMA, PMvc, PMdc, and CMAc (Takada et al., 2004). Most of these motor cortices, especially SMA, PMvc, PMdc, CMAc, and MI have their own somatotopy.

There have been two opposing views concerning how information originating from different cortical areas or different somatotopic regions is processed through the basal ganglia (**Figure 2**; Parent and Hazrati, 1995). One is the *parallel processing* hypothesis (Alexander et al., 1986; Hoover and Strick, 1993; Strick et al., 1995) proposing that information from different cortical areas is processed independently in the different parts of the basal ganglia (**Figure 2A**). The other is the *information convergence* hypothesis (Percheron and Filion, 1991; Percheron et al., 1994) proposing that information from different cortical areas converges and is integrated in the basal ganglia (**Figure 2B**). Recent studies suggest that both parallel processing and information convergence occur (**Figure 2C**). Information from cortical areas whose functions are distinct from each other terminates in the different regions in the basal ganglia. On the other hand, information from cortical areas whose functions are close to each other tends to converge in the basal ganglia. For example, projections from the motor, oculomotor, prefrontal, and limbic cortices terminate in different regions in the striatum. These striatal regions project to different regions of other basal ganglia nuclei. Thus, each nucleus of the basal ganglia can be segregated into motor, oculomotor, prefrontal, and limbic territories, and cortico-basal ganglia loops are composed of several independent and functionally distinct, but homological loops: motor, oculomotor, prefrontal, and limbic loops (**Figure 1**). Each loop controls brain functions independently (Alexander et al., 1986; Parent, 1990). Inside the motor loop, projections from the MI, SMA, and PM partly converge in the striatum, while projections from the MI and pre-SMA project to distinct regions of the striatum. Somatotopy is also well defined in each nucleus of the basal ganglia, and information from different body parts of the somatotopy is well preserved through cortico-basal ganglia loops.

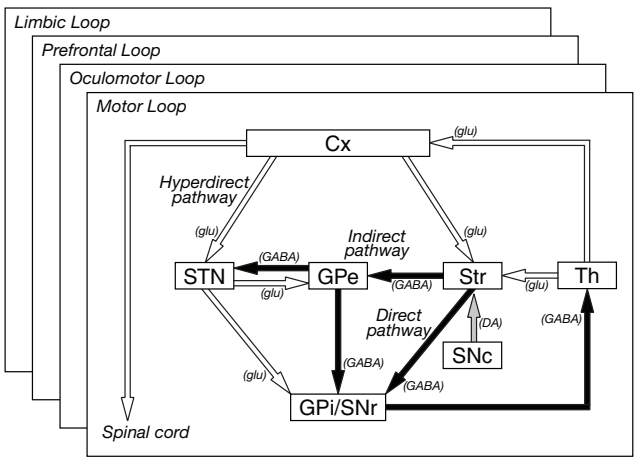

FIGURE 1 | Basic circuitry of the basal ganglia. Open and filled arrows indicate excitatory and inhibitory projections, respectively. Cx, cerebral cortex: DA, dopamine, GABA, gamma-aminobutyric acid; glu, glutamate; GPe and GPi, external and internal segments of the globus pallidus; SNc, substantia nigra pars compacta; SNr substantia nigra pars reticulata; STN, subthalamic nucleus; Str, striatum; Th, thalamus. Modified from Nambu et al. (2002b).

METHODS TO IDENTIFY SOMATOTOPY

Somatotopy of the basal ganglia reflects input and output connections of each nucleus, and can be investigated in several ways. The most basic method is an anatomical method examining fiber connections with other brain areas whose somatotopy is clearly identified. For example, anterograde tracers are injected into the orofacial, forelimb, and hindlimb regions of the MI, and then terminals in the striatum and STN are observed. Transsynaptic anterograde and retrograde tracing can be performed using herpes simplex virus (anterograde or retrograde) and rabies virus (retrograde) as tracers. Fiber connections can also be investigated by electrophysiological methods. Stimulation of the MI induces responses in the corresponding regions in the striatum, STN, GPe, and GPi.

Another useful electrophysiological method is recording neuronal activity in behaving animals. Neurons in the basal ganglia change activity during active movements of the corresponding body parts. These neurons usually respond to passive movements of the corresponding body parts as well, such as manipulations of joints and muscle palpations. Applying microstimulation through recording electrodes in some nuclei of the basal ganglia can induce movements of the corresponding body parts, although more pulses are necessary compared with that for intracortical microstimulation.

In the following sections, somatotopy in each nucleus of the basal ganglia will be discussed. "Cartoons" representing somatotopy will be drawn for each nucleus. However, they are metaphors, and readers should not take them too literally. For example, in **Figure 5**, the orofacial, forelimb, and hindlimb regions are represented in this order along the ventral-to-dorsal axis of the globus pallidus, but it is not known whether each finger is distinctly and orderly represented (Hamada et al., 1990).

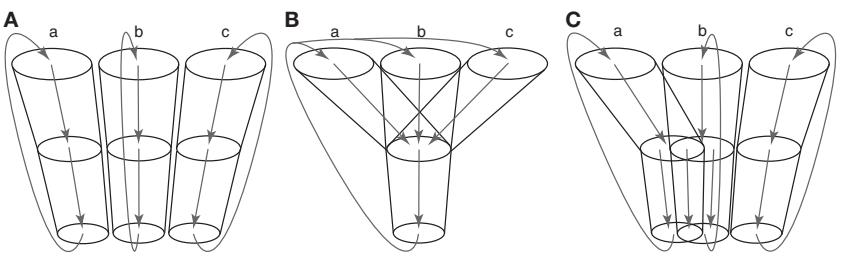

FIGURE 2 | Information processing in the basal ganglia. (A) *Parallel processing* hypothesis. Information originating from different areas (a, b, c) of the cerebral cortex is processed independently in the different parts of the basal ganglia, and returns to the original cortical areas. **(B)** *Information convergence* hypothesis. Information originating from different cortical areas converges and is integrated in the basal ganglia, and integrated information returns to all the cortical areas. **(C)** Intermediate hypothesis between parallel processing and information convergence hypotheses, which is supported by recent studies.

STRIATUM

The striatum, as an input station of the basal ganglia, receives excitatory inputs from all areas of the cerebral cortex except the primary visual cortex. The caudal aspect of the putamen, which is posterior to the anterior commissure, is considered to be the motor territory and shows clear somatotopy (**Figure 3A**). Distribution patterns of labeling in the striatum were observed after injection of anterograde tracers into the orofacial, forelimb, and hindlimb regions of the MI and SMA (Künzle, 1975; Flaherty and Graybiel, 1993; Takada et al., 1998b). Labeling consisted of dense and diffuse projection regions as recently proposed (Haber et al., 2006). The dense terminals were found in the lateral part (MI territory) after injection into the MI, and in the medial part (SMA territory) after injection into the SMA. The orofacial, forelimb, and hindlimb regions of the MI project to the ventral to dorsal parts of the lateral putamen. The corresponding regions of the SMA project to the ventral to dorsal parts of the medial putamen, which are mediodorsal to the MI territory. Therefore, the putamen has two sets of somatotopic representations in the medial and lateral parts. The diffuse terminals from the MI extend to the dorsomedial portion, and those from the SMA extend to the ventrolateral portion. Thus, the projections from the orofacial, forelimb, and hindlimb regions of the MI and those from the corresponding regions of the SMA converge in the medio-lateral central zone that occupies one-quarter of each territory. The forelimb region is widely represented in the MI territory. The proximal regions (elbow and shoulder) are located in the mediodorsal part, and the distal regions (wrist and digits) are located in the ventrolateral part (Tokuno et al., 1999).

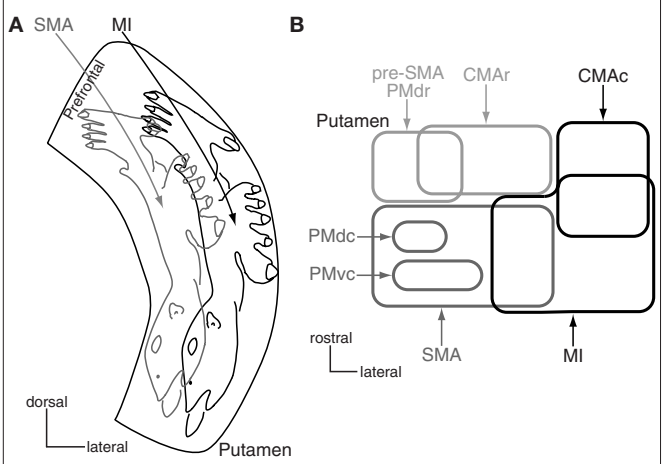

FIGURE 3 | Somatotopy of the putamen. (A) Somatotopy of the putamen is schematically shown in a frontal section. In the caudal aspect of the putamen, the lateral part receives somatotopic inputs from the primary motor cortex (MI), and the medial part from the supplementary motor area (SMA). The somatotopy in the SMA territory is located dorsomedially to that in the MI territory. Projections from the orofacial, forelimb and hindlimb regions of the MI and SMA converge in the medio-lateral central zone in the putamen. The most dorsomedial part receives inputs from the prefrontal cortex. Modified from Nambu et al. (2002a) **(B)** Input from motor cortices to the putamen is schematically shown in a horizontal section. CMAc and CMAr, caudal and rostral parts of the cingulate motor area; PMdc, PMdr, and PMvc, caudal part of dorsal premotor cortex, rostral part of dorsal premotor cortex, and caudal part of ventral premotor cortex. Modified from Takada et al. (2001).

This somatotopy reflects not only cortical inputs revealed by conventional tracers, but also putaminal outputs to the cortex through the direct and indirect pathways. Injection of rabies virus into the MI resulted in retrograde transsynaptic labeling of neurons in the putamen, which showed similar somatotopic organization, especially in its lateral side (Miyachi et al., 2006). Moreover, this study also showed no labeling of neurons in the SMA territory of the putamen, suggesting that the pathways originating from the MI territory of the putamen and from the SMA territory are independent of each other in the basal ganglia. The motor territory of the putamen also receives topographic inputs from the motor thalamus and centromedian and parafascicular nuclei, which are reciprocally connected with motor cortices (Nakano et al., 1990; Sadikot et al., 1992; McFarland and Haber, 2000; Jones, 2007). These projections are also considered to be somatotopically organized.

The somatotopy in the putamen is also confirmed by electrophysiological methods (Nambu et al., 2002a). Cortical stimulation of the forelimb regions of the MI and SMA orthodromically activates projection neurons in the corresponding MI (lateral) and SMA (medial) territories of the putamen, respectively, at a latency of 10–15 ms. Putaminal neurons in the central zones are activated by the stimulation of both the MI and SMA, and thus, convergence from the MI and SMA occurs at a single neuronal level. Putaminal neurons in the MI and SMA territories are activated by passive and/or active movements of the corresponding body parts on the contralateral side (Alexander and DeLong, 1985; Alexander and Crutcher, 1990b; Nambu et al., 2002a). However, putaminal neurons in the MI territory and those in the SMA territory show different activity patterns during task performance. Putaminal neurons in the MI territory are closely related to movements themselves, while neurons in the SMA territory are activated not only by movements themselves, but also during delay periods. Such activity differences of putaminal neurons seem to reflect the activity patterns of MI and SMA neurons that give rise to cortico-striatal projections. Microstimulation in the MI territory of the putamen produces movements of the corresponding body parts, while that in the SMA territory does not (Alexander and DeLong, 1985; Nambu et al., 2002a). The probable pathway for inducing movements by microstimulation is the direct pathway. Stimulation of the striatum may excite direct pathway neurons, inhibit GPi and finally disinhibit thalamic and cortical activity. The microstimulation studies suggest that putaminal neurons in the MI and SMA territories project independently to different territories in the nucleus of the basal ganglia, and that somatotopy is preserved through the basal ganglia circuitry.

Striatal projection neurons are classified into direct and indirect pathway neurons on the basis of the difference in receptors, peptides, and targets. The two groups of neurons may represent similar somatotopy and show similar activity patterns during task performance. The striatum also contains interneurons. Although cholinergic interneurons receive common cortical inputs with neighboring projection neurons, they show reward-related activity (Aosaki et al., 1995), which is different from that of neighboring projection neurons. Parvalbumin (PV)-positive GABAergic interneurons also receive cortical inputs and are thought to regulate the activity of projection neurons through feed-forward inhibition (Tepper et al., 2008). PV-positive interneurons showed task-related activity (Gage et al., 2010), suggesting that they share similar cortical inputs with neighboring

projection neurons. Activity patterns of other interneurons during task performance remain to be studied. The striatum is classified into μ-opiate receptor-rich patch compartment (or striosome) and matrix compartment (Graybiel, 1990), but the relationship between somatotopy and patch-matrix organization is unclear.

Other motor cortices also project to the striatum (**Figure 3B**; Takada et al., 1998a,b, 2001; Inase et al., 1999; Tachibana et al., 2004). The highest motor cortices, such as pre-SMA, PMdr, and CMAr, project to the anterior part of the striatum, especially to the bridge region connecting the caudate nucleus and putamen. The forelimb regions of the PMdc and PMvc project to two independent regions in the SMA territory of the putamen. On the other hand, the CMAc, which shows activity similar to that of the MI, projects to the MI territory. Projections from the primary somatosensory cortex also project to the MI territory (Flaherty and Graybiel, 1993). The projection patterns seem to obey the following rules: The motor cortices whose functions are distinct project to the different regions of the striatum, whereas the motor cortices whose functions are similar project to the common striatal regions in a convergent manner. The prefrontal cortex projects to the rostral part of the putamen anterior to the anterior commissure and the head of the caudate nucleus (prefrontal territory of the striatum), and the limbic cortex projects to the ventral striatum (limbic territory; Selemon and Goldman-Rakic, 1985; Haber et al., 1990; Parent, 1990). Eye movement-related neurons are located in the central part of the caudate nucleus (oculomotor territory; Hikosaka et al., 1989).

SUBTHALAMIC NUCLEUS (STN)

The STN, another input station of the basal ganglia, receives cortical inputs from the frontal lobe. The dorsal part of the STN is the motor territory and shows somatotopic organization (**Figure 4A**; Monakow et al., 1978; Nambu et al., 1996). The MI projects to the lateral part (MI territory), and the SMA projects to the medial part (SMA territory). The orofacial, forelimb, and hindlimb regions of the MI project to the lateral to medial parts of the lateral STN, while those of the SMA project to the medial to lateral parts of the medial STN. Therefore, two sets of somatotopic representations, which are mirror images of each other, are represented in the lateral and medial parts of the STN. The MI also partly projects to the somatotopically corresponding body parts in the SMA territory, and the SMA partly projects to the MI territory, vice versa. Thus, inputs from the MI and SMA partly converge in the STN. The forelimb regions of the PMdc and PMvc also project to the forelimb region of the SMA territory (**Figure 4B**; Nambu et al., 1997). The somatotopy of the STN reflects not only input organization, but also output organization, because similar somatotopy is observed after transneuronal retrograde labeling of rabies virus by its injection into the MI (Miyachi et al., 2006).

The somatotopy of the STN has also been confirmed by electrophysiological methods. Cortical stimulation of the MI and SMA induces a short latency excitation and a subsequent long latency excitation (Nambu et al., 2000), which are mediated by the cortico-STN (hyperdirect) and cortico-striato-GPe-STN (indirect) pathways, respectively. By observing cortically evoked responses, similar somatotopy can be drawn, with some neurons receiving convergent inputs from the MI and SMA. STN neurons in the MI territory change their activity (mostly excitation) in relation to active or passive movements of the corresponding body parts on the contralateral side (DeLong et al., 1985; Wichmann et al., 1994). STN neurons in the SMA territory may also show task-related activity. Microstimulation in the MI and SMA territories does not evoke movements, while that in the most lateral part of the STN often evokes movements probably because of the current spread to the internal capsule (Wichmann et al., 1994).

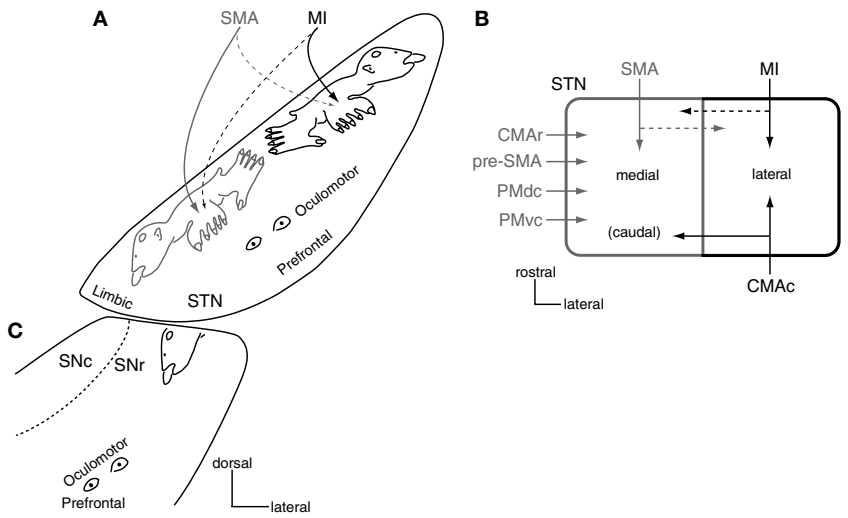

FIGURE 4 | Somatotopy of the subthalamic nucleus (STN) and substantia nigra (SN). (A) Somatotopy of the STN is shown in a frontal section. In the dorsal part of the STN, the lateral part receives somatotopic inputs from the MI, and the medial part from the SMA. The MI also projects partly to the medial part, and the SMA to the lateral part. Ventral to the motor territory, there exist the oculomotor and prefrontal territories. The most medial part is occupied by the limbic territory. Modified from Nambu et al. (2002b). **(B)** Input from motor cortices to the STN is schematically shown in a horizontal section. Modified from Takada et al. (2001). **(C)** Somatotopy of the SNr is shown in a frontal section. The orofacial region of the SNr is a continuation of that of the GPi (see **Figure 5**). Ventral to the motor territory, there exist the oculomotor and prefrontal territories.

Concerning other motor cortical inputs, the CMAc projects to the MI territory of the STN, and the pre-SMA and CMAr project to the SMA territory (**Figure 4B**; Inase et al., 1999; Takada et al., 2001). Thus, more convergence may occur in the cortico-STN projections than in the cortico-striatal projections (compare **Figure 4B** with **Figure 3B**), suggesting that the hyperdirect pathway assembles information from more wide areas of the motor cortices than the direct and indirect pathways do. Ventral to the motor territory in the STN, there exist the oculomotor territory (Matsumura et al., 1992) and the prefrontal territory (Monakow et al., 1978; Parent, 1990; **Figure 4A**). The most ventromedial part of the STN is occupied by the limbic territory (Parent, 1990).

EXTERNAL AND INTERNAL SEGMENTS OF THE GLOBUS PALLIDUS (GPe AND GPi)

The motor territory of the striatum (i.e., the caudal aspect of the putamen) projects to the ventral two-thirds of the caudal GPe and GPi, and thus, these areas are the motor territories of the globus pallidus (Smith and Parent, 1986; Parent, 1990) that show somatotopic organization (**Figure 5**). In GPe/GPi neurons, cortical stimulation evokes a triphasic response composed of early excitation, inhibition, and late excitation, which are mediated by the cortico-STN-GPe/GPi (cortico-STN-GPi: hyperdirect), cortico-striato-GPe/GPi (cortico-striato-GPi: direct), and cortico-striato-GPe-STN-GPe/GPi (cortico-striato-GPe-STN-GPi: indirect) pathways, respectively (Nambu et al., 2000; Kita et al., 2004; Tachibana et al., 2008). The somatotopy in the GPe/GPi can be drawn by observing responses evoked by the stimulation of the MI and SMA. Neurons responding to the orofacial, forelimb, and hindlimb regions of the MI are located along the ventral-to-dorsal axis in the GPe and GPi (MI territory, **Figure 5**; Yoshida et al., 1993). Neurons responding to the corresponding regions of the SMA are also located along the ventral-to-dorsal axis, but in more rostral and dorsal parts of the GPe/GPi (SMA territory). Stimulation of the PM also evokes responses in the SMA territory. GPe/GPi neurons rarely respond to cortical stimulation of multiple body parts, and thus, the orofacial, forelimb, and hindlimb regions of GPe/GPi are clearly and distinctly identified. On the other hand, many neurons respond to the stimulation of both the MI and SMA, and the somatotopic representation in the MI territory and that in the SMA territory are partly fused in the rostro-caudal central zone. Most GPe/GPi neurons show triphasic responses evoked by cortical stimulation, suggesting that the hyperdirect, direct, and indirect pathways originating from a certain body region in the cortex converge at a single GPe/GPi neuronal level.

The above-mentioned somatotopy is also supported by anatomical studies. The injection of anterograde tracers into the MI, SMA, and convergent territories in the putamen revealed the terminals in the GPe/GPi (Kaneda et al., 2002). Terminals from the SMA territory of the putamen are located more anterior and dorsal to those from the MI territory. The convergent territory of the putamen projects to the area in-between, and these three projection territories do not overlap. Transsynaptic anterograde and retrograde labeling studies by injecting herpes simplex virus into the MI reported similar results (Hoover and Strick, 1993, 1999; Strick et al., 1995; Akkal et al., 2007), although there is some discrepancy, such as that the PMv territory of the GPe/GPi is located ventrally to the MI territory. Dendritic fields of GPe/GPi neurons extend widely in the direction perpendicular to the striato-pallidal fibers, and this is considered to be the basis for information convergence (Percheron et al., 1984; Yelnik et al., 1984). However, somatotopic organization through the striato-pallidal projections is well preserved as described above.

Neurons in the MI and SMA territories of the GPe/GPi change their activity in relation to active and passive movements of the corresponding body parts on the contralateral side (DeLong, 1971; Georgopoulos et al., 1983; DeLong et al., 1985; Hamada et al., 1990). However, response patterns are different between these territories. Neurons in the MI territory show movement-related activity, while those in the SMA territory show delay-related activity (Nambu et al., 1990). On the other hand, response patterns in the GPe and GPi neurons during task performance are very similar. Microstimulation in the GPe/GPi does not induce any movements.

The prefrontal territory of the striatum projects to the rostral GPe and dorsal one-third of the caudal GPe/GPi, and thus, these areas are the prefrontal territory (Smith and Parent, 1986; Parent, 1990). Ventral striatum projects to the VP, the most rostral part of the GPe and the most medial part of the GPi, and thus, these areas correspond to the limbic territory (Haber et al., 1990; Parent, 1990).

SUBSTANTIA NIGRA (SN)

The SNr and GPi are the output nuclei of the basal ganglia and considered to be a continuum, which is divided into the SNr and GPi by the internal capsule. The motor territory of the striatum projects to the dorsal one-third of the SNr, and thus, this area is considered to be the motor territory of the SNr (**Figure 4C**; Smith and Parent, 1986; Parent, 1990). Neurons in the dorsolateral part

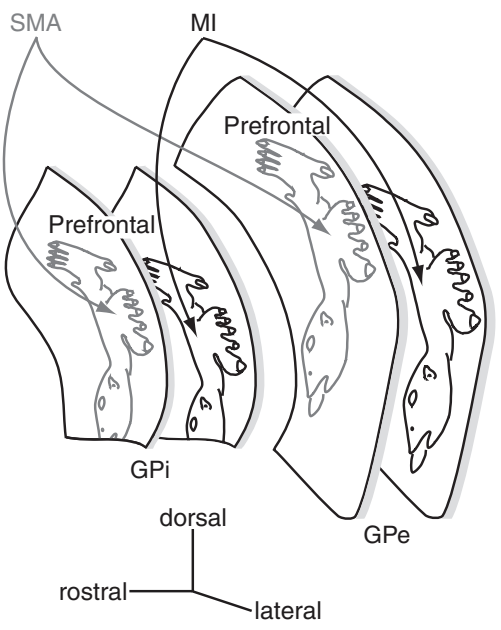

FIGURE 5 | Somatotopy of the external and internal segments of the globus pallidus (GPe and GPi). The GPe and GPi have their own somatotopic representations. The somatotopy in the SMA territory is located rostrodorsally to that in the MI territory. Two territories overlap partly in the rostro-caudal zone. Dorsal one-third of the GPe/GPi is occupied by the prefrontal territory.

of this area respond to the stimulation of the MI, especially to that of the orofacial region, and change their activity in relation to active or passive movements of the orofacial region (DeLong et al., 1983; Kitano et al., 1998). The orofacial region of the SNr is considered to be a continuation of the orofacial region of the GPi (see **Figures 4C and 5**). SNr neurons in the part ventral to the orofacial region receive inputs from the SMA territories of the putamen, and change their activity during task performance. However, the somatotopy is not clearly organized, and their activity is not so distinct compared to that of GPi neurons (Wichmann and Kliem, 2004). The prefrontal territory of the striatum projects to the rostromedial two-thirds of the SNr (Smith and Parent, 1986) that also include the oculomotor territory (Hikosaka and Wurtz, 1983; **Figure 4C**). The limbic territory of the striatum projects to the most medial part of the SNr (Haber et al., 1990).

The SNc is composed of dopaminergic neurons, and projects to the striatum and other basal ganglia nuclei. Dopaminergic projections from the SNc to the striatum display weak topography, and the terminal fields of a single dopaminergic neuron are large (Parent et al., 1983; Parent, 1990; Matsuda et al., 2009). SNc neurons do not respond to active or passive body part movements, but respond to novel sensory stimuli and/or rewards (DeLong et al., 1983; Schultz and Romo, 1990). Recent studies suggest that SNc neurons code the difference between the expected reward and the real reward (a temporal difference error in reinforcement learning). These observations suggest that the SNc has no clear somatotopy.

THALAMUS

The motor thalamus is a target structure of the basal ganglia, and also shows somatotopy (**Figure 6**). Subnuclei located in the rostral part of the motor thalamus receive inputs from the basal ganglia. The oral part of the ventrolateral nucleus (VLo) and the principal part of the ventroanterior nucleus (VApc) receive inputs from the GPi. The medial part of the ventrolateral nucleus (VLm) and the magnocellular part of the ventroanterior nucleus (VAmc) receive inputs from the SNr. On the other hand, subnuclei located in the caudal part, such as the oral part of the ventroposterolateral nucleus (VPLo), the caudal part of the ventrolateral nucleus (VLc) and area X, receive cerebellar inputs (Jones, 2007). Thus, projections from the SNr, GPi and cerebellar nuclei terminate in the rostral to caudal parts of the motor thalamus, and the overlap of their terminals is minimal. The VApc, VLo, VPLo, and VLc project to the motor cortices, and thus, most of the motor cortices receive inputs from both the basal ganglia and the cerebellum through the motor thalamus (Jones, 2007). The MI receives basal ganglia inputs through the VLo, and cerebellar inputs through the VPLo (Holsapple et al., 1991).

The VLo and VPLo display clear somatotopic organization (**Figure 6**). The orofacial, forelimb, and hindlimb regions are represented in the medial to lateral parts (Asanuma et al., 1983; Vitek et al., 1994). VLo neurons change their activity in relation to active movements of the corresponding body parts (Anderson and Turner, 1991; Nambu et al., 1991; Vitek et al., 1994). However, sensory inputs are not clearly identified, and the microstimulation in the VLo does not induce any movements (Buford et al., 1996; Vitek et al., 1996). On the other hand, VPLo neurons respond clearly to active and passive movements of discrete body parts (one to several joints) on the contralateral side. Microstimulation in the VPLo induces movements in the corresponding body parts, contralateral to the stimulation side. The somatotopy can also be confirmed by the anatomical study of the thalamo-cortical projections (Asanuma et al., 1983; Holsapple et al., 1991). Therefore, the thalamus has at least two sets of somatotopic representations: one in the GPi-receiving region (VLo) and the other in the cerebellar-receiving region (VPLo).

FUNCTIONAL SIGNIFICANCE OF THE SOMATOTOPY

Each nucleus of the basal ganglia shows clear somatotopic organization, and information originating from cortical regions representing different body parts rarely converges in the cortico-basal ganglia circuitry. These observations suggest that information related to different body parts, such as forelimb and hindlimb, is processed independently through the cortico-basal ganglia loop. On the other hand, information from different but related cortical areas, such as the forelimb regions of the MI and SMA, is processed in both convergent and non-convergent manners. However, the functional roles of such convergence remain to be elucidated.

SOMATOTOPY AND MOVEMENT DISORDERS

The pathophysiology of movement disorders can be explained by the changes of the firing rates and patterns in the basal ganglia, especially in the GPe, GPi, and STN. In addition, changes in the somatotopy have been reported in movement disorders. In a normal state, GPe and GPi neurons respond specifically to the movement of one direction of a single joint on the contralateral side. On the other hand, GPe/GPi neurons in a Parkinsonian state respond to multiple movements of multiple joints, sometimes of the upper and lower limbs and of both sides (Filion et al., 1988). Loss of functional segregation was also reported in the GPi-receiving thalamus (Pessiglione et al., 2005). Dopamine is considered to contribute to

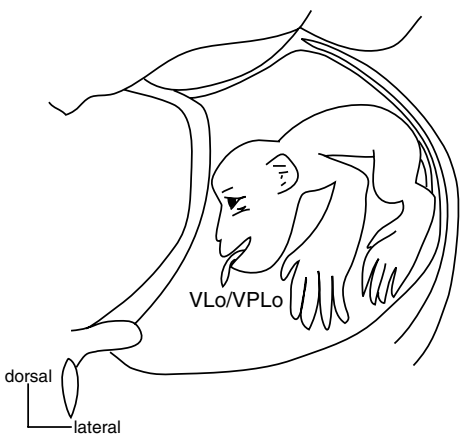

FIGURE 6 | Somatotopy of the thalamus. Somatotopy of the thalamus is shown in a frontal section. Both the oral part of the ventrolateral nucleus of the thalamus (VLo) receiving pallidal projections and the oral part of the ventroposterolateral nucleus of the thalamus (VPLo) receiving cerebellar projections have their own somatotopic representations. Their somatotopic representations are continuous rostrocaudally. Modified from Asanuma et al. (1983).

isolate information related to movements of specific body parts. Loss of dopamine may induce a crosstalk of information related to different body parts (Bergman et al., 1998).

In dystonia, somatotopy of the basal ganglia is also disorganized (Vitek et al., 1999; Chiken et al., 2008). GPe/GPi neurons respond to stimulation of multiple body parts, and they are intermingled. Dystonic patients show a phenomenon known as "motor overflow." Such a phenomenon may be explained by disorganization of the somatotopy. When patients try to move one body part, for example, a hand, not only the hand region, but also other regions, such as the neck region, of the GPi could be inhibited by somatotopic disorganization. This may lead to unintended movements of other body parts, such as the neck, that accompany intended movements of a hand.

Hemiballism is caused by lesions in the STN, such as a hemorrhage or infarction. Hemiballism in the lower limb is common, while that in the orofacial regions is rare (Carpenter et al., 1950; Hamada and DeLong, 1992). Hemiballism in the upper limb is associated with that in the lower limb. These characteristics can be explained by the mirror image organization of the somatotopy in the STN (**Figure 4A**; Nambu et al., 1996). It is supposed that the inactivation of both the MI and SMA territories of the corresponding body parts is necessary to produce hemiballism. Small lesions in the central STN affect both lower limb regions of the MI and SMA territories, and thus cause hemiballism in the lower limb. Large lesions affecting both upper limb regions of the MI and SMA territories also affect both lower limb regions, and thus hemiballism in the upper limb is accompanied by that in the lower limb. Lesions affecting both orofacial regions of the MI and SMA territories should be rare because they are remotely located.

Abnormal firing rates and patterns in the motor territory of the basal ganglia cause motor symptoms of the movement disorders. The target of stereotaxic surgery, including deep brain stimulation (DBS), for treatment of movement disorders aimed at the motor territory of the basal ganglia, such as the GPi and STN. The somatotopy gives us useful indices to identify the targets during stereotaxic surgery (Kaplitt et al., 2003). STN-DBS sometimes induces side effects of mood changes. This may be explained by the current spread from the motor territory to the limbic and prefrontal territories of the STN because of the small size of the STN. On the other hand, GP-DBS does not induce psychological side effects, probably because the motor territory is remotely located from the limbic and prefrontal territories in the GPi.

CONCLUSION

In this article, I have described that each nucleus of the basal ganglia shows clear somatotopic organization, and that information related to different body parts is processed independently through the cortico-basal ganglia loop. I would like to point out the following unsolved questions: In the topographic projections from one nucleus to another nucleus, what kind of information is added? What kind of information is originated? How do converging inputs from multiple motor cortices contribute to the execution of voluntary movements? How is the somatotopy in each nucleus of the basal ganglia organized during development? These are important questions closely related to the functions of the basal ganglia. The somatotopic perspective way of view will be a good clue and guide for further understanding of the basal ganglia.

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Functional anatomy: dynamic states in basal ganglia circuits

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The most appealing models of how the basal ganglia function propose distributed patterns of cortical activity selectively interacting with striatal networks to yield the execution of context-dependent movements. If movement is encoded by patterns of activity then these may be disrupted by influences at once more subtle and more devastating than the increase or decrease of neuronal firing that dominate the usual models of the circuit. In the absence of dopamine the compositional capabilities of cell assemblies in the network could be disrupted by the generation of dominant synchronous activity that engages most of the system. Experimental evidence about Parkinson's disease suggests that dopamine loss produces abnormal patterns of activity in different nuclei. For example, increased oscillatory activity arises in the GPe, GPi, and STN and is reflected as increased cortical beta frequency coherence disrupting the ability to produce motor sequences. When the idea of deep brain stimulation was proposed – it was supported by the information that lesions of the subthalamus reversed the effects of damage to the dopamine input to the system. However, it seems increasingly unlikely that the stimulation acts by silencing the nucleus as was at first proposed. Perhaps the increased cortical beta activity caused by the lack of dopamine could have disabled the patterning of network activity. Stimulation of the subthalamic nucleus disrupts the on-going cortical rhythms. Subsequently asynchronous firing is reinstated and striatal cell assemblies and the whole basal ganglia circuit engage in a more normal pattern of activity. We will review the different variables involved in the generation of sequential activity patterns, integrate our data on deep brain stimulation and network population dynamics, and thus provide a novel interpretation of functional aspects of basal ganglia circuitry.

Keywords: neuronal networks, striatum, cell assemblies, compositional properties, deep brain stimulation, calcium imaging, synaptic plasticity, intrinsic neuronal properties

INTRODUCTION

Global changes in the activity of specific basal ganglia structures generate devastating pathologies (e.g., Parkinson's disease; Huntington chorea). Interpreting the overall behavior of the group of structures called basal ganglia is fundamental for the understanding of motor disorders.

The most predominant model of basal ganglia function is based on the existence of two striatal pathways with antagonistic effects (Albin et al., 1989) the activation of the direct pathway facilitates the execution of movements, and the activation of the indirect pathway inhibits motion (Takakusaki et al., 2004a,b). So it was proposed that movement alterations in Parkinson's disease are caused by the over activation of the indirect pathway, whereas hyperkinetic disorders reflect the pathologically increased activity in the direct pathway. Initially it seemed that this simplification had some merit, since lesions in the indirect pathway helped Parkinson's disease symptoms (Bergman et al., 1990).

A MORE REALISTIC MODEL IS NEEDED

However, the reality of basal ganglia encoding is likely to be more complex. Not only is such simple coding unlikely to be versatile enough to encompass motor skills but the basal ganglia are also involved in learning such skills (Graybiel, 2000; Yin et al., 2009). We imagine that groups of neurons in several of the nuclei of the basal ganglia could work together in dynamic networks to accomplish these tasks. Recurrent and alternating activity of specific groups of cells could interact to form the substrate that gives rise to the processes associated with learning. Neuronal pools with these characteristics have been called cell assemblies (Hebb, 1949). In order to store and perform complex behaviors, cell assemblies must have diverse capabilities, such as the ability to synchronize activity and to compose sequences among different neuronal microcircuits (Hebb, 1949; Carrillo-Reid et al., 2009a). Compositionality is a central issue to cognitive processes; it refers to the ability to generate complex procedures from basic patterns represented by the coordinated activity of specialized neurons organized in hierarchies (Bienenstock and Geman, 1995; Hammer, 2003).

A BETTER SCHEME?

We would like to present the hypothesis that the compositional capabilities observed in neuronal microcircuits can explain normal and pathological behaviors of the basal ganglia. From this point of view, diverse functions can be represented by the continuous reorganization of specialized cell assemblies to accommodate new habit learning and automaticity in learned motor or cognitive tasks. It has been reported for instance that different striatal areas change their predominant activity as learning progresses (Lehericy et al., 2005). Indeed the final memory may be laid down outside the basal ganglia in cerebellum (Doyon et al., 2009). The next challenge is to develop novel analytical tools and experimental techniques

to define the dynamic functional anatomy of the basal ganglia. The study of how anatomy is related to the function of a system requires the understanding of different aspects that shape neural networks. The three main points that can describe the organization of specialized groups of neurons are: (1) the intrinsic properties of the neurons, (2) the synaptic dynamics, and (3) the compositional properties of several interconnected structures.

INTRINSIC PROPERTIES OF NEURONS

The most abundant cell type in the striatum is the medium spiny neuron which is characterized by a hyperpolarized resting membrane potential and low input resistance (Kita et al., 1984) as well as several types of potassium conductance that shape their firing patterns (Nisenbaum and Wilson, 1995). Membrane depolarization and spiking yield calcium influx, calcium influx activates small and large conductance calcium-activated potassium channels (Bargas et al., 1999) and therefore limits cell firing.

PLATEAU POTENTIALS

Medium spiny neurons display periodically changing firing patterns, referred to as "up and down" states, according to variations in their intrinsic membrane properties as well as excitatory drive from the cortex and thalamus (Wilson and Kawaguchi, 1996). The up state persists as long as sufficient excitatory drive is present to maintain depolarization (Wilson, 1993; Vergara et al., 2003).

Active conductances that give rise to plateau potentials play an important role in the generation of oscillations in several areas. A plateau potential is a stable membrane potential kept above the resting membrane potential. When a plateau potential is generated the cells can produce action potentials in the absence of sustained synaptic excitation (Grillner et al., 1981; Guertin and Hounsgaard, 1998a; Vergara et al., 2003). In this way, a transient depolarization of sufficient amplitude and duration can initiate a plateau potential. The plateau can last several seconds before ending spontaneously or it can be terminated by an inhibitory synaptic input. Moreover, plateau potentials are regulated by different neuromodulators for instance in spinal motor neurons (Guertin and Hounsgaard, 1998b).

A strong synchronous input is not always necessary to induce bursts of action potentials in several cells at the same time. It is often enough to have similar temporal sequences to induce prolonged activity even if the temporal sequences come from a different source (Carrillo-Reid et al., 2008).

Network dynamics that depend on plateau potentials have been observed in different structures of the basal ganglia (Vergara et al., 2003; Ibanez-Sandoval et al., 2007; Carrillo-Reid et al., 2008, 2009a,b). For example, it has been shown that plateau potentials underlie up and down state transitions in medium spiny projection neurons from the striatum (Vergara et al., 2003; Carrillo-Reid et al., 2008). *In vivo* experiments have revealed that the bursting activity of these neurons reflects specific motor patterns (Vautrelle et al., 2009). At present here are no ways to evaluate the presence and significance of plateau potentials in intact organisms, including humans (Kiehn and Eken, 1998).

SYNAPTIC DYNAMICS

Excitability changes at the presynaptic level that modify neurotransmitter release occur at all levels of the central nervous system with important consequences in synaptic communication. Activation of presynaptic receptors and modulation of secretion is a well-recognized event (de Jong and Verhage, 2009). In the basal ganglia presynaptic influences on dopamine release were reported more than thirty years ago (reviewed by Glowinski et al., 1979) followed some years later by presynaptic receptor mediated changes in axonal terminal excitability (Garcia-Munoz et al., 1991a,b). Since the first descriptions of corticostriatal synaptic plasticity were made on the corticostriatal terminals (Garcia-Munoz et al., 1992) the majority of presynaptic studies have examined synaptic plasticity and transmission at excitatory glutamatergic synapses on striatal output neurons (Ding et al., 2008; Kreitzer and Malenka, 2008). Short-term synaptic plasticity is also involved in the stabilization and reconfiguration of motor circuits and in the initiation, maintenance, and modulation of programs related to movement (Nadim and Manor, 2000). In the production of striatal short-term and long-term synaptic changes (potentiation and depression) the participation of glutamate and dopamine receptors has been demonstrated (Calabresi et al., 1992; Wickens et al., 1996; Kerr and Wickens, 2001; Tecuapetla et al., 2007).

In the model proposed by Hebb (1949) coincidental sustained firing is necessary to increase synaptic efficacy, a necessary characteristic to allow the transmission between short-term and long-term memory. With this in mind, the synchronous activation of specific microcircuits could modulate network synaptic efficacy promoting the long-term storage of mental representations (Tallon-Baudry et al., 2001).

Short-term synaptic plasticity can be used to dynamically select among different motor patterns. It can be used not only to select different patterns of movement, but also different motor programs (e.g., swimming, walking). Synaptic plasticity is dependent on activity and is regulated by neuromodulators, sensory experience or a combination of both (Nadim and Manor, 2000).

The induction of striatal short-term and long-term synaptic changes is related to habit formation but also to pathological states (Ingham et al., 1998; Costa et al., 2006; Day et al., 2006; Kreitzer and Malenka, 2008; Yin et al., 2009). In like manner the understanding of the synaptic dynamics between different structures of the basal ganglia could be a fundamental key to define the functional anatomy underlying motor related behaviors.

COMPOSITIONAL PROPERTIES AND BASAL GANGLIA FUNCTION

Procedural memories and habits can be represented by basic motor actions (Graybiel, 1998; Grillner, 2006). Basic modules can be combined to generate a broad repertoire of procedures used to perform specific tasks. The knowledge of the compositional rules that guide the formation of habits is fundamental to understanding the dynamic states underlying basal ganglia functions (Carrillo-Reid et al., 2008, 2009a). Nevertheless, newer techniques must be developed to investigate experimentally the functional connectivity between specialized groups of cells and behavioral events.

Repetitive practice can create procedural memories allowing the consolidation of specific patterns of motor activity. A memory system based on modular activity has the advantage that any module can retrieve the whole memory (Abeles et al., 2004; Carrillo-Reid et al., 2009a). Activation of recurrent sequences (i.e., neuronal patterns) has been related to working memory (Lewis et al., 2005a,b). Sustained frontal cortical activity represents working

memory processes (Kessler et al., 2005; Pollok et al., 2006) and the sustained corticostriatal activity has been proposed to participate in the planning and control of movements (Beiser et al., 1997). Distributed patterns of cortical activity selectively interacting with striatal networks may underlie the execution of context-dependent movements (Stern et al., 1998; Costa et al., 2006; Yin et al., 2009).

A REDUCED SYSTEM

In vitro preparations allow the easy access to neuronal assemblies making it possible to study the transfer of patterns of activity. By plating and growing cortical and striatal neurons in different compartments of a multielectrode array we have found that the activity patterns in the cortical compartment are followed by striatal neurons (**Figure 1**).

These preliminary experiments suggest that the behavior of cortical and striatal networks is present in greatly simplified systems but the results need to be expanded both in the details of the analysis of the activity and in applying similar methods to other basal ganglia networks. For instance these cultures have no dopamine input but it could be added.

There are many theories of brain function that depend on cell assemblies with the ability to reproduce sequential patterns of activity that can be "remembered" when a small sample of the network is ignited (Bienenstock and Geman, 1995; Abeles, 2003; Hammer, 2003; Abeles et al., 2004; Grillner, 2006). There are many fewer experiments showing that real nerve cells do produce such "compositional" networks (Ikegaya et al., 2004; Carrillo-Reid et al., 2008, 2009a).

In electrical recordings from cortical cells the subthreshold membrane potential state transitions are correlated with the slow rhythm of the electroencephalogram. Clear transitions seen in the anesthetized animal are replaced by oscillations in the firing of groups of neurons in "microzones" or "ensembles" that are time locked to specific movements (Jaeger et al., 1995).

CALCIUM IMAGING AS A METHOD TO EXAMINE ENSEMBLES

The use of calcium imaging techniques in corticostriatal slices has revealed that active up states of medium spiny neurons are accompanied by calcium influx (Vergara et al., 2003; Carrillo-Reid et al., 2008, 2009a). When NMDA is applied *in vitro* to striatal slices, it induces recruitment of active neurons and generates dispersed patterns of activity in the network. Neurons belonging to the network alternate their activity to generate spatiotemporal patterns of synchronization (Carrillo-Reid et al., 2008).

Research on how such striatal neuronal assemblies are modified by neurotransmitters and neuromodulators has just begun. So far we know that modifications in GABA and acetylcholine transmission have important consequences (**Figure 2**). $GABA_A$ receptor blockade locks the network in a recurrent pattern of activity. Activation of cholinergic muscarinic receptors induces a change in network dynamics by recruiting neurons into correlated firing without increasing the number of active neurons. The presence of acetylcholine seems to allow the formation of cell assemblies with different cycles of activity. These results are important inasmuch as they suggest that the cortical and thalamic glutamate inputs are not the only modulators of striatal network activity. GABA, dopamine and acetylcholine participate in typical sequential activation of cell assemblies, the key element in the generation of motor programs. Although we have looked at these in striatal neurons we expect similar properties to be present throughout the basal ganglia circuits.

It is proposed that activation of motor commands induces recruitment of active neurons and generates stable patterns of activity in striatal neurons that are preserved *in vitro*. Neurons belonging to specific network states alternate their activity to generate spatiotemporal patterns of synchronization (Carrillo-Reid et al., 2008, 2009a; Jaidar et al., 2010). Thus GABA, dopamine and acetylcholine participate in typical sequential activation of cell assemblies, the key element in the generation of motor programs (**Figure 2**).

CLINICAL IMPLICATIONS

The importance of these network properties for understanding basal ganglia function may not lie only in their intrinsic biological interest. In recent experiments on the underlying mechanism of the therapeutic effects of deep brain stimulation we were forced to conclude that the effect might be a consequence of action at the

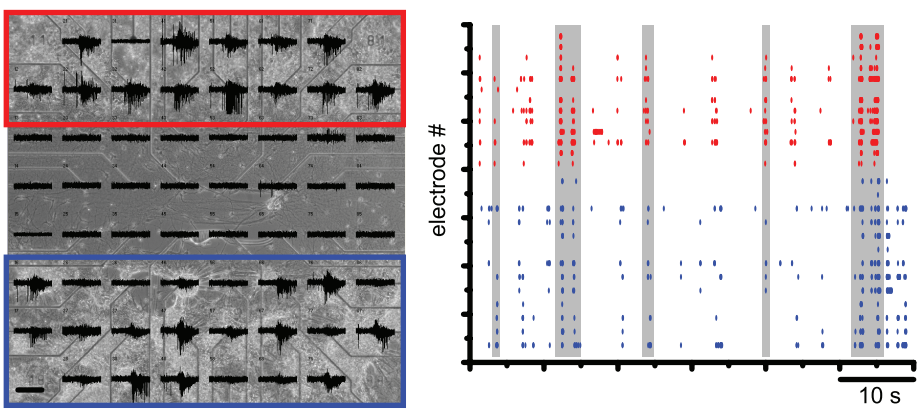

FIGURE 1 | Cortical networks can transfer information to striatal networks. Left: microelectrode array (MEA) recordings of a compartmentalized culture of cortical cells (red) and striatal cells (blue). Note that cortical activity can trigger the activity of striatal networks. Scale bar 100 μV/600 ms. Right: raster plot representing the activity recorded by the cortical electrodes (red) and the striatal electrodes (blue). The overall activity of the network seems to be defined by synchronous firing of the cortical neurons. Gray stripes indicate the entrainment of firing in the striatal compartment in time with the cortical activity.

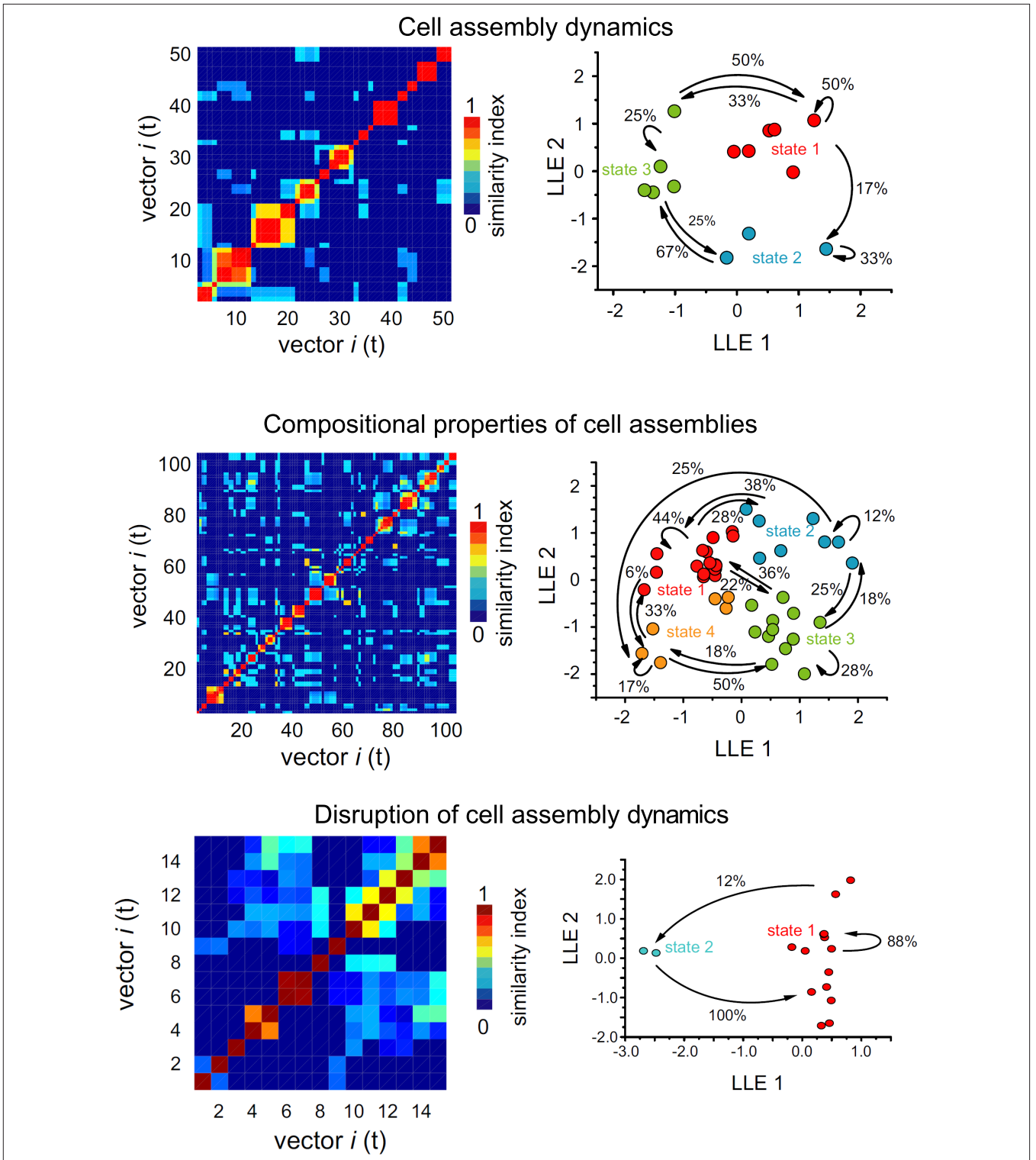

FIGURE 2 | Cell assembly dynamics in cortico-striatal slices. Left: maps showing the similarity index of the vectors representing network dynamics as a function of time. The vectors are generated from the firing of individual cells over time, each cell vector is compared with every other to generate the similarity index plot. Patterned structures indicate groups of cells firing together at different times. Right: two dimensional projection using locally linear embedding (LLE) of the vectors defining the states of the network. Arrows indicate transitions between different states. Top: NMDA receptor activation produced synchronous, recurrent and alternating activity in striatal neuronal pools. All these properties are characteristics of cell assemblies. Modified from Carrillo-Reid et al. (2009a). Middle: activation of the cholinergic system endows cell assemblies with compositional capabilities allowing the formation of complex sequences of activity from elemental patterns. Modified from Carrillo-Reid et al. (2009a). Bottom: blockade of $GABA_A$ receptors engages the striatal network in a dominant state. Note the drastic readjustment of cell assemblies from diverse cycles of activity with compositional properties to a fixed pattern that reduced the cell assembly diversity. Modified from Carrillo-Reid et al. (2008).

cortex – and not in the subthalamic nucleus, where the electrodes are aimed. One obvious effect of the stimulation on cortical activity is to suppress the beta waves in the ECoG that are induced by blocking dopamine receptors.

The drug effect is small compared to the result of lesions of the dopamine system (Mallet et al., 2008a,b), but the akinesia produced is profound and that also is alleviated by stimulation that reduces the beta frequency power in the ECoG (Dejean et al., 2009; **Figure 3**).

In contemporaneous but not directly related experiments Deisseroth's group (Gradinaru et al., 2009) used optogenetic techniques to influence the cells of the nucleus. They showed that inactivating much of the subthalamic nucleus, or activating the cells there, produced no effect on the symptoms caused by lesion of dopamine cells in the mouse. However, they could influence the behavioral effects of the lesion by applying light to activate the cells of layer V of the cortex. These are the same cells that it had been previously shown to be antidromically activated by deep brain stimulation in the rat subthalamic nucleus (Li et al., 2007; Lehmkuhle et al., 2009).

CORTICAL INVOLVEMENT IN DEEP BRAIN STIMULATION

Thus, disrupting the rhythmic activity in the cortex that gives rise to the beta rhythm in the ECoG is capable of relieving the symptoms of basal ganglia dopamine depletion. This suggests that the beta activity is somehow changing the basal ganglia dynamic in a way that disrupts its function (Arbuthnott et al., 2009). One interpretation of these results is that the patterned activity that we have imaged in striatal cultures and in slices of the striatum, is a vital part of the normal activity that underlies voluntary movement. Its disruption by cortical beta dominance blocks the compositional network activity, and so incapacitates the animal – or the patient.

Recovery after deep brain stimulation, or after magnetic stimulation of the cortex (Fregni and Pascual-Leone, 2007) may be a consequence of the disruption of this cortical rhythm and as a result removing its domination of the network activity in the striatum. Return the striatum to normal patterned activity and the animal can move and behave again; the Parkinsonian patient can move again. Of course the disruption of cortical activity itself has consequences that may entail "cognitive side effects" of stimulation. If thoughts and memories depend on compositional network activity then it is not a surprise that stimulation also interrupts those in cortex at least to some extent.

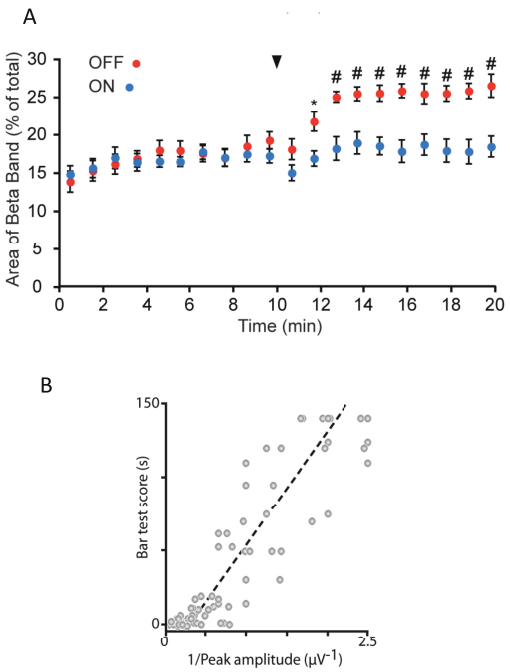

FIGURE 3 | Cortical effects and the recovery from akinesia. In **(A)** the average percentage power in the beta band of the EEG is plotted for all nine rats in the study. Each rat is recorded in two conditions. The red symbols are from a day when the rats were treated with DI and D2 blockers after 10 min in the recording chamber and for 10 min thereafter. The blue symbols represent 2 days later when the animals were again in the same recording chamber but with the stimulation ON (120 Hz and less than 80% of the threshold for movement). The beta power increase caused by the drugs is blocked. In **(B)** we show the inverse relationship of the effect to the stimulus strength. The threshold for a cortical evoked potential is just below 2 μA (0.5 on the scale). The animals come off the bar very quickly when the stimulus is above threshold and the recovery is directly related to the inverse of the stimulus power (more effective akinesia prevention is proportional to higher current ($r^2 = 0.84$)).

CONCLUSIONS

We have tried to introduce new concepts indicating that basal ganglia structures are capable of forming neuronal networks that can demonstrate compositional properties.

The concept of a basic stereotypic microcircuit formed by several neurons with common connections and dynamic operations can invigorate basal ganglia research. Storage and retrieval of learned patterns of movement could take place in the basal ganglia by the dynamic reorganization of cell assemblies. To make predictions of future events and generate appropriate behavior, our cortex and basal ganglia could store sequences of patterns and retrieve suitable sequences by their similarity with previously successful patterns.

The generation of predictive knowledge of the properties of the world around is vital to our ability to act upon it and within it. Not surprising then that the perceptual processes often thought of as vital for such knowledge of the body in the world should be visible within the basal ganglia. Informed, or disrupted by cortical influences, maintained by the action of different neurotransmitters, our normal lives depend on the compositional properties of diverse cell assemblies.

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Striatal spine plasticity in Parkinson's disease

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Striatal dopamine (DA) denervation results in a significant loss of dendritic spines on medium spiny projection neurons in Parkinson's disease. In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated parkinsonian monkeys, spines contacted either by cortical or thalamic glutamatergic terminals are severely affected on both direct and indirect striatofugal neurons. In rodents, indirect pathway neurons appear to be more sensitive, at least in early stages of acute dopamine denervation. The remaining corticostriatal and thalamostriatal axo-spinous synapses undergo complex ultrastructural remodeling consistent with increased synaptic activity in the DA-denervated primate striatum, which may explain the pathophysiological overactivity of the corticostriatal system reported in various animal models of parkinsonism. The calcium-mediated regulation of the transcription factor myocyte enhancer factor 2 was recognized as a possible underlying mechanism for striatal spine plasticity. Future studies to determine how alterations in striatal spine plasticity contribute to the symptomatology of parkinsonism are warranted.

Keywords: dendritic spine, plasticity, dopamine, glutamate, Parkinson's disease

INTRODUCTION

Since 1891 when Cajal described dendritic spines as morphologically distinct neuronal elements (Ramon y Cajal, 1891), studies of spine development, morphogenesis and plasticity have been at the forefront of research in various laboratories using modern techniques at the electron microscopic level for three-dimensional (3D) reconstructions of individual spines (Harris and Kater, 1994; DeFelipe, 2002; Smith et al., 2009; Villalba et al., 2010), single- and two-photon microscopy used in conjunction with fluorescent molecular tools (Yuste and Bonhoeffer, 2004; Knott and Holtmaat, 2008) or neurocomputational methods (Zhang et al., 2010) to study dynamic changes in spine morphology related to pathological or physiological regulation of neuronal plasticity (Yuste and Bonhoeffer, 2001).

Despite a broad range of experimental evidence showing changes in the density or morphology of dendritic spines in a large number of neurological and psychiatric diseases, the exact contribution of spine changes to brain diseases pathophysiology remains poorly understood (Ingham et al., 1989, 1998; Harris and Kater, 1994; Fiala et al., 2002; Picconi et al., 2005; Stephens et al., 2005; Zaja-Milatovic et al., 2005; Deutch et al., 2007; Surmeier et al., 2007; Bourne and Harris, 2008; Smith and Villalba, 2008; Villalba et al., 2009, 2010).

In the striatum, the principal neuronal subtypes are the medium spiny neurons (MSNs), which represent 95% of all striatal neurons (Kemp and Powell, 1971; Chang et al., 1982). In rats, each MSN harbors approximately 5000 dendritic spines (Wickens et al., 2007). Each of these spines receives glutamatergic inputs from the cerebral cortex or specific thalamic nuclei (Kemp and Powell, 1971; Smith et al., 2004, 2009; Raju et al., 2006). Spines are also an important target of midbrain dopaminergic inputs that play a critical role in the regulation of cortical glutamatergic afferents (Smith and Bolam, 1990; Smith et al., 1994; Nicola et al., 2000). In both animal models of parkinsonism and Parkinson's disease (PD) patients, MSNs lose as much 30–50% dendritic spines, which results in complex anatomical, neurochemical and electrophysiological changes of striatal glutamatergic transmission (Ingham et al., 1989; Stephens et al., 2005; Zaja-Milatovic et al., 2005; Smith and Villalba, 2008; Villalba et al., 2009).

The goal of this review is to provide an overall assessment of anatomical and functional evidence for spine loss in the striatum, and discuss the potential mechanisms involved in this pathophysiological phenomenon in parkinsonism. The readers are referred to other recent reviews for additional information (Deutch et al., 2007; Smith and Villalba, 2008; Smith et al., 2009).

SPINE LOSS IN PARKINSON'S DISEASE AND PD MODELS

Over the past decades, it became clear that the progressive loss of striatal dopamine in PD patients and animal models of parkinsonism results in a significant loss of dendritic spines on MSNs of the dorsal striatum (**Figure 1**).

The first clear evidence for striatal spine loss came from studies showing that unilateral degeneration of the nigrostriatal dopaminergic system results in about 20% spine loss in the caudate-putamen complex of rats treated with 6-hydroxydopamine (6-OHDA) (Ingham et al., 1989). Since then, corresponding degrees of spine pruning have been demonstrated in postmortem striatal tissue of PD patients (Stephens et al., 2005; Zaja-Milatovic et al., 2005) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated parkinsonian monkeys (Smith and Villalba, 2008; Villalba et al., 2009). In monkeys and humans, the extent of spine loss correlates with the relative degree of striatal dopamine denervation (Zaja-Milatovic et al., 2005; Smith and Villalba, 2008; Villalba et al., 2009). For instance, in MPTP-treated parkinsonian monkeys, MSNs in striatal regions most severely dopamine-depleted, like the postcommissural putamen (i.e., the sensorimotor striatum) lose as much as 50% spines, while spine loss in less affected striatal areas like the nucleus accumbens (i.e., the limbic striatum) ranges between 20% to 25% of total striatal spines (Villalba et al., 2009) (**Figure 2**).

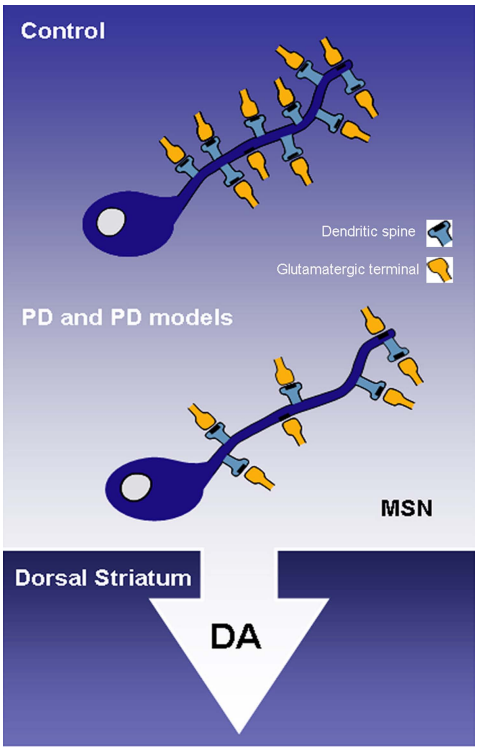

FIGURE 1 | The progressive striatal dopaminergic depletion in Parkinson's disease (PD) patients and animal models of PD results in a severe spine loss in medium spiny neurons (MSNs) of the dorsal striatum. DA, dopamine; MSN, medium spiny neuron; PD, Parkinson's disease.

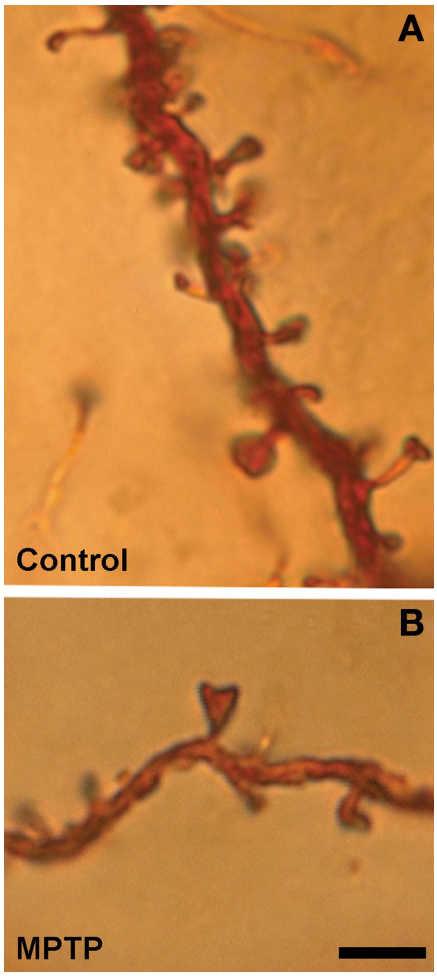

FIGURE 2 | Dendrites from Golgi-impregnated MSNs in the caudate nucleus of a control (A) and a MPTP-treated (B) monkey. Note the dramatic spine loss on the dendrite of the MSNs from the MPTP-treated monkey compared with control. Scale bar in **(B)** [valid for **(A)**]: 5 μm.

This correlation has also been established in asymptomatic MPTP-treated monkeys with partial lesion of the nigrostriatal dopaminergic system, thereby suggesting that striatal spine loss may be an early pathology of parkinsonism that does not correlate with the severity of parkinsonian motor dysfunctions (Villalba et al., 2009).

ARE BOTH "DIRECT" AND "INDIRECT" STRIATOFUGAL NEURONS AFFECTED?

The basic model of direct and indirect pathways introduced in the late 1980s has been instrumental in our understanding of the basal ganglia circuitry, the pathophysiology of PD and the development of new therapeutic approaches for basal ganglia-related movement disorders (Albin et al., 1989; Bergman et al., 1990; Wichmann and Delong, 2007). Studies in animal models of PD suggest that striatal DA depletion results in an imbalance of activity between these two pathways in favor of an increased GABAergic striatal outflow from indirect pathway neurons combined with a decreased striatal output from direct pathway neurons, thereby leading to an overall increased tonic inhibition of thalamocortical neurons by basal ganglia outflow and resulting parkinsonian motor symptoms (Albin et al., 1989; DeLong, 1990; Obeso et al., 2000). Thus, taking into consideration that the activation of striatal MSNs rely on extrinsic glutamatergic inputs (Wilson, 1995), the possibility that spine loss affects preferentially one population of striatal MSNs over the other in parkinsonism has generated considerable interest in recent years.

Data obtained so far about this issue remain controversial. On one hand, observations gathered from reserpine-treated mice and 6-OHDA-treated rats suggested that D2-containing striatopallidal neurons, but not D1-positive striatonigral neurons, selectively lose spines following dopamine depletion (Day et al., 2006). These observations are at odds with a large number of studies in both human parkinsonians and animal models of parkinsonism showing a rather homogeneous loss of spines across large populations of Golgi-impregnated striatal MSNs (Ingham et al., 1989, 1998; Stephens et al., 2005; Zaja-Milatovic et al., 2005; Smith and Villalba, 2008; Villalba et al., 2009). Furthermore, recent data from rhesus monkeys chronically treated over many months with MPTP demonstrated a comparable loss of both D1-immunoreactive and D1-immunonegative spines in the DA-denervated putamen (Villalba et al., 2009), suggesting that spine pathogenesis affects both direct and indirect pathway striatofugal neurons in this animal model (Villalba et al., 2009). However, other monkey studies suggested a decrease in D2-positive spines accompanied with a

corresponding increase in D1-immunoreactive spines in the caudate nucleus of MPTP-treated cynomolgus monkeys (Scholz et al., 2008). These apparent discrepancies may be due to the use of different animal models, different regimens of neurotoxin administration, different quantitative methods to determine striatal spine loss and different striatal regions being examined.

DOES STRIATAL SPINE LOSS AFFECT PREFERENTIALLY CORTICAL- OVER THALAMIC-RECIPIENT TARGETS?

The striatum receives glutamatergic inputs from the cerebral cortex and the thalamus. Although cortical inputs target almost exclusively dendritic spines, a substantial component of the thalamostriatal system that originates from the caudal intralaminar nuclei, center median and parafascicular nuclei, target preferentially dendritic shafts, whereas inputs from rostral intralaminar, relay and associative thalamic nuclei mainly innervate dendritic spines (Smith et al., 2004, 2009; Raju et al., 2008). Although these two glutamatergic inputs cannot be differentiated from each other solely based on structural features, they can be categorized as of cortical or thalamic origin based on their specific expression in vesicular glutamate transporter 1 (vGluT1) or vGluT2, respectively (Raju et al., 2006, 2008; Smith et al., 2009). Taking advantage of these specific markers, we recently reported a significant increase in the overall density of vGluT1-positive terminals, but no significant change in the prevalence of vGluT2-positive boutons in the striatum of MPTP-treated parkinsonian monkeys (Raju et al., 2008). These findings are supported by postmortem human data showing an increased vGluT1 protein expression in the striatum of parkinsonians (Kashani et al., 2007).

At first glance, these observations are paradoxical to the fact that striatal MSNs undergo significant spine loss in parkinsonism, and that the bulk of synaptic inputs to striatal spines originate from cortical terminals (Kemp and Powell, 1971; Smith and Bolam, 1990; Raju et al., 2008). However, they are consistent with a large number of electrophysiological and neurochemical studies that suggest an increased glutamatergic transmission at corticostriatal synapses and augmented neuronal excitability in the striatum of rodent models of parkinsonism (Gubellini et al., 2002; Mallet et al., 2006, but see Day et al., 2006). Recent evidence suggests that such increased striatal activity may also occur in MPTP-treated monkeys (Liang et al., 2008). Although there is no clear explanation for this apparent discrepancy between morphological and functional data related to the corticostriatal system in parkinsonism, there are various indications that the striatum undergoes complex compensatory changes in glutamate receptors expression (Betarbet et al., 2000) and synaptic architecture that may account for increased glutamatergic transmission in parkinsonian condition (see Smith et al., 2009, for a review).

ULTRASTRUCTURAL REORGANIZATION OF GLUTAMATERGIC CORTICOSTRIATAL AND THALAMOSTRIATAL SYNAPSES IN PARKINSONISM

Early rodent and human data, indeed, suggested morphological changes of asymmetric synapses consistent with increased synaptic activity in the dopamine-denervated striatum (Ingham et al., 1998; Meshul et al., 1999, 2000). These observations were recently supported an expanded by detailed 3D ultrastructural analysis (**Figure 3**).

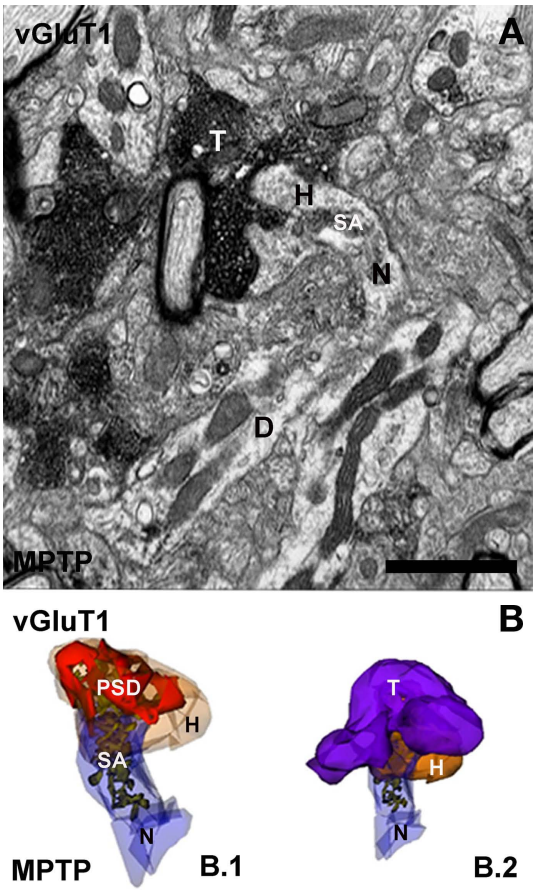

FIGURE 3 | Electron micrograph and three-dimensional (3D) reconstruction of a corticostriatal (vGluT1-immunoreactive) axo-spinous synapse in the striatum of a MPTP-treated monkey. (A) Electron micrograph of a synapse between the spine of a MSN and a vGluT1-positive terminal (T). **(B)** 3D-reconstructed images from serial electron micrographs of the head (H) and neck (N) of the spine (B.1) and the axo-spine synapse (B.2) shown in **(A)**. In (B.1), the spine is partially transparent to illustrate the complexity and distribution of the spine apparatus (SA) and the postsynaptic density (PSD). D, dendrite. Scale bar in **(A)**: 1 μm.

Quantitative analysis of 3D-reconstructed axo-spinous cortico- and thalamostriatal analysis showed that the size of the postsynaptic density (PSD) area at both glutamatergic synapses is increased in the non-human primate model of PD, thereby suggesting that both thalamic and cortical inputs undergo plastic changes consistent with increased synaptic strength in parkinsonism (Smith et al., 2009; Villalba et al., 2010) (**Figure 4**).

Our data also revealed that the complexity and the total number of perforations at PSDs at corticostriatal and thalamostriatal glutamatergic synapses are increased in parkinsonism, a type of structural modification associated with increased synaptic efficacy and learning in the hippocampus (Nieto-Sampedro et al., 1982).

The exact mechanisms underlying these functional changes are not known, but it has been hypothesized that the compartmentalization of multiple transmission zones impedes the saturation of postsynaptic receptors and allow multiple transmitter

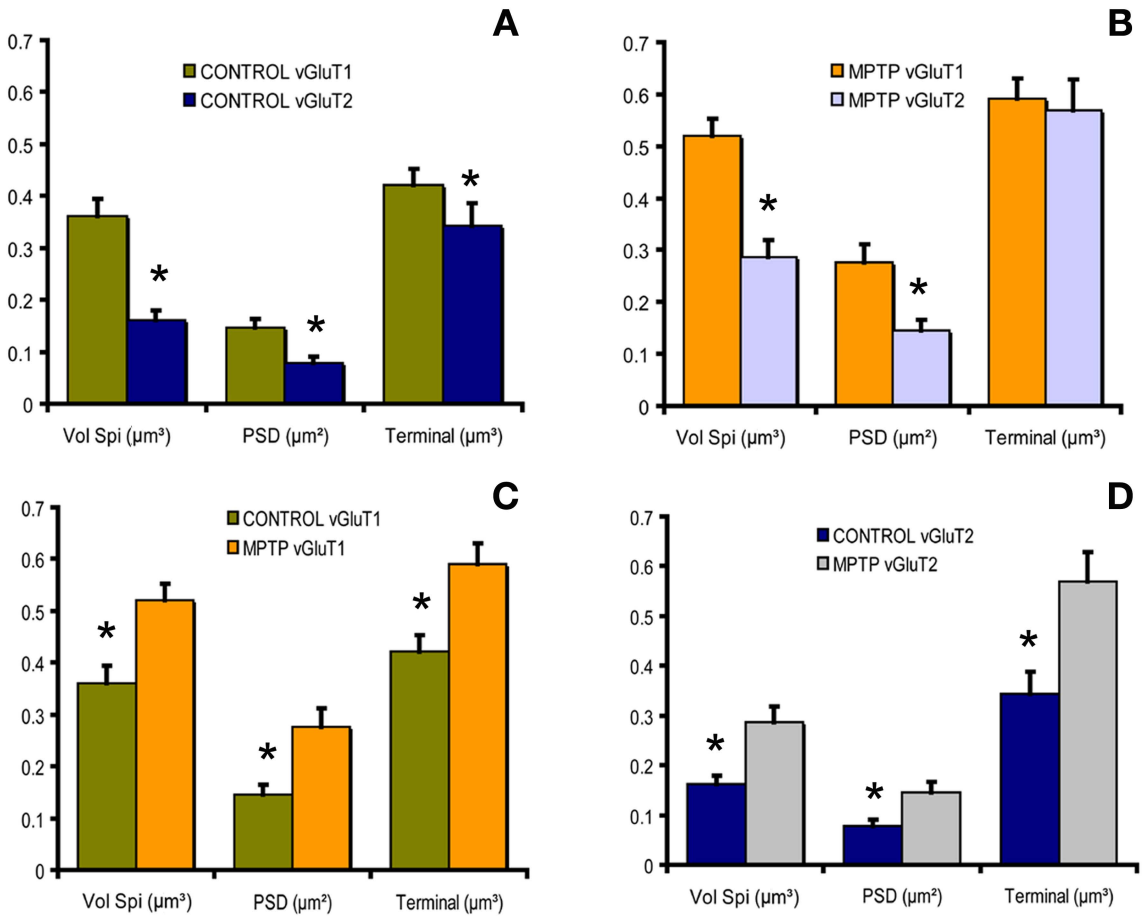

FIGURE 4 | Histograms comparing the morphometric measurements (spine volume, PSD area, terminal volume) of structural elements at corticostriatal and thalamostriatal glutamatergic synapses using the 3D reconstruction method of serial ultrathin sections collected from 30 axo-spinous synapses in normal and MPTP-treated monkeys. (A) In control monkeys, the spine volume (Vol. Sp), the PSD areas and the size of pre-synaptic terminals at corticostriatal synapses are significantly larger than those at thalamostriatal synapses (*$P < 0.001$ for Vol. Sp. and PSD; *$P = 0.016$ for terminal; *t*-test). **(B)** The same is true for MPTP-treated monkeys, except for the pre-synaptic terminals that do not display any significant size differences in this condition. **(C,D)** The spine volumes, the PSD areas and the volume of vGluT1- and vGluT2-containing terminals are significantly larger in MPTP-treated parkinsonian monkeys than in controls (*$P < 0.001$; *t*-test).

quanta to be effective at the same postsynaptic spine, thereby enhancing the strength of individual synapses (Lisman and Harris, 1993). Thus, the extensive remodeling of PSDs at glutamatergic axo-spinous synapses in the striatum of parkinsonian monkeys provides another mechanism whereby cortical and thalamic glutamatergic afferents may increase their synaptic strength in parkinsonism (**Figure 5**).

Another striking change observed in spines that receive vGluT1-containing cortical inputs in the striatum of MPTP-treated monkeys is the massive growth of the spine apparatus at corticostriatal synapses (Villalba et al., 2010) These changes are consistent with increased protein synthesis and increased buffering of intraspinous calcium at glutamatergic synapses in other brain regions (Fifkova et al., 1983; Bourne and Harris, 2008), thereby providing further evidence for increased corticostriatal glutamatergic transmission in parkinsonian condition (Gubellini et al., 2002).

POTENTIAL CELLULAR, MOLECULAR AND GENETIC MECHANISMS FOR STRIATAL SPINE LOSS IN PARKINSON'S DISEASE

Despite clear evidence for major structural and functional plastic changes in glutamatergic transmission at axo-spinous synapses in the parkinsonian striatum, the mechanisms underlying this phenomenon remain poorly understood. However, there is some indication that calcium likely contributes to this plastic event (Segal et al., 2000; Sabatini et al., 2001; Oertner and Matus, 2005; Day et al., 2006; Deutch et al., 2007; Surmeier et al., 2007). Findings in support of a calcium-regulated mechanism were recently gathered from transgenic mice with EGFP-labeled D1 or D2 DA receptors. In EGFP-D2 mice, spine loss can be prevented by genetic deletion of Cav1.3α1 subunits or the pharmacological blockade of L-type Cav1.3 channels (Day et al., 2006). Knowing that D2 dopamine receptor signaling targets only the channels that contain the Cav1.3α1 subunit (Surmeier et al., 2007), these data suggest

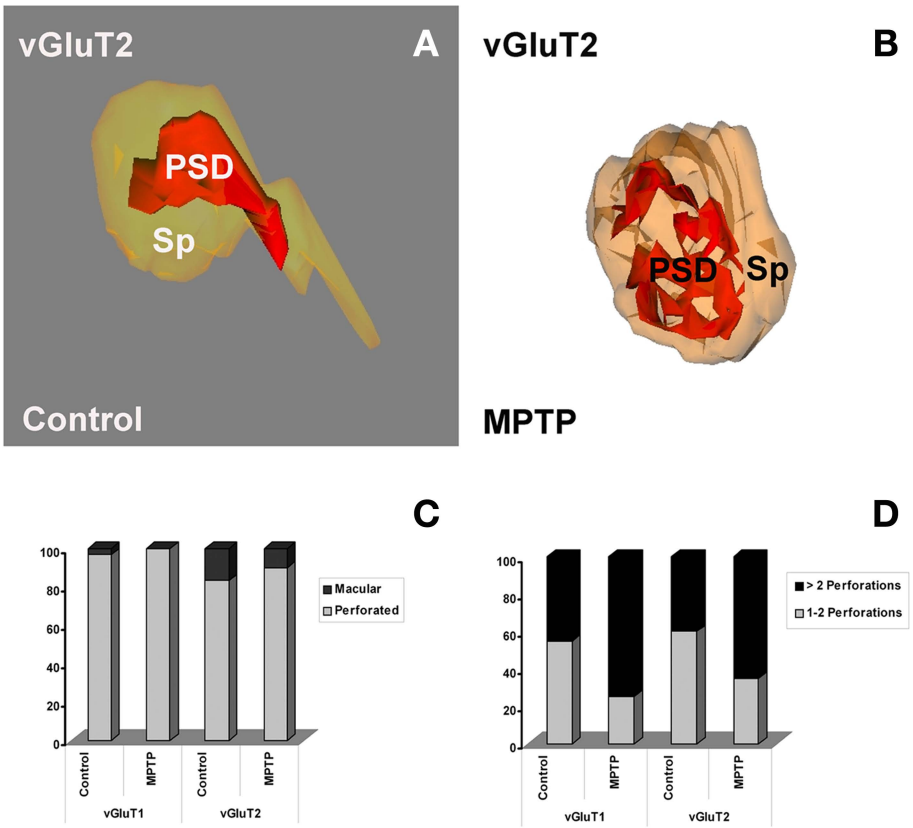

FIGURE 5 | Morphology of three-dimensionally reconstructed dendritic spines showing different types of postsynaptic densities (PSDs). (A,B) Macular **(A)** and fenestrated **(B)** PSDs from spines contacted by vGluT2-IR terminals in control **(A)** and MPTP-treated **(B)** monkeys. **(C)** Quantitative analysis of the two types of PSDs (perforated vs. macular) at corticostriatal and thalamostriatal axo-spinous synapses in control and MPTP-treated monkeys. **(D)** Comparative analysis of the relative percentages of PSDs with different number of perforations in spines contacted by vGluT1- or vGluT2-IR terminals in control and MPTP-treated parkinsonian monkeys. The proportion of complex PSDs (more than two perforations) is significantly increased *(Pearson Chi-square* analysis, χ^2) in MPTP-treated animals at both corticostriatal ($\chi^2 = 4.78$; $P = 0.03$) and thalamostriatal ($\chi^2 = 4.34$; $P = 0.035$) axo-spinous synapses. Sp, spine; PSD, postsynaptic density.

that a dysregulation of calcium concentrations, preferentially in D2-containing striatopallidal neurons, may ultimately lead to specific spine loss on indirect pathway neurons in the rodent striatum (Day et al., 2006; Deutch et al., 2007; Surmeier et al., 2007). Further support for the involvement of calcium in this process comes from observations showing that striatal areas poor in calbindin D-28k (CaB) (Francois et al., 1994), such as the post-commissural putamen (sensorimotor striatal territory), are those that display the most severe striatal spine loss in MPTP-treated monkeys (Villalba et al., 2009). Recent data from co-culture studies suggest that the pruning of glutamatergic synapses and spines in the striatum, dependent upon calcium entry through L-type calcium channels, involves the activation of the Ca^{+2}-dependent protein phosphatase, calcineurin and the up-regulation of the transcriptional activity of the myocyte enhancer factor 2 (MEF2). In turn, MEF2 up-regulation leads to increased expression of the Nurr77 and Arc genes linked to synaptic remodeling (Tian et al., 2010), thereby providing a signaling cascade through which striatal MSNs may undergo structural plasticity in parkinsonism (Tian et al., 2010).

It is noteworthy that MEF2 down-regulation is required for abnormal outgrowth of spines on striatal MSNs in cocaine-treated animals (Pulipparacharuvil et al., 2008). Together, these data indicate that MEF2 plays a critical role in regulating striatal spine plasticity in pathological conditions. Cholinergic signaling through M1 muscarinic receptors and Kir2 potassium channels is another essential trigger for the pruning of glutamatergic synapses in models of parkinsonism (Shen et al., 2009).

Despite the lack of direct evidence that any of the gene mutations characterized in PD underlie striatal spine loss, it is worth noting that PD-associated mutations that result in an increased kinase activity of Lrrk2 reduces neurite outgrowth in primary neuronal cultures and in the intact rat CNS, whereas LRRK2 deficiency leads to increased neurite length and branching (MacLeod et al., 2006). These findings, combined with evidence for strong Lrrk2 expression in striatal MSNs (Lee et al., 2010) and Lrrk2 enzymatic activity toward protein complexes involved in the formation of actin-enriched precursors of dendritic spines during neural development (Parisiadou et al., 2009), suggest that LRRK2 mutation in PD may contribute to striatal spine pathology in these patients.

CONCLUDING REMARKS

Striatal spine loss is a cardinal pathological feature of PD. Despite clear evidence that nigrostriatal dopaminergic lesion induces significant striatal spine loss in various animal models of PD, there is no direct evidence that dopamine denervation is the causal factor of striatal spine pathology. In fact, recent *in vitro* and *in vivo* studies have indicated that striatal spine pruning of MSNs in response to dopamine denervation is significantly attenuated by cortical lesions (Neely et al., 2007; Garcia et al., 2010), suggesting that glutamatergic cortical inputs are essential to mediate striatal spine loss in parkinsonism. Thus, whether striatal spine loss is the consequence of the lack of dopamine-mediated effects on corticostriatal glutamatergic transmission or primarily induced by dysfunction of the corticostriatal system remains to be established (Smith et al., 2009).

Another poorly understood aspect of this striatal pathology is its functional significance in PD symptomatology. Our recent observations that partially lesioned MPTP-treated monkeys with 30–40% spine loss in the sensorimotor striatum (i.e., the postcommissural putamen), do not display any significant parkinsonian symptoms suggest that the loss of spines in the motor striatum is not sufficient to elicit abnormal motor behaviors in parkinsonism (Smith et al., 2009; Villalba et al., 2009). Based on various findings from our laboratory and others showing the complex ultrastructural compensatory changes that affect remaining glutamatergic synapses in the dopamine-denervated striatum, we suggest that striatal MSNs are endowed with a high level of synaptic plasticity that allows sensorimotor-related information to be properly transmitted and integrated at the striatal level despite a major reduction in the number of corticostriatal synapses, at least early in the disease process (Smith et al., 2009; Villalba et al., 2009). Whether this level of plastic compensation also applies to non-motor striatal areas to alleviate cognitive and limbic dysfunctions remains to be established.

In conclusion, striatal spine pathology provides further evidence that early striatal dysfunctions in dopaminergic and glutamatergic transmission are key elements of the complex pathophysiology of parkinsonism.

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Basal ganglia circuits underlying the pathophysiology of levodopa-induced dyskinesia

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Involuntary movements or dyskinesia, represent a debilitating complication of levodopa therapy for Parkinson's disease. Dyskinesia is, ultimately, experienced by the vast majority of the patients. Despite the importance of this problem, little was known about the cause of dyskinesia, a situation that has dramatically evolved in the last few years with a focus upon the molecular and signaling changes induced by chronic levodopa treatment. Departing from this, we here review the progress made in functional anatomy and neuroimaging that have had a tremendous impact on our understanding of the anatomo-functional organization of the basal ganglia in Parkinsonism and dyskinetic states, notably the demonstration that dyskinesia are linked to a pathological processing of limbic and cognitive information.

Keywords: Parkinson's disease, levodopa, basal ganglia, abnormal involuntary movements

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, which neuropathological hallmark is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc). The loss of dopaminergic input to the striatum results in the depletion of dopamine that causes a cascade of functional modifications that involves all components of the basal ganglia circuitry. These changes are thought to represent the neural substrate for parkinsonian motor symptoms such as bradykinesia (slowness of movement), rigidity (stiffness), and tremor. However, other neurotransmitter systems (e.g., cholinergic, adrenergic, serotoninergic) also degenerate and cell loss is seen in other brain stem nuclei and the cortex (Braak et al., 2002; Chaudhuri et al., 2006). This non-dopaminergic degeneration is thought to be the major cause of the non-motor symptoms of PD (e.g., cognitive decline, autonomic dysfunction). Dopaminergic drugs (e.g., dopamine precursor drug, L-3,4-dihydroxyphenylalanine – levodopa), dopamine agonists and the inhibitors of dopamine catabolism are the main therapeutic options for alleviating the parkinsonian motor symptoms.

INCIDENCE AND PHENOMENOLOGY OF DYSKINESIA

However, as PD patients receive chronic treatment with levodopa upon a progressive disease, they gradually develop two clinical phenomena requiring changes in their clinical management: fluctuations in motor response and a variety of abnormal involuntary movements, known as levodopa-induced dyskinesia (LID) (Yahr et al., 1968). The frequency of these motor complications has been estimated between 40 and 50%, after 4–6 years of levodopa treatment (Ahlskog and Muenter, 2001) but increases to 90% after 10 years of treatment (Rascol et al., 2000). Despite its frequency and clinical significance, the pathophysiology and the clinical risk factors causing dyskinesia in PD are not understood. The incidence is estimated at about 10% per year after initiating levodopa therapy. Some people exhibit severe dyskinesia very rapidly, whereas others do not develop this complication despite many years of levodopa treatment. The phenomenology of dyskinesia encompasses various forms: chorea, athetosis, dystonia, stereotypy, ballismus, or a combination of these. In addition to levodopa-induced motor fluctuations, PD patients can frequently experience affective, motivational, and cognitive disorders (Ahlskog and Muenter, 2001). The development of dyskinesia has been reported to depend on several clinical risk factors, such as duration of disease, severity of disease, duration of levodopa treatment and actual, or cumulative levodopa dose. Numerous important advances have been made in understanding of the etiopathogenesis, pathology and clinical phenomenology of PD and LID over the past 10 years.

BASAL GANGLIA ANATOMICAL ORGANIZATION

The basal ganglia comprise a group of interconnected subcortical nuclei located at the base of the cerebral hemispheres, with parts belong to the forebrain, diencephalon, and midbrain. The basal ganglia nuclei include the striatum (caudate and putamen), the globus pallidus pars externa (GPe) and pars interna (GPi), the subthalamic nucleus (STN) and the substantia nigra, divided into its pars compacta (SNc) and pars reticulata (SNr). Current knowledge suggests that the basal ganglia constitute a highly organized network, whose functional organization is complex. There is a clear consensus in considering that input to the basal ganglia from different cortical areas terminates within specific basal ganglia territories, which are connected to similarly specific portions of the thalamus.

Abbreviations: CM, centromedian nucleus; 2-DG, 2-deoxyglucose; D1 dopamine receptor; D2R, D2 dopamine receptor; FDG, fluorodeoxyglucose; GABA, g-amino-butyric acid; GAD, glutamic acid decarboxylase; GPe, globus pallidus pars externa; GPi, globus pallidus pars interna; IT, intratelencephalic corticostriatal neurons; LID, levodopa-induced dyskinesia; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MSN, medium spiny neurons; 6-OHDA, 6-hydroxydopamine, PD, Parkinson's disease; PDRP, Parkinson's disease-related pattern; PET, positron emission tomography; Pf, parafascicular nucleus; PPE-A, preproenkephalin A; PPE-B, preproenkephalin-B; PPT, pedunculopontine tegmental nucleus; PT, pyramidal tract; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SPECT, single photon emission computed tomography; STN, subthalamic nucleus.

These thalamic areas, in turn, project back to the same areas of the cortex from which the circuit originates (Alexander et al., 1986; Kelly and Strick, 2004; DeLong and Wichmann, 2007). Numerous data suggest that the basal ganglia nuclei are involved in movement control, as well as associative learning, planning, working memory, and emotion (Hikosaka et al., 2002; Pasupathy and Miller, 2005; Yin and Knowlton, 2006).

At present, there is a classical model of movement disorders in basal ganglia disease, developed in the late 1980s (Alexander et al., 1986; Crossman, 1987; Albin et al., 1989), both describing the neural mechanisms underlying parkinsonian akinesia and explaining the appearance of abnormal involuntary movements (dyskinesia). These represent two diametrically opposed mechanisms. However, the limitations and pitfalls of these models have also been discussed extensively on several occasions (Wichmann and DeLong, 1996; Obeso et al., 1997; Rodriguez-Oroz et al., 2009). The classical box and arrows basal ganglia model (Crossman, 1987; Albin et al., 1989; DeLong, 1990) proposes a motor circuit consisting of two input structures, comprising the striatum and STN, two output structures (GPi and SNr) and two intrinsic structures, including GPe and SNc (Mink, 1996). The striatum and the STN receive topographically organized input from the cerebral cortex (Monakow et al., 1979; Nambu et al., 1997; Lei et al., 2004) whereas the GPi and the SNr provide basal ganglia output to the thalamus and brainstem (Carpenter et al., 1976; Parent and De Bellefeuille, 1982; Francois et al., 1984; Oertel and Mugnaini, 1984).

The striatum receives massive cortical excitatory inputs (Kemp and Powell, 1970; Kitai et al., 1976; McGeer et al., 1977; Cherubini et al., 1988) and is densely innervated by dopamine from the SNc (Faull and Mehler, 1978; Beckstead et al., 1979). In the striatum, the major neuronal population is represented by medium spiny neurons (MSNs), accounting for almost 95% of total striatal cells (Kemp and Powell, 1971). MSNs use g-amino-butyric acid (GABA) as a inhibitory neurotransmitter (Kita and Kitai, 1988). They form two main populations of projection neurons (striatofugal system) that differ in their expression of the receptors that mediate the effect of dopamine. The striatonigral MSNs that monosynaptic project to GPi and the SNr (direct pathway) express preferentially dopamine D1 receptors (D1R) and produce the neuropeptides dynorphin and substance P whereas the striatopallidal MSNs that project to GPe (indirect pathway) express dopamine D2 receptors (D2R) and enkephalin (Gerfen et al., 1990). Although this strict "segregation" was supported by previous studies, a significant number of D1R and D2R-coexpressing neurons (about 5–10%) were found in rat (Le Moine and Bloch, 1995) and primate (Aubert et al., 2000) using double *in situ* hybridization technique. In this same line, anatomical studies clearly show that single striatofugal axons arborize in both pallidal segments in rodents (Kawaguchi et al., 1990; Castle et al., 2005) and in primates (Parent and Hazrati, 1995; Levesque and Parent, 2005; Nadjar et al., 2006). These data indicate that the striatofugal system is not as functionally segregated in rodents and primates as previously considered in the current model of basal ganglia. Dopamine modulates glutamatergic effects on corticostriatal inputs by exerting a dual effect on striatal neurons, exciting D1R neurons in the direct pathway and inhibiting D2R neurons in the indirect circuit. Within this general context, activation of direct-pathway circuits has been proposed to facilitate or select appropriate movements, whereas activity in the indirect pathway may inhibit unwanted or inappropriate movements (Albin et al., 1989; Alexander et al., 1990). Clearcut demonstration of such roles has just been released using optogenetic approaches (Kravitz et al., 2010).

The GPe and the STN are classically viewed as part of the so-called indirect pathway (Parent and Hazrati, 1995). The GPe, principally sends GABAergic projections to the STN (Albin et al., 1989; Alexander et al., 1990; DeLong, 1990) but anatomical studies have revealed the existence of new efferent projections of the GPe to the two output structures of the basal ganglia (Hazrati et al., 1990; Kincaid et al., 1991). The STN is an important control structure of basal ganglia circuits, being the only glutamatergic nucleus of the network (DeLong and Wichmann, 2007). The STN, like the other components of the basal circuit, is subdivided into different territories, motor, oculomotor, associative, and limbic, each with different connections and functions (Parent and Hazrati, 1995; Bevan et al., 2006). The large dorsolateral portion of the STN corresponds to the motor territory; the ventromedial portion to the associative territory and the medial tip to the limbic territory of the STN.

Most STN neurons are glutamatergic projection neurons and provide a powerful excitatory input to the GPe (Van Der Kooy and Hattori, 1980; Kita and Kitai, 1987; Parent et al., 2000; Castle et al., 2005) and to the two output structures of the basal ganglia (Parent and Smith, 1987; Smith et al., 1990). Additionally a subpopulation of efferent STN neurons innervate directly the ventral motor thalamic nuclei (Nauta and Cole, 1978; Rico et al., 2010). The STN also has important reciprocal connections with the pedunculopontine tegmental nucleus (PPT) (Hammond et al., 1983; Jackson and Crossman, 1983; Kita and Kitai, 1987; Granata and Kitai, 1989; Steininger et al., 1992) as well as the cerebral cortex (Jackson and Crossman, 1981; Nambu et al., 2002; Degos et al., 2008). Furthermore, as inputs to both striatum and STN arise from the intralaminar thalamic nuclei, the centromedian nucleus (CM), and the parafascicular nucleus (Pf) (Wilson et al., 1983; Sadikot et al., 1992; Feger et al., 1994; Lanciego et al., 2004; Castle et al., 2005), the STN is now viewed as a key entry to the basal ganglia circuit, probably as important as the striatum itself.

The GPi and SNr share many histological characteristics, as well as similar afferent and efferent connections. Although the projection neurons of the basal ganglia output nuclei are generally considered to be GABAergic (Penney and Young, 1981; Rajakumar et al., 1994), there is evidence that projection neurons within the entopeduncular nucleus (ENT, the rodent homolog of GPi) also express other markers such as markers of cholinergic (Parent et al., 1981) and glutamatergic neurons (Kha et al., 2000), as well as peptides like somatostatin and substance P (Murakami et al., 1989a,b). Both nuclei project to the ventral motor thalamus, caudal intralaminar nuclei (Sidibe et al., 1997, 2002) and PPT (Steininger et al., 1992; Grofova and Zhou, 1998). Finally the thalamic nuclei then send glutamatergic projections to the motor cortex, thus closing the loop.

THE STRIATUM, FOCUS OF MOST STUDIES

Dyskinesia in PD seems to be mediated by alterations in basal ganglia activity that are the opposite of those occurring in PD (Vidailhet et al., 1999; Obeso et al., 2000; Boraud et al., 2001). Current

models of LID suggest that excessive decrease in GPi activity in turn disinhibits the motor thalamus and the cortex, giving rise to abnormal increase in cortical drive and consequent excessive motor movements (Wichmann and DeLong, 1996; Bezard et al., 2001a).

The first site of interest is of course the striatum with a particular emphasis upon the MSNs. Over the past few years, LID have been associated with a number of molecular changes, including regulation of striatal dopamine receptors, downstream changes in striatal proteins and genes, abnormalities in non-dopaminergic transmitter systems, etc., all changes that go beyond the topic of the present review (Bezard et al., 2001a; Jenner, 2008). Changes are not simply the consequence of chronic treatment since the first levodopa dose would induce expression changes of numerous proteins in the dopamine depleted striatum that equate those induced by chronic exposure as evidenced using a proteomic approach in the MPTP macaque model (Scholz et al., 2008).

DENDRITIC SPINE PRUNING OCCURS ON D2-EXPRESSING MSNs

A simple but long ignored question was the possible changes in the connections in the basal ganglia circuit in both the parkinsonian and dyskinetic states. Recently, Nadjar et al. (2006) showed that both the phenotype and the targets of striatofugal neurons, and therefore their relative influence on target structures, is preserved after dopamine denervation in the parkinsonian state and after additional chronic levodopa treatment in both non-dyskinetic and dyskinetic groups (Nadjar et al., 2006). This suggests that the phenotypic plasticity of the striatofugal system is not affected by the experimental condition. It does not mean however that plastic changes do not occur in the striatum. For instance, it has been shown that the size of the dendritic tree and the density of dendritic spines of MSNs is significantly reduced in the caudate nucleus and the putamen of PD patients compared with controls (Stephens et al., 2005), confirming previous data in rodents (Ingham et al., 1998). Such pruning was observed in MPTP-primates as well (Scholz et al., 2008; Villalba et al., 2009). The MSNs submitted to this dramatic plastic change were recently characterized as the D1R-immunonegative neurons, i.e., the D2R-expressing neurons (Day et al., 2006). Unfortunately, the impact of such spine pruning on MSN physiology is still unclear. These data nevertheless support the idea of plastic changes in the corticostriatal network but with no consequence on the phenotype and organization of projections of striatal neurons. Thus, loss of cortical afferents appears unlikely to affect the phenotypic pattern of striatal neurons, but rather might alter their activity or mRNA processing (Day et al., 2006). Altogether, these changes contribute in the development of adverse events related to levodopa therapy, because they would alter information flow through the striatum and rest of the basal ganglia nuclei.

IS THE DIRECT/INDIRECT PATHWAY IMBALANCE CAUSED BY DIFFERENTIAL CORTICOSTRIATAL INPUT?

Many studies have investigated the pathophysiology of the basal ganglia after dopamine denervation. Imbalances between neural activity in the two major output pathways of striatum have been proposed to underlie the profound motor deficits observed in PD, such as the hypokinesia (Albin et al., 1989; DeLong, 1990; Bezard et al., 2001b). This imbalance was first documented in anatomo-functional studies (Gerfen et al., 1990; Gerfen, 2000) and, surprisingly, only very recently confirmed with an electrophysiological approach (Mallet et al., 2006). Such an imbalance could be generated locally within the striatum or caused by a complex interaction with the corticostriatal excitatory. Retrograde tract-tracing experiments in the rat have shown that striatonigral neurons are preferentially innervated by cortical neurons that project inside the telencephalon (intratelencephalic (IT)-type), in both the ipsilateral and contralateral striatum, whereas striatopallidal neurons receive a greater input from cortical neurons that send their main axon into the pyramidal tract (PT) and their collateral axons only in the ipsilateral striatum (Lei et al., 2004). Although such a clearly segregated corticostriatal organization has recently been challenged (Ballion et al., 2008), a deficit in specific cortical inputs might also contribute to selectively depress the activity of striatonigral neurons (Mallet et al., 2006). Both the spontaneous activity and the sensitivity to cortical stimulation of striatonigral neurons were reduced by the lesion, whereas the reverse effects were observed for striatopallidal neurons (Mallet et al., 2006). However, elegant electrophysiological studies have shown that the decreased IT neuron activity associated with the dopaminergic depletion does not contribute to the striatal imbalance (Ballion et al., 2008).

STRIATAL GLUTAMIC ACID DECARBOXYLASE mRNA LEVELS IN PD AND LID

While electrophysiological investigations are scarce, anatomo-functional studies have documented the specific changes in the transcriptional activity of subpopulations of striatal GABA neurons in PD and LID conditions. Outside the scope of this review, a number of studies have indirectly confirmed the anatomo-functional organization of the striatal territories. Immediate-early genes have been extensively studied and expression patterns of c-fos and FosB proteins clearly relate a given behavioral phenotype with an increased expression/signal in a sub-territory of the striatum (Saka et al., 1999; Cenci, 2002; Jenner, 2008).

For instance, a number of studies using *in situ* hybridization studies have unraveled changes in glutamic acid decarboxylase (GAD) mRNA levels, the rate-limiting enzyme in the synthesis of GABA, in parkinsonian and dyskinetic animal models. Studies carried out during the last 25 years have shown the existence of two GAD isoforms, GAD_{65}, and GAD_{67}, each encoded by a different gene, and differing in molecular size and intraneuronal distribution (Denner and Wu, 1985; Kaufman et al., 1991; Martin et al., 1991; Martin and Rimvall, 1993). In MPTP-treated primates, GAD_{65} mRNA and GAD_{67} mRNA are increased in the striatum (Pedneault and Soghomonian, 1994; Soghomonian et al., 1994; Levy et al., 1995). Levodopa treatment significantly normalizes GAD_{67} mRNA expression in the putamen and caudate nucleus to levels similar to those found in control monkeys (Levy et al., 1995). Other studies, however, showed not significant changes in the distribution of both isoforms in the cortex, caudate, and putamen of parkinsonian and dyskinetic primates (Stephenson et al., 2005). In rats bearing a unilateral 6-OHDA lesion, GAD gene expression is increased in the striatum on the side of the lesion (Lindefors et al., 1989; Soghomonian et al., 1992; Consolo et al., 1999; Bacci et al., 2002). By contrast, the administration of levodopa leads to further increases in striatal GAD_{67} mRNA levels (Cenci et al., 1998; Consolo et al., 1999; Carta et al., 2001, 2003; Bacci et al., 2002; Nielsen and Soghomonian, 2004).

OPIOID PEPTIDE PRECURSOR mRNA AND OPIOID RECEPTOR LEVELS IN PD AND LID

Besides GAD, expression levels of precursors of the opioid peptides have been extensively investigated. Investigations in rodents (Gerfen et al., 1990; Engber et al., 1992; Duty et al., 1998), primates (Herrero et al., 1995; Morissette et al., 1999; Tel et al., 2002) and humans (Nisbet et al., 1995; Calon et al., 2002; Henry et al., 2003) have shown that Parkinsonism is associated with an increased expression of the opioid precursor preproenkephalin-A (PPE-A) messenger RNA (mRNA) in striatal neurons projecting to the GPe and a decreased preproenkephalin-B (PPE-B) mRNA expression in striatal neurons projecting to the GPi. In the dyskinetic state, however, the expression of PPE-B mRNA is increased (Cenci et al., 1998; Duty et al., 1998; Henry et al., 1999; Westin et al., 2001; Tel et al., 2002; Winkler et al., 2002; Henry et al., 2003), whereas that of PPE-A mRNA is either unchanged or further increased (Herrero et al., 1995; Morissette et al., 1997; Duty et al., 1998; Henry et al., 1999; Morissette et al., 1999; Zeng et al., 2000; Westin et al., 2001; Calon et al., 2002; Tel et al., 2002). These data suggest a role for enhanced endogenous opioid peptide transmission in striatal output pathways for the generation of LID. However, none of these studies has regarded basal ganglia nuclei other than the striatum as potential sources and those opioid precursors have almost never been quantified, simultaneously with the levels of opioid receptors, at the peak of dyskinesia severity, a quite surprising observation. Recently, Aubert and colleagues, studying a comprehensive brain bank of control, parkinsonian and dyskinetic monkeys terminated at the peak of levodopa-induced antiparkinsonian efficacy and dyskinesia manifestation, found a reduction in κ and μ opioid receptor binding in the GPi correlating with dyskinesia severity. Such decrease also correlated with an enhanced expression of PPE-B mRNA, but not that of PPE-A, in both the striatum and the STN, known to also express peptide precursors (Merchenthaler et al., 1997). This abnormal PPE-B-derived transmission could therefore be involved in LID manifestation with increased peptide levels arising from both the striatum and the STN (Aubert et al., 2007).

TRANSCRIPTOMIC CHANGES AFFECTING PALLIDAL COMPLEX AND STN IN PD AND LID

In the 6-OHDA-lesioned rat model of PD, the profound dopamine depletion in the striatum resulted in significant increases in the percentage of GPe neurons that expressed GADs mRNA and in the amount of GADs mRNA per GPe neuron (Kincaid et al., 1992; Soghomonian and Chesselet, 1992). Similar results were described MPTP-treated monkeys, the expression of GAD_{67} but not GAD_{65} was augmented in the GPe, along with a significant increases in number of GAD_{67} neurons, while no significant difference in the number of GAD_{65} neurons was observed (Stephenson et al., 2005). Levodopa treatment did not significantly change the number of GAD_{65} or GAD_{67}-expressing pallidal neurons following MPTP (Stephenson et al., 2005).

In the GPi of MPTP-treated monkeys, i.e., the main output structure, the expression of GAD_{67} and GAD_{65} mRNAs is increased (Pedneault and Soghomonian, 1994; Soghomonian et al., 1994; Herrero et al., 1996). Similar results were described in MPTP-lesioned cats (Schroeder and Schneider, 2001). Interestingly the increase in GAD_{67} mRNA is abolished by levodopa treatment in MPTP-treated monkeys (Herrero et al., 1996). These data fit with the observation that there is no difference in the levels of GAD_{67} mRNA between levodopa-treated PD patients and control subjects (Herrero et al., 1996), i.e. that levodopa treatment normalizes GAD_{67} mRNA levels. In rats, an ipsilateral marked up-regulation of $GAD_{65}/_{67}$ mRNA expression in the ENT nucleus has been reported following 6-OHDA lesion (Soghomonian and Chesselet, 1992; Barroso-Chinea et al., 2008). Continuous or intermittent levodopa administration is equally effective at reversing the lesion-induced increase in GAD_{67} mRNA expression in the ENT nucleus (Nielsen and Soghomonian, 2004). Altogether, these results indicate that the level of GAD_{67} mRNA is increased in the cells of the GPi after nigrostriatal dopaminergic denervation and that this increase can be reversed by levodopa therapy (Herrero et al., 1996).

One should however keep in mind that a transcriptional regulation does not necessarily mean a change in electrical activity. Parallel to these observations, there are evidences of an increase in mitochondrial respiratory chain enzyme activity in ENT nucleus in the lesioned hemisphere of 6-OHDA rats suggesting increased synaptic activity, perhaps due to increased firing of the STN (Porter et al., 1994). The enzymatic activity or the changes in the expression of cytochrome oxidase-I (COI) have indeed been shown to correlate with changes in the firing activity of several structures (Wong-Riley and Welt, 1980; Wong-Riley, 1989). "*In situ*" hybridization of cytochrome oxidase-I (COI) mRNA in the MPTP monkey model of PD has shown increased levels parallel increased firing of the STN (Bergman et al., 1994; Vila et al., 1996; Bezard et al., 1999). Comparably, increased levels in the GPi correlate with an increased firing frequency of GPi neurons (Bezard et al., 1999; Boraud et al., 2000, 2001). As expected, levodopa treatment reversed such COI mRNA overexpression in all affected structures (Vila et al., 1996). Similar results were obtained in the 6-OHDA rat model (Vila et al., 1999). No changes were however detected in levodopa-treated PD patients compared to control subjects, a situation that could either reflect the levodopa-induced normalization of the COI mRNA expression in PD patients or the inescapable poor quality of human post-mortem samples (Vila et al., 1996). In conclusion, these anatomo-functional evidences correlate with the observed hypoactivity of both the STN and GPi during levodopa or apomorphine-induced dyskinesia in MPTP-treated monkeys (Filion et al., 1991; Boraud et al., 2001), in dyskinetic PD patients (Merello et al., 1999) and in patients with generalized dystonia and hemiballismus (Suarez et al., 1997; Vitek et al., 1999).

"PARKINSON'S DISEASE-RELATED PATTERN" IN PD AND LID

Considerable efforts have been devoted to develop neuroimaging methods to study the basal ganglia (Eidelberg and Edwards, 2000; Feigin et al., 2001; Eckert et al., 2005; Asanuma et al., 2006; Trost et al., 2006; Eidelberg, 2009). These techniques have been developed with the hope that they could be used as biomarkers to help the diagnosis, to detect early stages of the disease, later on to grade the disease severity of the disease, and, finally, to serve as a surrogate marker for progression of the underlying disease. Positron emission tomography (PET) and single photon emission computed tomography (SPECT), which is less sensitive but more widely available than PET, are capable to provide an objective measure of PD severity as both techniques depict the loss of neurotransmitter

function and can detect changes in striatal dopamine levels after levodopa administration in relationship with the motor responses. Such investigations of the dopamine transmission have brought extremely important insights, even unraveling the physiological basis for the placebo effect (de la Fuente-Fernandez et al., 2001), but they do not impact our understanding of the functional anatomy of PD and LID. Metabolic PET studies, however, have demonstrated that PD is characterized by a set of reproducible functional brain networks that correlate with its clinical features (Huang et al., 2007). Using [^{18}F] fluorodeoxyglucose (FDG) and PET, changes in a so-called Parkinson's disease-related pattern (PDRP) expression have been observed. Disease progression is associated with increasing metabolism in the STN and GPi, as well as in the dorsal pons and primary motor cortex. Advancing disease is associated with declining metabolism in the prefrontal and inferior parietal regions (Huang et al., 2007). Changes in a cognition-related network paralleled these motor-related changes. At present, there is a clear consensus in considering that the PDRP is characterized by increased pallido-thalamic and pontine activity, associated with relative reductions in cortical motor and premotor areas in PD patients (Carbon et al., 2003; Eidelberg, 2009) although no changes are reported in the thalamus. The impact of dopaminergic therapy upon PDRP has also been investigated (Feigin et al., 2001; Asanuma et al., 2006). The changes in the pallidal metabolism and the overall PDRP network activity correlated significantly with clinical improvement of PD symptoms during dopaminergic treatment (Feigin et al., 2001; Asanuma et al., 2006). Interestingly, a recent study (Hirano et al., 2008) showed a highly significant dissociation between levodopa-mediated PDRP changes in cerebral blood flow and glucose metabolic scans. This phenomenon was accentuated in PD patients with LID, reflecting excessive dopaminergic-induced vasodilatation in these subjects.

What is clear from these studies is that not only the PDRP is affected by dopaminergic treatments but the cognition-related pattern as well. Such findings have lead to dig in the experimental literature when researchers used the then-popular 2-deoxyglucose (2-DG) accumulation technique for studying brain metabolism. Alan Crossman and his colleagues extensively studied the metabolic changes induced by dopamine depletion and further dopaminergic treatments. Of particular interest for this review, they found that the STN showed a dramatic increase in 2-DG uptake in animals exposed to dopamine agonist immediately prior to the terminal procedure, especially in ventromedial "limbic/associative" STN, along with relative greater levels of 2-DG uptake in GPi (Mitchell et al., 1989, 1992). This suggested that a major effect of dopaminergic treatment was to affect the limbic/associative network more than the motor network. Since consistent funneling of information takes place between the sensorimotor, limbic, and associative cortico-basal ganglia domains (Haber et al., 1993, 2000), we hypothesized that non-motor domains play a role in these manifestations and studied the changes in 2-DG accumulation in the sensorimotor, limbic, and associative domains of basal ganglia and thalamic nuclei of four groups of non-human primates (Guigoni et al., 2005): normal, parkinsonian, parkinsonian chronically treated with L-dopa without exhibiting dyskinesia and parkinsonian chronically treated with levodopa and exhibiting overt dyskinesia. While non-dyskinetic animals displayed a rather normalized metabolic activity, dyskinetic animals were distinguished by significant changes in 2-DG accumulation in limbic and associative-related structures and not simply in sensorimotor-related ones, suggesting that dyskinesia are linked to a pathological processing of limbic and cognitive information (Guigoni et al., 2005). These metabolic changes likely reflect the underlying neural mechanisms of not simply motor dyskinesia but also affective, motivational, and cognitive disorders associated with long-term exposure to levodopa.

CONCLUDING REMARKS

The anatomical and functional organization of the basal ganglia circuitry has received considerable attention in the last two decades. This has led to a better understanding of the physiological and pathophysiological aspects involved in PD and LID. To our opinion, the most fundamental consequence of the recent findings is that we cannot continue analyzing LID by investigating the only motor areas, thus rendering unreliable all studies that do not pay attention to the anatomo-functional organization of cortico-basal ganglia loops. Seeing LID as either "caused" by unwanted involvement of associative and limbic areas or simply as having their cognitive and limbic abnormal counterparts ("consequence") as often reported in hyperkinetic disorders might have different clinical consequences. For instance, if electrophysiological investigations support the causative hypothesis, modulating the activity of non-motor regions would reduce LID severity, thereby offering new drug targets for treatment of this debilitating condition.

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Pulvinar projections to the striatum and amygdala in the tree shrew

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Visually guided movement is possible in the absence of conscious visual perception, a phenomenon referred to as "blindsight." Similarly, fearful images can elicit emotional responses in the absence of their conscious perception. Both capabilities are thought to be mediated by pathways from the retina through the superior colliculus (SC) and pulvinar nucleus. To define potential pathways that underlie behavioral responses to unperceived visual stimuli, we examined the projections from the pulvinar nucleus to the striatum and amygdala in the tree shrew (*Tupaia belangeri*), a species considered to be a prototypical primate. The tree shrew brain has a large pulvinar nucleus that contains two SC-recipient subdivisions; the dorsal (Pd) and central (Pc) pulvinar both receive topographic ("specific") projections from SC, and Pd receives an additional non-topographic ("diffuse") projection from SC (Chomsung et al., 2008). Anterograde and retrograde tract tracing revealed that both Pd and Pc project to the caudate and putamen, and Pd, but not Pc, additionally projects to the lateral amygdala. Using immunocytochemical staining for substance P (SP) and parvalbumin (PV) to reveal the patch/matrix organization of tree shrew striatum, we found that SP-rich/PV-poor patches interlock with a PV-rich/SP-poor matrix. Confocal microscopy revealed that tracer-labeled pulvino-striatal terminals preferentially innervate the matrix. Electron microscopy revealed that the postsynaptic targets of tracer-labeled pulvino-striatal and pulvino-amygdala terminals are spines, demonstrating that the pulvinar nucleus projects to the spiny output cells of the striatum matrix and the lateral amygdala, potentially relaying: (1) topographic visual information from SC to striatum to aid in guiding precise movements, and (2) non-topographic visual information from SC to the amygdala alerting the animal to potentially dangerous visual images.

Keywords: blindsight, superior colliculus, striosome, matrix, synapse

INTRODUCTION

The primary visual pathway (from the retina to the lateral geniculate nucleus to the striate cortex or V1) is chiefly responsible for the conscious perception of visual stimuli. A second visual pathway relays presumably unconscious visual information from the retina to the superior colliculus (SC), then to the pulvinar nucleus. The pulvinar nucleus subsequently projects to visual areas of the parietal and temporal cortex (Stepniewska et al. 1999, 2000; Wong et al., 2009; Berman and Wurtz, 2010; Lyon et al. 2010) as well as to the striatum (Beckstead, 1984; Lin et al., 1984; Takada et al., 1985a,b; Harting et al., 2001a,b; Harting and Updyke, 2006; Kunzle, 2006) and to the amygdala (Linke et al., 1999; Shi and Davis, 2001). Although the exact functions of the pulvinar nucleus remain obscure, a variety of studies suggest that it plays important roles in directing spatial attention as well as linking visual stimuli with context-specific motor responses. Damage to, or inactivation of the pulvinar nucleus can result in inattention to the visual hemifield contralateral to the affected pulvinar, and/or impaired ability to guide movements in relation to visual signals (spatial neglect; Grieve et al. 2000; Arend et al., 2008; Snow et al., 2009; Wilke et al. 2010). Spatial neglect can also result from damage to either the cortical or subcortical targets of the pulvinar nucleus. Lesion sites within the right parietal or temporal cortex can cause neglect symptoms (Verdon et al., 2010) and the subcortical damage sites most often associated with visual neglect are the right caudate and putamen (Karnath et al., 2002, 2004).

Visual pathways through the SC and pulvinar nucleus may also be responsible for (1) the ability of patients who have sustained V1 damage to continue to use visual cues to guide their movements even in the absence of conscious visual perception, a phenomenon referred to as "blindsight" (for review Cowey, 2010), and (2) the finding that fearful images can elicit emotional responses in the absence of their conscious perception, a phenomenon referred to as "unseen fear" or "subliminal fear" (Liddell et al., 2004; Williams et al., 2006). Findings supporting this function of the SC and pulvinar nucleus include evidence that lesions of the pulvinar impair responses to threatening visual images (Ward et al., 2005) and that the SC, pulvinar, and amygdala are activated during the brief presentation of fearful faces masked by neutral faces (Morris et al., 1999, 2001; Liddell et al., 2005).

The idea that components of the second visual pathway are involved in linking vision with action is also supported by correlating the anatomical organization of these pathways in different species with the behavioral effects of V1 lesions. In particular, in a

prototypical primate, the tree shrew, the majority of the pulvinar nucleus receives input from the SC (Luppino et al., 1988; Chomsung et al., 2008), and visually guided movements are amazingly unrestricted even after complete removal of V1 (Diamond and Hall, 1969; Snyder et al., 1969). The tree shrew therefore provides an excellent model for the study of the organization of secondary visual pathways and their potential role in neglect, blindsight and/or unseen fear.

In the tree shrew, the dorsal (Pd) and central (Pc) pulvinar both receive restricted, topographic ("specific") projections from the SC, and the Pd receives an additional widespread, non-topographic ("diffuse") SC projection (Luppino et al., 1988; Chomsung et al., 2008). Further distinctions between these pathways were provided by ultrastructural studies, which revealed a greater involvement of pulvinar interneurons in the specific pathway, when compared to the diffuse pathway (Chomsung et al., 2008). However, the function of these two pathways remains obscure; we found the Pd and Pc targeted similar regions of the temporal cortex (Chomsung et al., 2010).

In the current study, we sought to determine whether the Pd and Pc project differentially to the striatum and amygdala in order to gain insight into the potential function of the specific and diffuse tectopulvinar pathways. Within the striatum, we examined the relationship of Pd and Pc projections to the striosome and matrix compartments to further assess the function of these two pathways. Graybiel and Ragsdale (1978) originally described the unique organization of striatum sections stained for acetylcholinesterase (AChE) as a mosaic of pale staining patches (or striosomes) distributed in a more densely stained matrix. Subsequent studies of the connection patterns of these compartments suggest that they participate differentially in limbic-based (striosome) and sensorimotor/associative (matrix) circuits (for review, see Graybiel, 2008). Our results indicate that the specific tectopulvinar pathway relays topographic visual information to the striatum matrix while the diffuse tectopulvinar pathway relays non-topographic visual information to the amygdala. We suggest that the specific pathway may aid in the visual guidance of precise movements, while the diffuse pathway may function as an "alarm" pathway to potentially alert the animal to dangerous visual images.

MATERIALS AND METHODS

A total of 9 adult tree shrews (*Tupaia belangeri*); 4 male and 5 female, (140–270 g) were used for these experiments. To label pulvinar cells by retrograde transport, 2 tree shrews received an iontophoretic injection of the beta subunit of cholera toxin (CTB; List Biological Laboratories, Inc., catalog #105) or fluorogold (FG; Fluorochrome LLC, Denver, CO, USA) in the putamen (1, CTB) or amygdala (1, CTB and FG on opposite sides). To label pulvinar projections by anterograde transport, 7 tree shrews received an iontophoretic injection of biotinylated dextran amine (BDA; 3,000 MW, Molecular Probes, Eugene, OR, USA) in the pulvinar nucleus (other pulvinar projections in these 7 cases were reported in our earlier publications (Chomsung et al., 2008, 2010). Selected sections from injected tree shrew brains were stained for acetylcholinesterase (AChE) to identify pulvinar subdivisions, or substance P (SP), parvalbumin, or the potassium channel interacting protein (KChIP) to identify the patch matrix compartments of the striatum. All methods were approved by the University of Louisville Animal Care and Use Committee and conform to the National Institutes of Health guidelines.

TRACER INJECTIONS

Tree shrews that received injections were initially anesthetized with intramuscular injections of ketamine (100 mg/kg) and xylazine (6.7 mg/kg). Additional supplements of ketamine and xylazine were administered approximately every 45 min to maintain deep anesthesia through the completion of the injections. Prior to injection, the tree shrews were placed in a stereotaxic apparatus and prepared for sterile surgery. The heart rate was continuously monitored with a MouseOx pulse oximeter (STARR Life Sciences Corp., Pittsburgh, PA, USA). A small area of the skull overlying the putamen, amygdala or pulvinar nucleus was removed and the dura reflected. A glass pipette containing BDA (5% in saline, tip diameters approximately 3 μm) or FG (2% in saline, tip diameter approximately 10 μm) or CTB (1% desalted CTB in 0.1 M phosphate buffer (PB), pH 6.0; tip diameters approximately 2 μm) was lowered vertically and the tracer was ejected iontophoretically (2 μA positive current for 15–30 min).

After a 7-day survival period, the tree shrews were given an overdose of ketamine (600 mg/kg) and xylazine (130 mg/kg) and were perfused through the heart with Tyrode solution, followed by a fixative solution of 2% paraformaldehyde and 2% glutaraldehyde or 4% paraformaldehyde in 0.1 M PB (pH 7.4). The brain was removed from the skull, sectioned to a thickness of 50 μm using a vibratome, and placed in PB.

HISTOCHEMISTRY TO REVEAL TRACERS

The BDA was revealed by incubating sections in a 1:100 dilution of avidin and biotinylated horseradish peroxidase (ABC; Vector Laboratories, Burlingame, CA, USA) in phosphate-buffered saline (0.01 M PB with 0.9% NaCl, pH 7.4; PBS) overnight at 4°C. The sections were subsequently rinsed three times in PB (10 min each), reacted with nickel-intensified 3,3′-diaminobenzidine (DAB) for 5 min, and washed in PB. DAB-labeled sections were either mounted on slides or prepared for electron microscopy as described below. To reveal BDA for confocal microscopy (in combination with immunocytochemical labeling of the patch/matrix compartments, described below) sections were incubated in a 1:100 dilution of streptavidin conjugated to Alexa Fluor 488 or 546 (Molecular Probes, Eugene, OR, USA).

The FG was revealed with ultraviolet light, or by incubation with a rabbit anti-FG antibody (Fluorochrome) diluted 1:50,000. The antibody was revealed by either a biotinylated goat-anti-rabbit antibody (1:100, Vector), ABC and DAB reaction, or by a goat-anti-rabbit antibody conjugated to Alexa Fluor 488 or 546 (Molecular Probes). The CTB was revealed using a rabbit-anti-cholera toxin antibody (Sigma) diluted 1:10,000, followed by a biotinylated goat-anti-rabbit antibody (Vector), ABC, and DAB reaction or a goat-anti-rabbit antibody conjugated to Alexa Fluor 488 or 546 (Molecular Probes).

A Neurolucida system and tracing software (MicroBrightField, Inc., Williston, VT, USA) was used to plot the distributions of pulvino-putamen and pulvino-amygdala cells and terminals. Adjacent sections stained for AChE (described below) were used to delineate the Pd and Pc.

AChE STAINING TO REVEAL PULVINAR SUBDIVISIONS

Using a protocol modified from Stepniewska and Kaas (1997), the tissue was rinsed in deionized water, placed in a solution of AChE for 3 h, and then rinsed in saline, followed by deionized water, before reacting with a 1.25% sodium sulfite solution for 1 min. Following deionized water rinses, the tissue was then incubated in a 1% silver nitrate solution for 5 min, rinsed with deionized water and placed in a 5% sodium thiosulfite solution to adjust the contrast of the tissue staining (approximately 5 min). Finally, the tissue was rinsed in saline, and mounted on slides for light microscope examination.

IMMUNOCYTOCHEMISTRY TO REVEAL PATCH/MATRIX PROTEINS

To reveal the patch/matrix organization within the tree shrew striatum, antibodies shown to bind to proteins that are differentially expressed in the patch or matrix compartments of the striatum in various species were employed. The following antibodies and dilutions were used: rat-anti-substance P (SP; Accurate Chemical, Westbury, NY, USA used at a dilution of 1:250), mouse-anti-paravalbumin (Sigma, St Louis, MO, USA used at a dilution of 1:10,000), mouse-anti-potassium channel interacting protein (KChIP; NeuroMab, UC Davis, used at a dilution of 1:5). Selected sections were incubated overnight in one of the above antibodies. The next day sections were rinsed in 0.1 M phosphate buffer, incubated 1 h in the appropriate secondary antibody at a 1:100 dilution (biotinylated goat-anti-rat for substance P; biotinylated goat-anti-mouse for parvalbumin and KChIP; Vector Laboratories, Burlingame, CA, USA), rinsed again and incubated for 1 h in avidin and biotinylated-horseradish peroxidase (ABC solution) at a dilution of 1:100, before reacting with nickel-enhanced diaminobenzidine (DAB).

CONFOCAL MICROSCOPY

To determine if pulvino-striatal axons innervate the patch or matrix compartments or the striatum, sections containing terminals labeled by the anterograde transport of BDA injected in the pulvinar nucleus were incubated overnight in a 1:100 dilution of streptavidin conjugated to Alexa 546 (Molecular Probes, Carlsbad, CA, USA) and either the rat-anti-SP or mouse-anti-parvalbumin antibodies. The following day the sections were rinsed and incubated for 1 h in secondary antibodies (anti-rat for substance P; anti-mouse for parvalbumin) conjugated to Alexa Fluor 488 at a dilution of 1:100. Sections were then mounted and viewed with a laser scanning confocal microscope (Olympus 3 Laser Scanning Confocal Microscope Canter Valley, PA, USA). To illustrate the interlocking pattern of the patch and matrix compartments, sections were incubated in rat-anti-SP 1:250 and mouse-anti-parvalbumin 1:10,000 overnight. The following day the sections were incubated in biotinylated goat-anti-rat for 1 h, followed by streptavidin conjugated to Alexa Fluor 546 and anti-mouse conjugated to Alexa Fluor 488 for 1 h. Sections were then mounted and viewed with the confocal microscope.

ELECTRON MICROSCOPY

Striatum and amygdala sections that contained terminals labeled by the anterograde transport of BDA were postfixed in 2% osmium tetroxide, dehydrated in an ethyl alcohol series, and flat embedded in Durcupan resin between two sheets of Aclar plastic (Ladd Research, Williston, VT, USA). Durcupan-embedded sections were first examined with a light microscope to select areas for electron microscopic analysis. Selected areas were mounted on blocks, ultrathin sections (70–80 nm, silver–gray interference color) were cut using a diamond knife, and sections were collected on Formvar-coated nickel slot grids. Selected sections were stained for the presence of gamma amino butyric acid (GABA). A postembedding immunocytochemical protocol described previously (Patel and Bickford, 1997) was employed. The GABA antibody (Sigma, catalog # A2052, used at a dilution of 1:1000–1:2000) was tagged with a goat-anti-rabbit antibody conjugated to 15-nm gold particles (Amersham, Arlington Heights, IL, USA). The sections were air dried and stained with a 10% solution of uranyl acetate in methanol for 30 min before examination with an electron microscope.

All labeled terminals involved in a synapse were photographed within each examined section. The pre- and postsynaptic profiles were characterized on the basis of size (measured using SigmaScan software, SPSS Inc., Chicago, IL, USA) and the presence or absence of synaptic vesicles.

RESULTS

PD AND PC PROJECT TO THE STRIATUM; PD PROJECTS TO THE AMYGDALA

Following large BDA injections that involved both the Pd and Pc (**Figure 1A**), terminals labeled by anterograde transport fill large regions of the postcommisural putamen (**Figure 1B**) caudate (**Figure 1C**), as well as the lateral amygdala (**Figure 1F**). No pulvino-striatal terminals were distributed in regions of the caudate and putamen rostral to the anterior commissure. Smaller injections, confined to either the Pd or Pc (example Pc injection illustrated in **Figure 1D**; all other injection sites illustrated in Chomsung et al., 2008, 2010), labeled more discrete clusters of terminals in the caudate (**Figures 2A–C**) and putamen (**Figures 1E**, **2A–C, E–G**), and injections of the Pd, but not the Pc, also labeled diffusely distributed axons in the lateral amygdala (**Figures 2E–G**).

To confirm the differential projection patterns of the Pd and Pc, we placed injections of CTB or FG in the putamen or amygdala (**Figure 2I**). Confirming our anterograde tracing results, an injection in the putamen (**Figure 2I**, pink) that overlapped the distribution of terminals labeled by Pc BDA injections (**Figures 2A–C**) labeled clusters of cells in the Pc (**Figure 2L**) that were located in regions very similar to the location of the Pc BDA injections (**Figure 2D**). Similarly, injections in the amygdala (**Figure 2I**, green and orange) labeled cells in the Pd (**Figures 2J,K**) that were located in regions very similar to the location of the Pd BDA injections (**Figure 2H**).

THE PATCH MATRIX ORGANIZATION OF THE TREE SHREW STRIATUM

As illustrated in **Figures 3 and 4**, several different antibodies reveal the striosomes and matrix of the tree shrew caudate and putamen. An antibody against substance P stains dense patches of terminals (3A-C, G), while an antibody against parvalbumin intensely stained cells and neuropil within the matrix (3D, E). Interdigitating substance P and parvalbumin staining patterns are illustrated in

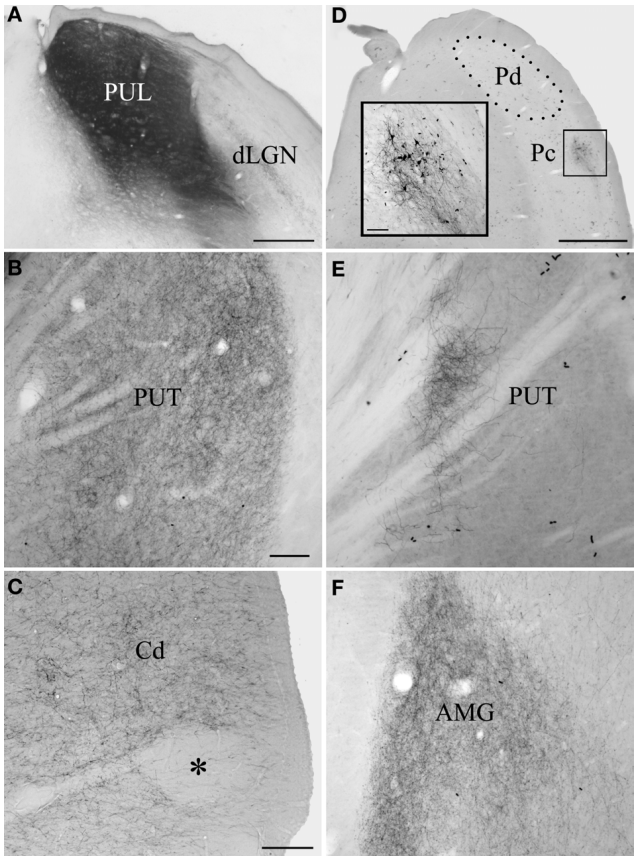

FIGURE 1 | The pulvinar nucleus projects to the striatum and lateral amygdala. Large BDA injections that involved both the dorsal (Pd) and central (Pc) pulvinar **(A)** labeled terminals throughout putamen (PUT, **B**) and caudate nucleus (Cd, **C**), although conspicuous patches (asterisk) were devoid of label. The injection illustrated in **(A)** also labeled terminals throughout the lateral amygdala (AMG, **F**). Smaller BDA injections confined to the Pd or Pc labeled restricted clusters of terminals in the Cd and PUT. An example Pc injection is illustrated in **(D)** (inset illustrates the injection site at higher magnification) and the resulting terminal distribution in the PUT in **(E)**. Scale bar in **A** = 500 μm. Scale bar in **B** = 100 μm and also applies to **(E)**. Scale bar in **C** = 100 μm and also applies to **(F)**. Scale bar in **D** = 1 mm (inset scale bar = 100 μm). dLGN, dorsal lateral geniculate nucleus; OT, optic tract. **A** was previously published in Chomsung et al., 2010.

Figures 4B–D. As recently demonstrated in primate tissue (Mikula et al., 2009), an antibody against KChIP stained cells throughout the matrix of the caudate and putamen and dense patches of terminals in striosomes (3F) in the tree shrew striatum.

THE PD AND PC PROJECT TO THE MATRIX OF THE STRIATUM

The labeling patterns observed following the large injections of BDA in the pulvinar nucleus suggest that pulvino-striatal axons innervate the matrix. These injections labeled axons that innervated large regions of the caudate and putamen, but conspicuous patches were devoid of labeled axons (**Figure 1C**). To confirm whether these unlabeled zones corresponded to striosomes, we stained tissue containing BDA-labeled pulvino-striatal axons for substance P or parvalbumin. As illustrated in **Figures 4E–J**, all observed pulvino-striatal axons originating from either the Pd or Pc innervated the matrix, as defined by substance P or parvalbumin staining. We therefore conclude that the majority of pulvino-striatal axons innervate the matrix.

THE PD AND PC CONTACT SPINES IN THE STRIATUM AND AMYGDALA

Using electron microscopy, we examined a total of 181 pulvino-putamen terminals (labeled by the anterograde transport of BDA injected into the Pd and/or Pc) that were involved in synaptic contacts. No size differences were detected in the populations of terminals labeled by Pd, Pc, or combined Pd/Pc injections (Pd injections: 0.48 ± 0.30 μm^2, Pc injections: 0.47 ± 0.24 μm^2, Pd/Pc injections: 0.47 ± 0.27 μm^2). Most terminals formed single synapses (62%, **Figures 5A,B**), but smaller numbers of perforated (34%, **Figure 5C**) or multiple (4%) synaptic contacts were also identified. Pulvino-putamen terminals primarily contacted spines (Pc, 100%; Pd, 94%; Pd/Pc, 93.3%) as illustrated in **Figures 5A–C**. This suggests that pulvino-striatal terminals contact GABAergic spiny projection neurons. We attempted to confirm the GABAergic nature of the postsynaptic targets by using postembedding immunocytochemical techniques to reveal the presence of GABA. However, we did not observe any accumulation of GABA within spines, although GABAergic terminals were easily detected by a qualitative assessment of gold particle density (**Figures 5A and G**).

We examined a total of 123 pulvino-amygdala terminals (labeled by a combined Pd/Pc injection) that were involved in synaptic contacts. The size of the labeled terminals (0.42 ± 0.17 μm^2) was similar to the size of pulvino-putamen terminals. Most terminals formed single synapses (55%, **Figures 5D, F and G**), but smaller numbers of perforated (35%) or multiple (10%; **Figure 5E**) synaptic contacts were also identified. All pulvino-amygdala terminals were found to contact spines, as illustrated in **Figures 5D–G**.

DISCUSSION

Figure 6 schematically illustrates the potential influence of pulvinar projections on basal ganglia circuits and behavior initiated by the SC. The current results and those of our previous study of tectopulvinar projections (Chomsung et al., 2008) indicate that visual information from the SC is relayed via the pulvinar nucleus to the spiny output cells of the striatum matrix and the lateral amygdala. As discussed below, we suggest that the specific projections from the SC to the Pd and Pc relay topographic visual information to the striatum to aid in guiding precise movements related to pursuit of non-threatening objects, while the diffuse projections from the SC to the Pd relay non-topographic visual information to the amygdala to alert the animal to potential danger and initiate escape behavior.

POTENTIAL RELATION OF PD AND PC PROJECTIONS TO APPROACH AND AVOIDANCE BEHAVIOR

Stimulation studies in rats revealed that the SC initiates at least two distinct behaviors: stimulation of the lateral SC (representing the lower visual field) initiates orienting/approach behavior, and stimulation of the medial SC (representing the upper visual field) initiates escape/avoidance behavior (Sahibzada et al., 1986). Subsequent lesion studies revealed that these behaviors are mediated by two anatomically distinct cell groups (Redgrave et al.,

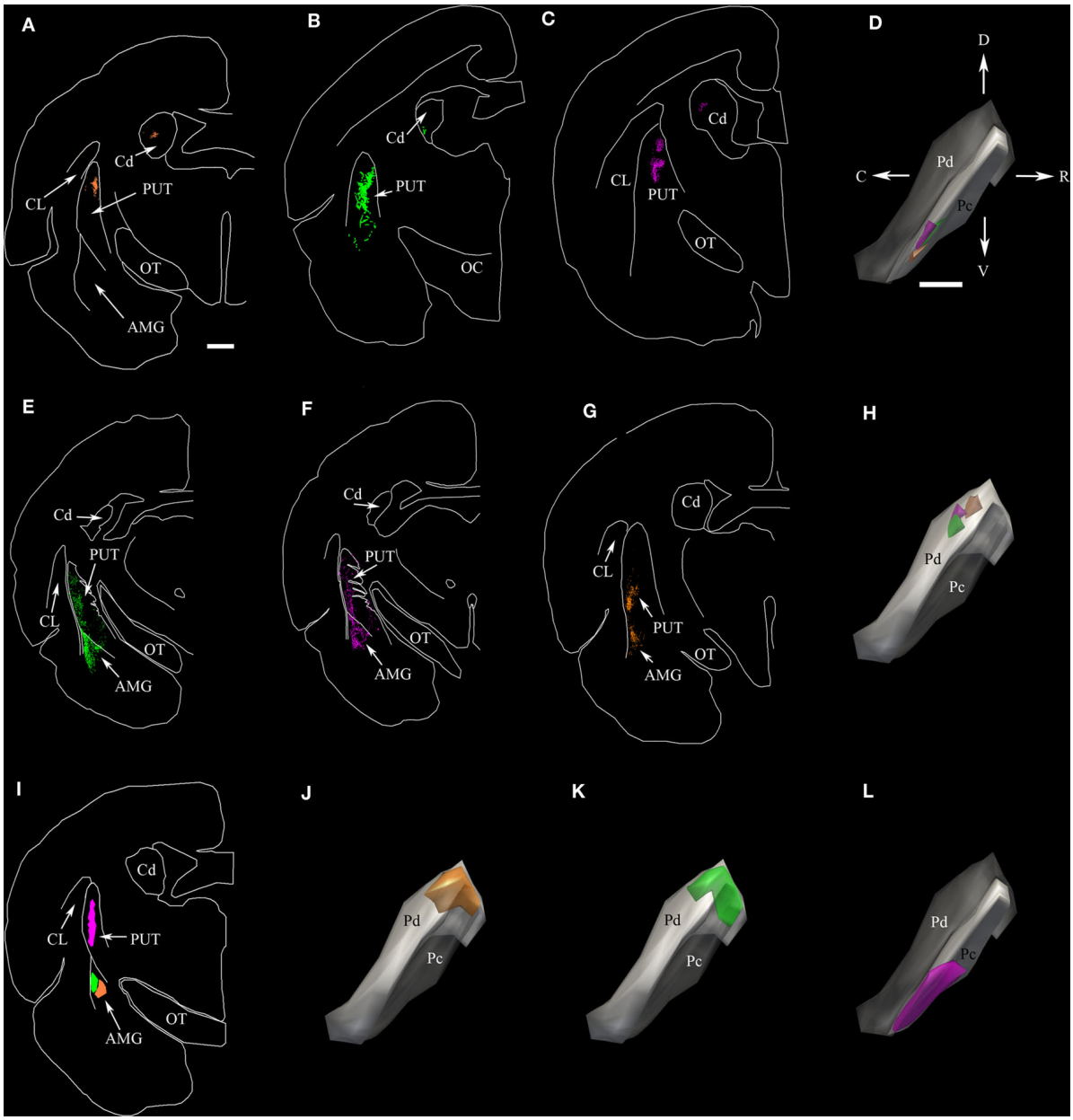

FIGURE 2 | The dorsal (Pd) and central (Pc) pulvinar project to the striatum; only the Pd projects to the amygdala. (A–C) Two dimensional (2D) neurolucida plots of the distribution of terminals labeled by anterograde transport in the caudate (Cd) and putamen (PUT) following three separate injections of BDA in the Pc. **(D)** Injection sites for **(A–C)** depicted in three-dimensional (3D) neurolucida reconstructions of the pulvinar nucleus. The color of each injection site corresponds to the color of the terminals in **(A–C)**. **(E–G)** Two-dimensional plots of the distribution of terminals labeled by anterograde transport in the PUT and amygdala (AMG) following three separate injections of BDA in the Pd. **(H)** Three-dimension reconstructions of injection sites for **(E–G)**. Because the full extent of the Pd is not viewable in the orientation depicted, the Pc subdivision was made transparent in this panel as well as in **(J,K)**. **(I)** Two-dimension plots of the location of FG and CTB injections in the PUT and AMG. **(J,K,L)** Three-dimension reconstructions of the distribution of cells labeled in the pulvinar nucleus by retrograde transport from the injections illustrated in I. The color of the cell distributions corresponds to the injection site colors in I. Scale bar in **A** = 1 mm and applies to all 2D plots. Scale bar in **D** = 1 mm and applies to all 3D pulvinar reconstructions. C, caudal, CL, claustrum, D, dorsal, OC, optic chiasm, OT, optic tract, R, rostral, V, ventral. Orientation arrows in D also apply to all 3D pulvinar reconstructions.

1986; Bickford and Hall, 1989) within the deep layers: approach movements by the crossed descending tecto-reticulo-spinal projection (predorsal bundle, PDB) and avoidance behavior by the ipsilateral descending (cuneiform, CNF) pathway (Dean et al., 1986, 1989). The primary sensory responses of these two cell groups: PDB cells to somatosensory stimulation within the lower visual field, and CNF cells to large "looming" visual stimuli in the upper visual field, suggest that the PDB initiates

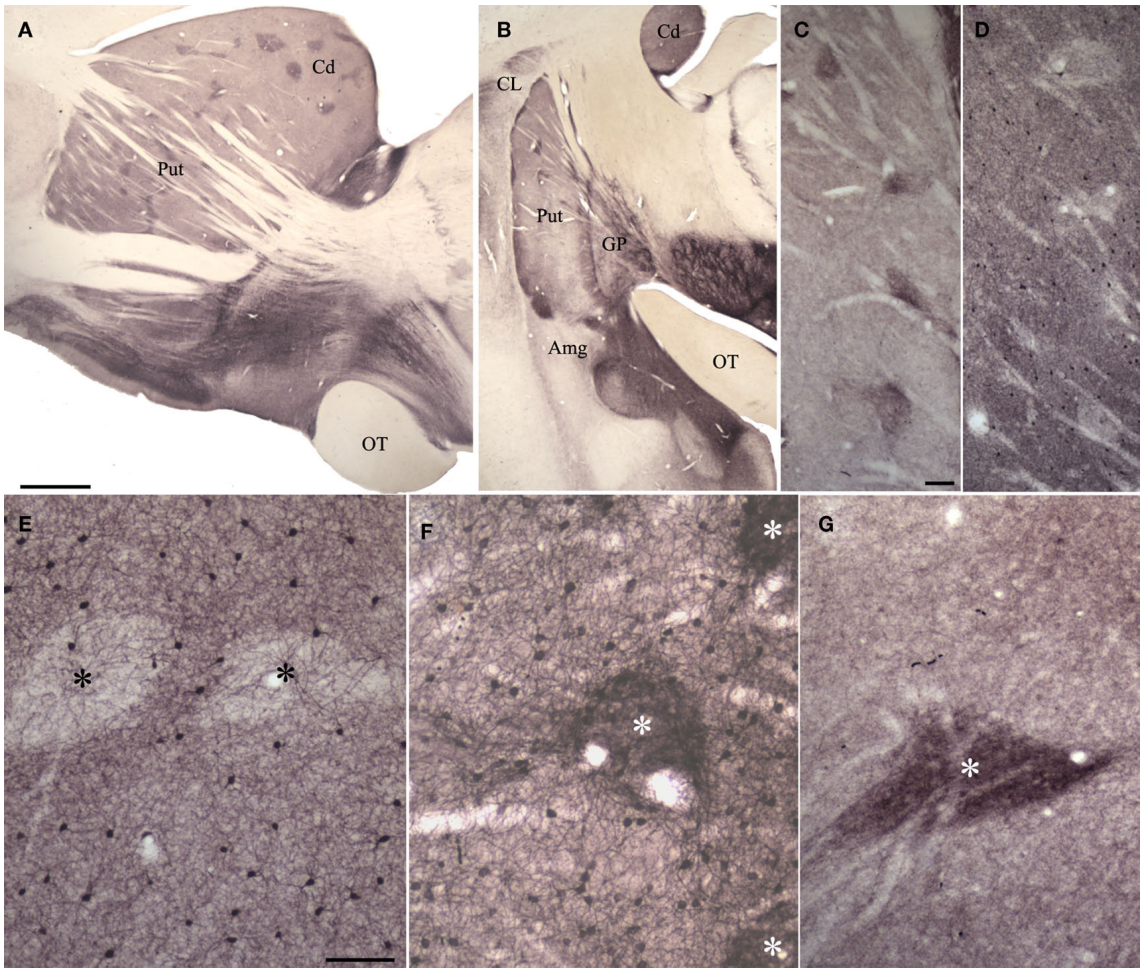

FIGURE 3 | Substance P (SP), parvalbumin (PV) and potassium channel interacting protein (KChIP) antibodies reveal patch/matrix organization of tree shrew striatum. (A,B) Sections (parasagittal, **A**; coronal, **B**) through the striatum stained for SP. Dark SP-positive patches are seen throughout the tree shrew striatum. **(C,D)** Adjacent sections through the putamen showing SP staining of patches **(C)** and PV staining in the matrix **(D)**. **(E)** PV stains cells within the matrix but not within patches (black asterisks). **(F)** KChIP stains clusters of terminals in patches (white asterisk) and cells in the matrix. **(G)** SP stains densely distributed terminals in patches (white asterisk). In **(A)**, scale bar = 1 mm and applies to **B**. Scale bar in **C** = 100 μm and applies to **(D)**. Scale bar in **E** = 100 μm and applies to **(F,G)**. Amg, amygdala, Cd, caudate, CL, claustrum, GP, globus pallidus, OT, optic tract, Put, putamen.

approach toward novel, non-threatening, stimuli while the CNF initiates avoidance in response to stimuli that represent danger (Westby et al., 1990).

In contrast to what would be expected from their common name, most species of tree shrews (family Tupaiidae) are terrestrial and forage within 1.5 m of the ground (Kawamichi and Kawamichi, 1979; Langham, 1982; Emmons, 2000). Predators vary depending on local region, but most always include mammalian carnivores and birds of prey. From her field studies, Emmons (2000) noted that tree shrews may appear indifferent to threats from below, but almost always emitted strong alarm signals to perceived threats from above. With these aspects of their behavior in mind, our previous findings of the projections of the visual field on the SC are relevant. Specifically, we found that the tree shrew SC projects topographically to the Pd and Pc, with the medial SC (upper visual field) projecting to the Pd, and the lateral SC (lower visual field) projecting to the Pc ("specific" projections, schematically illustrated on the left side of **Figure 6**). The Pd receives additional non-topographic convergent input from the entire SC ("diffuse" projections, schematically illustrated on the right side of **Figure 6**). We suggested that specific tectopulvinar projections could function in coordinating precise movements to capture insects or fruit, while the diffuse tectopulvinar projections may mediate rapid responses to visual stimuli that would activate large regions of the visual field which would be considered threatening to tree shrews.

Our current results, which indicate that both the Pd and Pc project to the caudate and putamen (red arrows, left side of **Figure 6**) while the Pd, but not the Pc, projects to the amygdala (black arrow, right side of **Figure 6**) further support our proposed division of function of the tecto-pulvinar pathways through the Pd and Pc. Projections from the Pd and Pc to the GABAergic output neurons of the striatum could initiate inhibition of GABAergic nigrotectal cells which densely innervate PDB cells (Bickford and Hall, 1992). Disinhibition of PDB cells has been well established

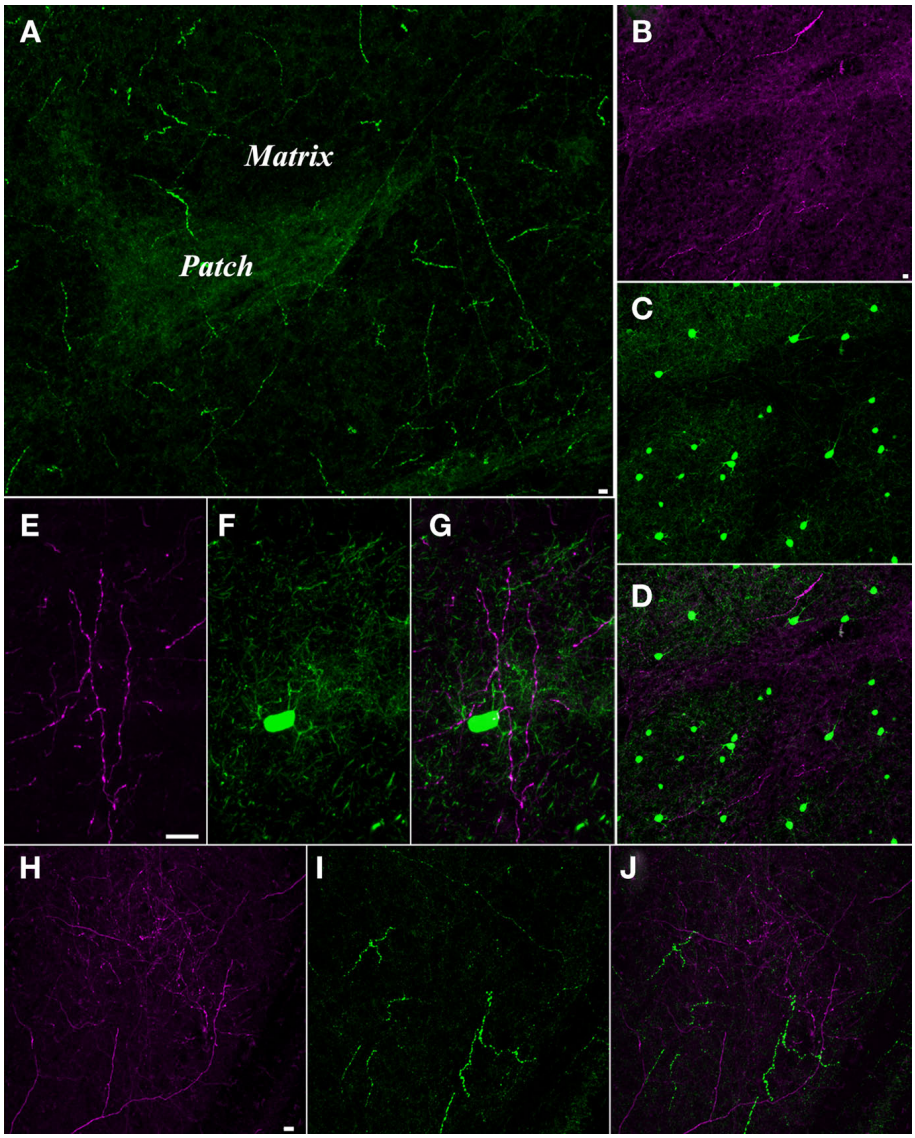

FIGURE 4 | Pulvinar axons innervate the striatum matrix. (A) Confocal microscope image of substance P (SP, green) staining in the putamen. The matrix is sparsely innervated by thick SP axons, while the patches are densely innervated by fine SP axons. **(B–D)** Confocal image of a section stained for SP and parvalbumin (PV). The fine SP axons (purple, **B**) that innervate the patches interdigitate with a dense distribution of cells in the matrix that contain PV **(C)**. **(D)** Merged image of **(B)** and **(C)** illustrates the interlocking arrangement of the SP-rich patches and the PV-rich matrix. **(E–G)** Pulvino-striatal axons innervate the PV-rich matrix. **E** illustrates BDA-labeled pulvino-striatal axons (purple) in the putamen. **(F)** shows a PV stained cell and dendrites (green) in the same section. **(G)** Merged image of **(E)** and **(F)** shows that pulvino-striatal axons target the PV-rich matrix. **(H–J)** Pulvino-striatal axons innervate the SP-poor matrix. **(H)** shows BDA-labeled pulvino-strital axons (purple) in the putamen. **(I)** shows SP staining (green) in the same section. Only the sparse SP axons of the matrix are stained. **(J)** A merged image of **(H)** and **(I)** illustrates that pulvino-striatal axons innervate the SP-poor matrix. All scale bars = 10 μm. Scale bar in **(B)** also applies to **(C,D)**. Scale bar in **(E)** also applies to **(F,G)**. Scale bar in **(H)** also applies to **(I,J)**.

as a primary mechanism in the initiation of saccades and orienting movements, and the importance of SC-basal ganglia loops in this process has been clearly demonstrated (Hikosaka and Wurtz, 1983, 1985, 1989; Wurtz and Hikosaka, 1986; McHaffie et al., 2006; Schulz et al., 2009).

The Pd projections to the lateral amygdala (activated by diffuse tectopulvinar projections and/or specific projections from regions of the SC representing the upper visual field) could function to signal potential danger. A wide variety of studies have indicated that the amygdala is activated by threatening visual images (e.g., Morris et al., 2001; Vuilleumier et al., 2003; Liddell et al., 2005). As indicated on right side of **Figure 6**, the lateral amygdala (La) projects to both the striatum and the central amygdala (Ce, Amorapanth et al., 2000; LeDoux, 2007). The Ce projects densely to the dopaminergic substantia nigra pars compacta (Gonzales and Chesselet, 1990; Fudge and Haber, 2000) which may serve to amplify or inhibit subsequent motor responses, dependent on their context (Amorapanth et al., 2000; Dommett et al., 2005; Redgrave and Gurney, 2006; Cohen and Castro-Alamancos, 2010).

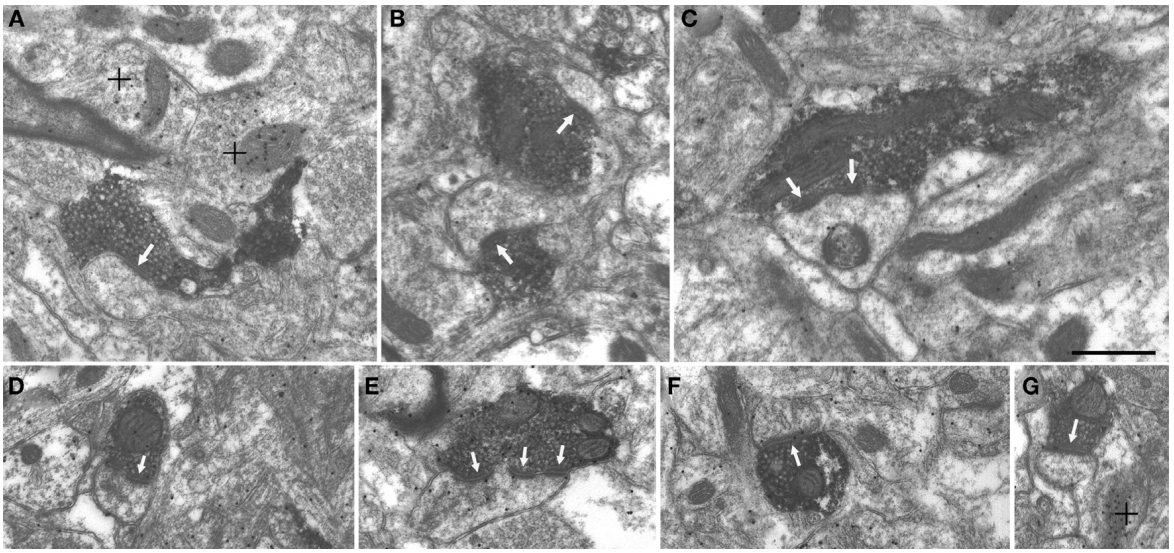

FIGURE 5 | Pulvino-striatal and pulvino-amygdala terminals contact spines. Terminals labeled by the anterograde transport of BDA injected in the pulvinar nucleus contact (white arrows) spines in the putamen **(A–C)** and the amygdala **(D–G)**. Single **(A,B,D,F,G)** perforated **(C)** and multiple **(E)** contacts were observed. Postembedding immunocytochemical staining for GABA revealed surrounding GABAergic terminals (high density of gold particles; + denotes) but BDA-labeled terminals and postsynaptic spines contained a low density of gold particles. Scale bar = 0.5 μm and applies to all panels.

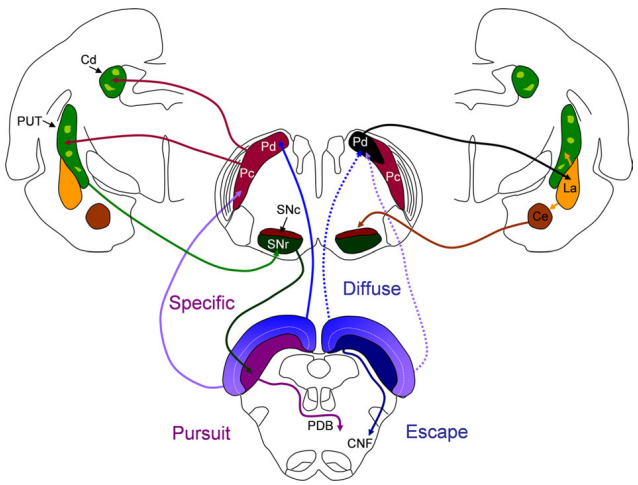

FIGURE 6 | The potential influence of dorsal (Pd) and central (Pc) pulvinar projections on basal ganglia circuits and behavior initiated by the superior colliculus (SC). Left side of figure: the Pd and Pc receive topographically arranged inputs from the SC ("specific") and project to spiny output cells in the matrix (dark green) of the caudate (Cd) and putamen (PUT). GABAergic spiny cells of the Cd and PUT project to the substantia nigra pars reticulate (SNr) and GABAergic nigrotectal cells densely innervate the cells of origin of the crossed predorsal bundle (PDB). Tecto-pulvino-striatal projections could initiate pursuit movements mediated by the PDB. Right side of figure: the Pd receives additional non-topographic, convergent projections from the SC ("diffuse") and projects to spiny output cells of the lateral amygdala (La). The La projects to the PUT and central amygdala (Ce). The Ce projects to the dopaminergic substantia nigra pars compacata (SNc). Tecto-pulvino-amygdala projections could serve to amplify escape movements mediated by the ipsilateral cuneiform (CNF) pathway. See text for details.

VISUAL CAPABILITIES IN THE ABSENCE OF V1: RELATIONSHIP TO TECTO-PULVINAR PROJECTIONS

The proportion of the visual thalamus innervated by the superficial (retinorecipient) layers of the SC varies with species. In nocturnal rodents, which rely primarily on somatosensory signals to navigate their environment, a relatively small region of the lateral posterior nucleus (LPN) receives input from the superficial SC and these projections are non-topographic (Mooney et al., 1984; Masterson et al., 2009). In contrast, in diurnal squirrels and tree shrews, fast moving species which rely heavily on visual information to navigate their habitats, the SC is proportionally larger and the superficial layers innervate relatively large regions of the pulvinar nucleus in a topographic manner (Robson and Hall, 1976, 1977; Luppino et al., 1988; Harting et al., 1991; Van Hooser and Nelson, 2006; Chomsung et al., 2008). These differences in tectothalamic innervation patterns are also reflected in the density of projections from the visual thalamus to the striatum. In rats, the tectorecipient LPN contributes relatively sparse input to the striatum (Doron and Ledoux, 1999; Cheatwood et al., 2003, 2005; Kamishina et al., 2008), while the tectorecipient pulvinar nucleus of the squirrel (Lin et al., 1984) and tree shrew projects densely to the striatum.

The density of tecto-pulvinar connections in the tree shrew and squirrel is likely related to their surprisingly robust visual capabilities in the absence of V1. Snyder et al. (1969) and Diamond and Hall (1969) tested tree shrew and squirrel visual behavior after complete ablation of the striate cortex (with corresponding degeneration of the lateral geniculate nucleus) and discovered that both species exhibited relatively normal visual behavior. Their detailed studies of the tree shrew, and others that followed (e.g., Snyder et al., 1969; Ward and Masterton, 1970; Killackey and Diamond, 1971; Killackey et al., 1971; Ware et al., 1972, 1974),

revealed that tree shrews with V1 lesions were still able to make visual pattern discriminations, but that extension of the ablations to include the cortical targets of the pulvinar (i.e., temporal cortex) severely compromised the animals' visual ability and visual discrimination learning. Furthermore, ablations of the pulvinar targets alone (leaving striate areas intact) produced deficits in visual pattern discrimination and in visual "reversal learning" not seen following striate lesions. Because the temporal cortex lesions caused severe degeneration of the pulvinar nucleus, the pulvino-striatal and pulvino-amygdala projections were also likely affected in these experiments. Finally, lesions of the SC were found to compromise some color vision tasks in addition to producing deficits in pattern discrimination (Casagrande et al., 1972; Casagrande and Diamond, 1974). Taken together, these studies clearly demonstrate the significant role of the tecto-pulvinar system in many aspects of tree shrew vision.

In primates, V1 lesions produce a profound loss of visual perception, yet the phenomena of "blindsight" has revealed that even in humans, conscious perception of visual stimuli is not a prerequisite for the detection of visual stimuli and the initiation of reactive movements (Cowey, 2010). Although it is still unclear what pathways mediate blindsight, many studies suggest that projections from the pulvinar or dorsal lateral geniculate nucleus to the middle temporal cortex (area MT) are involved (Sincich et al., 2004; Berman and Wurtz, 2010; Lyon et al., 2010; Schmid et al., 2010). This idea remains controversial however, because V1 lesions severely diminish the responsiveness of area MT while the effects of SC lesions on area MT responses are relatively minor (Rodman et al., 1989, 1990; Girard et al., 1992; Kaas and Krubitzer, 1992; Collins et al., 2003, 2005). Furthermore, although inactivation of the pulvinar nucleus severely disrupts visually guided behavior (Wilke et al., 2010), it is still unclear whether this is primarily due to disruption of cortical or subcortical circuits. Our results raise the possibility that projections of the pulvinar nucleus to the striatum and amygdala contribute to the detection of visual signals and the initiation of appropriate behavioral responses, and may also play a role in blindsight.

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Inputs to the dorsal striatum of the mouse reflect the parallel circuit architecture of the forebrain

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The basal ganglia play a critical role in the regulation of voluntary action in vertebrates. Our understanding of the function of the basal ganglia relies heavily upon anatomical information, but continued progress will require an understanding of the specific functional roles played by diverse cell types and their connectivity. An increasing number of mouse lines allow extensive identification, characterization, and manipulation of specified cell types in the basal ganglia. Despite the promise of genetically modified mice for elucidating the functional roles of diverse cell types, there is relatively little anatomical data obtained directly in the mouse. Here we have characterized the retrograde labeling obtained from a series of tracer injections throughout the dorsal striatum of adult mice. We found systematic variations in input along both the medial–lateral and anterior–posterior neuraxes in close agreement with canonical features of basal ganglia anatomy in the rat. In addition to the canonical features we have provided experimental support for the importance of non-canonical inputs to the striatum from the raphe nuclei and the amygdala. To look for organization at a finer scale we have analyzed the correlation structure of labeling intensity across our entire dataset. Using this analysis we found substantial local heterogeneity within the large-scale order. From this analysis we conclude that individual striatal sites receive varied combinations of cortical and thalamic input from multiple functional areas, consistent with some earlier studies in the rat that have suggested the presence of a combinatorial map.

Keywords: striatum, retrograde, tracing, basal ganglia, mouse

INTRODUCTION

In the mammalian telencephalon four interconnected nuclei comprise the basal ganglia: the striatum, the globus pallidus (GP), the subthalamic nucleus (STN), and the substantia nigra (SN; Haber, 2003). Pathological or experimental disruptions of neuronal activity in the basal ganglia cause profound deficits in motor control and learning throughout the chordate phylum, from amphibians to primates (Reiner et al., 1998). The vast majority of excitatory projections enter the basal ganglia via the striatum (Tepper et al., 2007). As a result, the circuitry of the striatum in vertebrates has been the subject of intense study since the pioneering application of techniques for tract tracing by Nauta and colleagues in the middle of the twentieth century (Paxinos, 2004).

It was observed in the earliest tract tracing studies that axons from subregions and lamina of the neocortex were inhomogeneously distributed in the dorsal striatum of rats (Gerfen, 1984) or the caudate–putamen (CPu) of primates (Goldman and Nauta, 1977). Careful registration of labeling patterns across subjects and the development of techniques for simultaneous use of multiple labels provided clear evidence for a topographical organization of the corticostriatal projection (Malach and Graybiel, 1986; Gerfen, 1992). Input to the dorsal striatum follows the relative position of neurons within the neocortex such that projection neurons from more lateral and dorsal cortical structures projecting to more lateral and dorsal aspects of the striatum (Paxinos, 2004). In addition to the corticostriatal projection, it has also been demonstrated that the thalamostriatal and nigrostriatal projections are topographically organized (Haber, 2003; Paxinos, 2004; Haber and Calzavara, 2009). In addition, anterograde tracing has demonstrated that the topographic organization of the striatum is maintained in a more compact form at its output targets in the ventral midbrain (Tulloch et al., 1978; Deniau et al., 2007).

The profound effects of pathological disruptions have indicated a critical role for the processing of excitatory input within the striatum (Albin et al., 1989). The topographical organization of inputs to and outputs from the striatum has long played a central role in both theory and the interpretation of functional data in the basal ganglia (Ragsdale and Graybiel, 1990; Mink, 1996). The core functional property of the basal ganglia from this perspective is the processing of information in largely non-overlapping "loops" that connect functionally related regions of the cortex, basal ganglia, and thalamus (Graybiel et al., 1994; Haber, 2003). An important support for this model was a series of elegant studies in the monkey that revealed a convergence of functionally related, but anatomically distant, neocortical regions (Flaherty and Graybiel, 1991, 1995; Parthasarathy et al., 1992; Parthasarathy and Graybiel, 1997). However, it is not clear that the convergence of functionally related cortical regions is a general feature across vertebrates. For example, functional mapping studies (Brown, 1992; Brown and Sharp, 1995; Brown et al., 1996, 1998) and anatomical tracing experiments (Alloway et al., 2000, 2006; Hoffer and Alloway, 2001; Ramanathan et al., 2002; Hoffer et al., 2005) have revealed that

sensory and motor cortical regions project to diverse targets within the rodent striatum. While convergence is clearly present, it may be diminished compared to that detected in primates with <5–10% of overlap in functionally related somatosensory and motor corticostriatal projections (Hoffer and Alloway, 2001).

Although the extent and importance of functional convergence is unclear in rats, and unknown in mice, in the last several years it has become clear that anatomically distinct regions of the striatum have dissociable functional properties (Packard and Knowlton, 2002; Balleine et al., 2009 Redgrave, 2010 #256). It has been elegantly demonstrated in rats that a profound dissociation between goal-directed and habitual behavior could be mapped onto circuits in the dorsomedial (Yin et al., 2005a,b) and dorsolateral striatum (Yin et al., 2006), respectively. More recently, an analogous dissociation between the dorsomedial and dorsolateral striatum during motor learning has also been demonstrated in mice (Yin et al., 2009). These recent behavioral results have shed light on the critical and specific roles of the dorsal striatum in instrumental conditioning and motor control that complement and extend earlier ideas about roles of striatal subregions in modality specific learning (Graybiel et al., 1994; Packard and Knowlton, 2002). However, these results in rodents raise the question of whether a finer scale organization of specific cortical and thalamic inputs is present in addition to the large-scale separation of medial and lateral input patterns.

The above mentioned differences between the anatomy of the dorsal striatum in rats and primates highlights the need for careful anatomical studies in different species. Despite deep similarities in the anatomy of the basal ganglia in vertebrates, significant differences are nonetheless present (Reiner et al., 1998). At best, analogies between closely related species (such as the rat and the mouse) need to be transformed into precise anatomical localizations in each. At worst, the failure of such analogies has the potential to cause misinterpretation of functional data. Yet, despite the substantial literature on the vertebrate striatum, there have been relatively few anatomical studies of the macrocircuitry of the mouse (White and DeAmicis, 1977; Hersch and White, 1982; Porter and White, 1983; Bernardo and Woolsey, 1987; Mattiace et al., 1989; Hofstetter and Ehret, 1992; Cepeda et al., 2003; Miura et al., 2007; Brazhnik et al., 2008; Usunoff et al., 2009; Ibáñez-Sandoval et al., 2010; Tlamsa and Brumberg, 2010) and to our knowledge an examination across multiple topographic regions has not been previously conducted.

To further establish the relationship between defined anatomical circuits and behavior we suggest that a detailed accounting of the similarities and differences between the anatomy of the mouse striatum and other vertebrates will be of continued importance. As an initial attempt at creating a more comprehensive anatomical description of the basal ganglia in mice, here we analyze a series of injections of retrograde tracer throughout the dorsal striatum. We focus on the topographic organization of the dorsal striatum and its connectivity with both cortical and subcortical structures.

MATERIALS AND METHODS

PREPARATION AND STEREOTACTIC INJECTION OF FLUORESCENT LATEX MICROSPHERES

A total of 35 male mice aged 2–4 months were used. All work involving animals was approved by the Janelia Farm Institutional Animal Care and Use Committee (Protocol Number 08-36), and met the standards of the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) accredited program. Mice were housed in ventilated cage racks (Allentown, Inc), provided ad lib irradiated feed (Lab Diet 5053), reverse osmosis water chlorinated to 2–3 ppm, and were kept on a 12:12 light cycle. For the duration of the surgical exposure of the brain, implantation of cannulae, and intracranial injections mice were maintained at a surgical plane of anesthesia using isoflurane (1.5–2.5% in O_2). In several injections, after the mouse was deeply anesthetized and secured in the stereotaxis, guide cannulae spanning the depth of the cortex were implanted in order to minimize the leak of fluorescent beads during insertion of the injection needle into the brain. For guide cannulae we used one or two sterile, 30 gage (26 gage in some cases) stainless-steel guide cannula. After lowering the guide cannula to the appropriate depth dental cement was used to secure the cannulae to the skull.

Injections were targeted to a range of coordinates centered around (1) posterior, (–0.2 posterior, 2.5–2.8 lateral, 2.5–3.5 ventral; all units are mm), (2) medial anterior (+1.0 anterior, 1.2 lateral, 2.5–3.5 ventral), and (3) lateral anterior (+1.0 anterior, 2.5 lateral, 2.5–3.5 ventral) striatal targets. In some experiments injections were targeted to the motor (+1.0 anterior, 0.7 lateral, 0.4–0.7 ventral) and somatosensory cortical areas (–0.55 posterior, 3.0 lateral, 0.4–0.7 ventral). A fine needle (140 μm external diameter and 40 μm internal diameter) made by silica capillary tubing (VS-140-40, Scientific Glass Engineering) was used as injection needle, which was held by an attached stainless-steel collar. The tip of needle extended 1–2 mm beyond the tip of guide cannula. The needle was filled with red or green Lumafluor beads (Lumafluor beads, Lumafluor, Inc.) or a mixture of both and mated to a 1 μl Hamilton syringe with plastic tubing. Following implantation of the needle 80–100 nl of beads were infused into the target areas with the speed of 100 nl/min using a microinjection driver. The needle was left in place for 10 min before being removed from the brain.

PREPARATION AND STEREOTACTIC INJECTION OF VIRUSES

Stereotactic injection of viruses was accomplished using procedures equivalent to those described above for injection of retrograde tracer. The virus was a modified adeno-associated virus (serotype 2/1) engineered to drive the over expression of enhanced green fluorescent protein (eGFP). The sequence for the expression construct and details of viral production are described in more detail in subsequent manuscript (Mao et al., in preparation). Injection volumes for virus were 20–40 nl per injection site with 1–2 sites per target region.

HISTOLOGY

In initial experiments we found that similar results were obtained after allowing axonal transport of the retrograde tracer for as few as 3 days or as many as 14 days after injection. For the experiments reported here, 3–7 days following tracer injection and 1–3 weeks following viral infection, mice were deeply anesthetized under constant inhalation of isoflurane (>2% in O_2) and transcardially perfused with 4% paraformaldehyde in phosphate buffered saline (0.1 M, pH = 7.4). The brains were removed and left in a 4% paraformaldehyde solution for 1–7 days for the injection of retrograde tracers or 24 h for viral infection. Each brain was sectioned on a

vibrating microtome at 50–100 μm. The sections were mounted onto slides and allowed to air dry. Preliminary examination of labeling was accomplished using a dissecting microscope equipped with filter sets for epifluorescence detection (Olympus MVX10). Images were obtained using acquisition with a digital camera or in a small number of cases using a Zeiss LSM710 confocal microscope.

ANALYSIS

For mapping of the injection sites and cell labeling areas, the photos of slices were checked through. The injection and labeling sites were determined according to a standard stereotaxic atlas (Paxinos and Franklin, 2004). To do quantitative analyses, an ideal method is to count the percentage of all labeled cells in a given brain region area. However, this analysis is complicated by a challenge in defining a brain region as well as the challenge of producing high quality stereological counts of all cells in a brain area. Finally, we find that the signal-to-noise of labeled cells can vary widely in some regions (especially the neocortex) and thus, the definition of a positive labeled cell is not rigorous. We also found that it was impossible to accurately define the exact extent or magnitude of the injection site. Thus, here we settled upon a semi-quantitative method to quantify labeling in a given brain region. For a particular labeled region, the relative intensity of labeling of an area was defined by counting the numbers of labeled somata. The intensity marker "+" was give for the numbers of labeling cells were counted as 1–10, "++" for 11–20, "+++" for 21–30, and "++++" for more than 31.

Tabulated results from manual annotation and counting were analyzed using custom written routines in Igor Pro (Wavemetrics, Eugene, OR, USA) and Matlab (Mathworks, Natick, MA, USA). Correlation and covariance analysis was accomplished using standard Matlab functions applied to our annotated database of all labeling intensity. All plotting and curve fitting was accomplished using custom scripts written for Igor Pro.

RESULTS

Retrograde tracers were injected under stereotaxic control into the dorsal striatum of mice using volume displacement (**Figure 1**). Our injection sites were distributed throughout the dorsal striatum with particular focus on the less studied posterior striatum. In preliminary experiments we tested both the injection of latex microspheres adsorbed with fluorescent small molecules (Lumafluor beads, Lumafluor, Inc.) and high molecular weight dextrans conjugated with fluorescent small molecules (10,000 MW, Invitrogen). For a given injection of tracer, labeled somata were found in relatively extensive regions of the neocortex, and more focal regions of the thalamus and midbrain as early as 2 days following injection into the dorsal striatum. We found that latex microspheres ("beads") gave more reliable labeling with improved signal-to-noise and thus all of the data reported here will focus on our injections using beads. We found qualitatively similar results using both tracers, however, a detailed comparison was not conducted.

QUALITATIVE DESCRIPTION OF LABELED SOMATA

As expected, the majority of labeled somata were found in the neocortex, discrete nuclei in the thalamus, and nuclei of the ventral midbrain reflecting the corticostriatal, thalamostriatal, and nigrostriatal pathways, respectively (**Figure 1**). The complete distribution of labeled somata, an indication of the maximal cell count, and the frequency of detection (see Materials and Methods for details) from all of the injections of fluorescent beads examined are collected in **Figure 2A** and **Table 1**. All abbreviations follow the conventions of the standard mouse brain atlas (Paxinos and Franklin, 2004). Consistent with canonical descriptions of the striatal circuitry, we found these three pathways to have core features that are shared between primates (Haber, 2003) and rodents (Paxinos, 2004). One, labeled somata were found in deep layers of a wide distribution of frontal and parietal neocortex (**Figures 1 and 3**). Two, the intralaminar nuclei of the thalamus were a major source of neurons projecting to the striatum (**Figure 4**). Three, the dopaminergic input to the dorsal striatum originated from neurons with somata located predominantly in the substantia nigra *pars compacta* (SNc), but also found in the ventral tegmental area (VTA) and the retrorubral field of the caudal midbrain (A8; **Figure 5**).

In our dataset we regularly observed more intensely labeled and larger numbers of cortical neurons in the ipsilateral hemisphere as compared to the contralateral hemisphere. Additionally, posterior and lateral injections regularly yielded less contralateral labeling than more anterior and medial injections. As a result the quantification of labeled subregions of cortex and their quantification were derived entirely from ipsilateral projections. While labeled cortical somata were confined almost exclusively to deep layers, there were substantial apparent differences in the distribution of labeled somata between frontal cortices, temporal cortices, and sensory areas of parietal cortices. In well-defined six-layer neocortex labeled somata were clearly confined to distributions of cells throughout the entire extent of layer V and often concentrated in both a narrow superficial band consistent with layer Va and deeper layers (**Figures 1 and 3**). However, in frontal and temporal cortices labeled neurons appeared distributed throughout a wider range of cortical layers extending from the border of layer III to layer VI (**Figure 3**); reminiscent of the broad laminar distribution corticostriatal neurons identified with anterograde tracing in the frontal cortex of the rat (Gerfen, 1989).

In the thalamus we reliably observed retrograde labeling of neurons not only in the parafascicular (PF) and centromedian (CM) intralaminar nuclei (Smith et al., 2004), but also within sensory nuclei located more laterally such as the posterior (PO), ethmoid (Eth), medial (MG), and lateral geniculate (LG) nuclei (**Figure 4**). Across the entire collection of retrogradely labeled somata we found that individual injection sites have both idiosyncratic and focal distributions of somata in the thalamus (**Table 1**; **Figures 2 and 4**).

Injection sites throughout dorsal striatum were associated with intensely labeled somata in the basolateral (BLA) and central (CEA) nuclei of the amygdala (**Figure 6**). Our labeling was consistent with early studies in which injection of tritiated amino acids into the CEA and BLA revealed labeled axons across a large extent of the dorsal striatum in the rat (Kelley et al., 1982; Price, 1986). In addition to an amygdalostriatal projection, there has been considerable interest in the interaction between the extended amygdala and striatum. Although to our knowledge there have been no prior report of projections from the extended amygdala to the dorsal

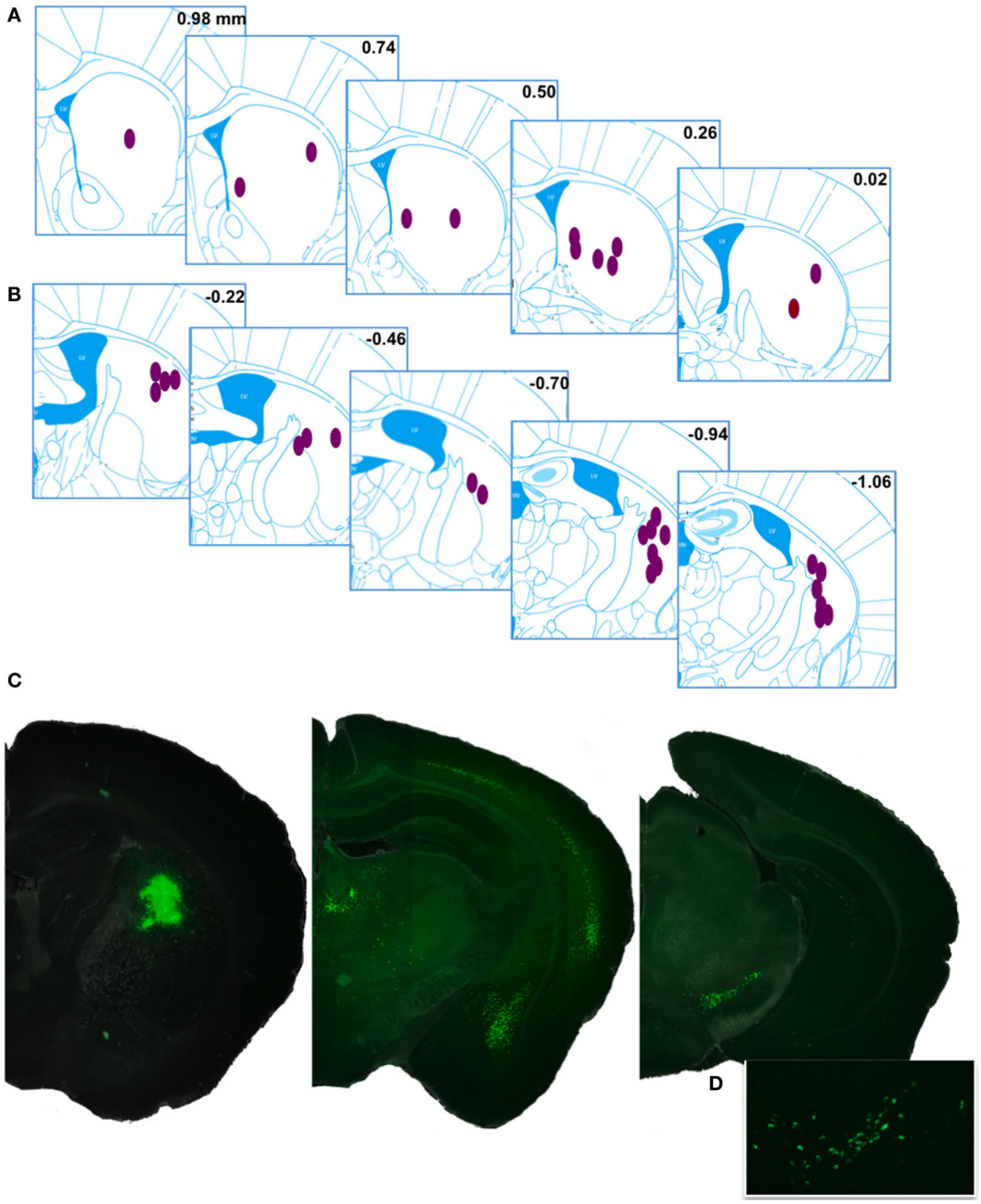

FIGURE 1 | Injection sites and representative retrograde labeling. (A,B) The approximate central points for reported injections are indicated by ellipses. Inset numbers are position (mm) of the coronal plane relative to bregma, Anterior and posterior injection sites are displayed in **(A,B)**, respectively. Histological references are modified from Paxinos and Franklin, 2004. **(C)** An example injection site with major planes of labeled somata. **(D)** Higher magnification fluorescence image showing the typical punctuate labeling in the ventral midbrain.

striatum we found clear labeling of somata in the interstitial nucleus of the posterior limb of the anterior commissure (IPAC) at many anterior injection sites (**Figure 6**). Finally, there have been a series of previous studies focused on the projection from midline brainstem nuclei to the striatum (Di Matteo et al., 2008). Consistent with these results we found clear labeling of large somata in medial and dorsal raphe nuclei of the brainstem, a putative serotonergic projection to the dorsal striatum (**Figure 7**).

ANTEROGRADE TRACING CONFIRMS A NON-CANONICAL PROJECTION

Although retrograde labeling of more lateral thalamic nuclei such as PO was previously observed in the rat (Erro et al., 2002; Alloway et al., 2006), we were nonetheless concerned that such labeling could result from leakage of some tracer in the neocortex during the insertion of the injection needle. Moreover, as described above, we also observed retrograde labeling in areas of the amygdala and midbrain that, while these sites have also been observed

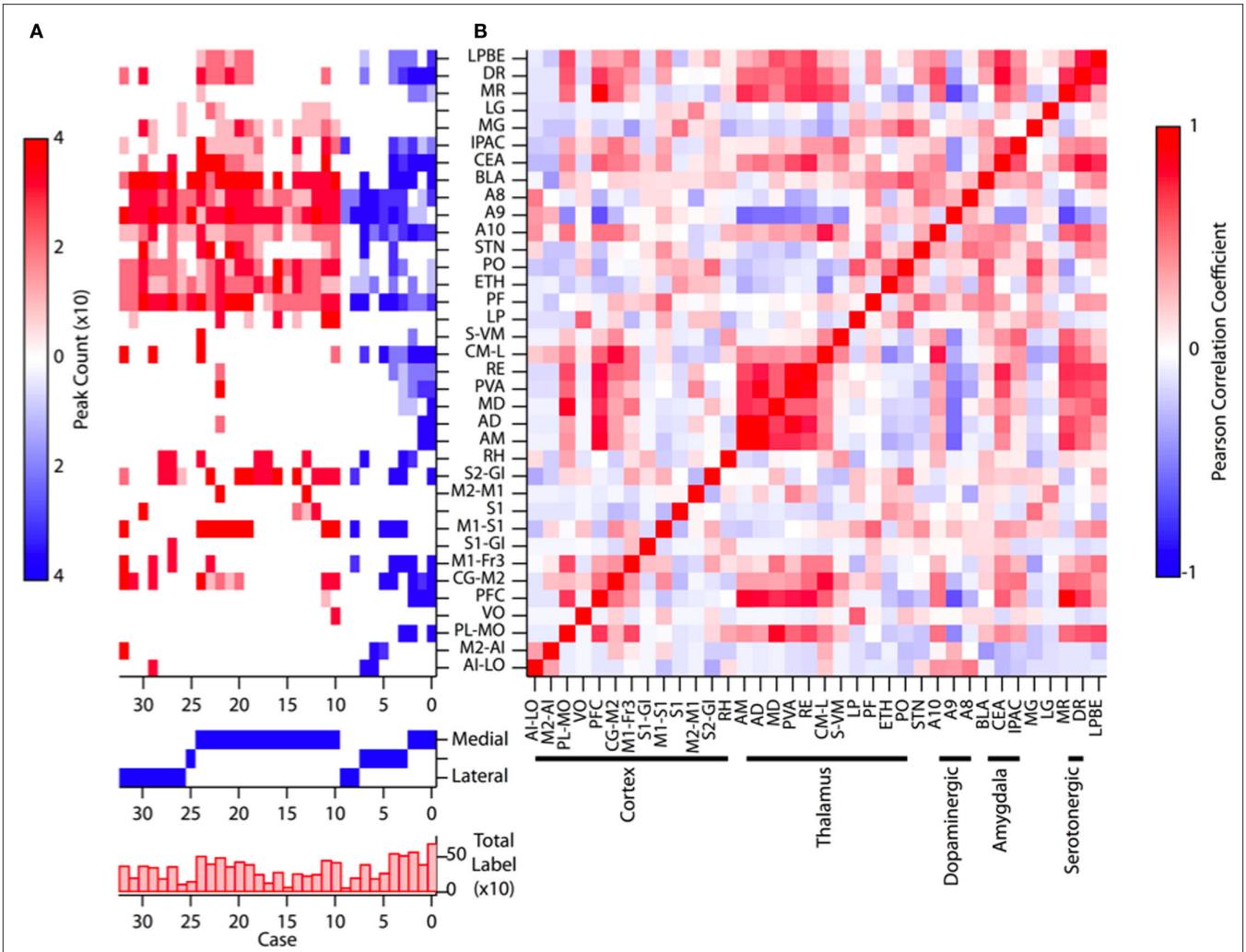

FIGURE 2 | Summary of all injections of retrograde tracer [(A), upper] Plot of labeling intensity for each injection site ("case") with peak count represented by the color scale at left. Labels for each manually annotated brain area (after Paxinos and Franklin, 2004) are indicated along the y axis. Cases plotted in blue are the most anterior injections and cases plotted in red are derived from the more posterior injections. [**(A)**, middle] The approximate medial to lateral position of the injection in each case is indicated by a solid blue square. [**(A)**, lower] The sum of labeling across all brain areas is plotted for each case. **(B)** The complete pairwise correlation matrix for all injections and all annotated brain areas. Annotated brain areas are grouped according to their principal features along the x axis. For select correlation values see text. In this and subsequent figures brain region annotations follow the conventions of Paxinos and Franklin, 2004.

previously, were less commonly reported in the literature. We took two steps to ensure the accuracy of our labeling. One, we found that implanting cannulae spanning the neocortex and largely prevented leakage of tracer during insertion and did not change our observed retrograde labeling in the thalamus and cortex (an example is shown in **Figure 3C**). Two, we sought to directly confirm the thalamostriatal projection from the more lateral nuclei using anterograde tracing.

To visualize the projection from the PO nucleus of the thalamus we infected neurons with an adeno-associated virus driving the expression of the gene encoding enhanced green fluorescent protein (GFP). Two weeks after infection expression of the GFP was sufficient to detect both somata localized to the thalamus as well as clear axonal projections coursing through the internal capsule and terminating in the cortex (**Figure 8**). As expected, the vast majority of axons from the posterior and ventral posterior thalamus formed a dense columnar plexus of axonal ramifications in layer IV of the neocortex (**Figure 8B**). However, with careful examination we found that axon collaterals from the thalamus exited the internal capsule and ramified in a band in the posterior dorsolateral striatum (**Figures 8C,D**) consistent with our retrograde labeling. Axons observed in the striatum appeared both branched and lined with varicosities consistent with the formation of local synaptic contacts (**Figures 8D,E**). The massive labeling of fibers terminating in the cortex compared with those in the striatum are consistent with sparse retrograde labeling we observed (**Figure 4**) and with the suggestion that only a subpopulation of neurons in lateral, posterior thalamic nuclei project to the striatum (Erro et al., 1999, 2001; Barroso-Chinea et al., 2008).

Table 1 | Summary of retrograde labeling results.

Labeling sites	Anterior–medial injections ($n = 4$)	Anterior–lateral injections ($n = 8$)	Posterior injections ($n = 21$)
FOREBRAIN			
PrL-MO	++++(2/4)	++++(1/8)	–(0/21)
LO-AI	–(0/4)	++++(2/8)	–(0/21)
PrL-IL	++++(3/4)	–(0/8)	–(0/21)
Cg1-M2	++++(4/4)	++++(2/8)	–(0/21)
M1-Fr3	++++(2/4)	++++(3/8)	+++(4/21)
M1-S1	++++(1/4)	++++(4/8)	++++(8/21)
S2-GI	++++(1/4)	++++(3/8)	+++(13/21)
EcT-PRh	++++(1/4)	++++(3/8)	++(4/21)
IPAC	+++(4/4)	+++(3/8)	+++(11/21)
AMYGDALA			
CEA	++++(4/4)	++++(2/8)	++(10/21)
BLA	++++(2/4)	++++(3/8)	++++(18/21)
THALAMUS			
AM	++++(2/4)	–(0/8)	–(0/21)
AD-IAD	++++(2/4)	–(0/8)	+(1/21)
MD	+++(2/4)	+(1/8)	–(0/21)
PVA	+++(3/4)	+(1/8)	+(1/21)
RE	++(3/4)	+(1/8)	–(0/21)
CM-CL	++++(4/4)	++++(4/8)	+++(3/21)
AM-AV	+++(1/4)	–(0/8)	–(0/21)
Sub-VM	+++(2/4)	–(0/8)	–(0/21)
LP	–(0/4)	++(1/8)	++(8/21)
PF	+++(4/4)	++++(6/8)	+++(20/21)
PO	+(2/4)	++(3/8)	+++(20/21)
Eth	+(4/4)	+(4/8)	++(19/21)
MG	–(0/4)	–(0/8)	+(14/21)
LG	–(0/4)	–(0/8)	+(6/21)
MIDBRAIN			
A10	+++(4/4)	+++(6/8)	++(19/21)
A9	+(2/4)	++++(8/8)	+++(21/21)
A8	+++(2/4)	+++(8/8)	++(20/21)
BRAIN STEM			
DRV	++++(4/4)	+++(2/8)	++(7/21)
MnR	++(4/4)	–(0/8)	–(0/21)
LPBE	++(3/4)	++(3/8)	++(5/21)

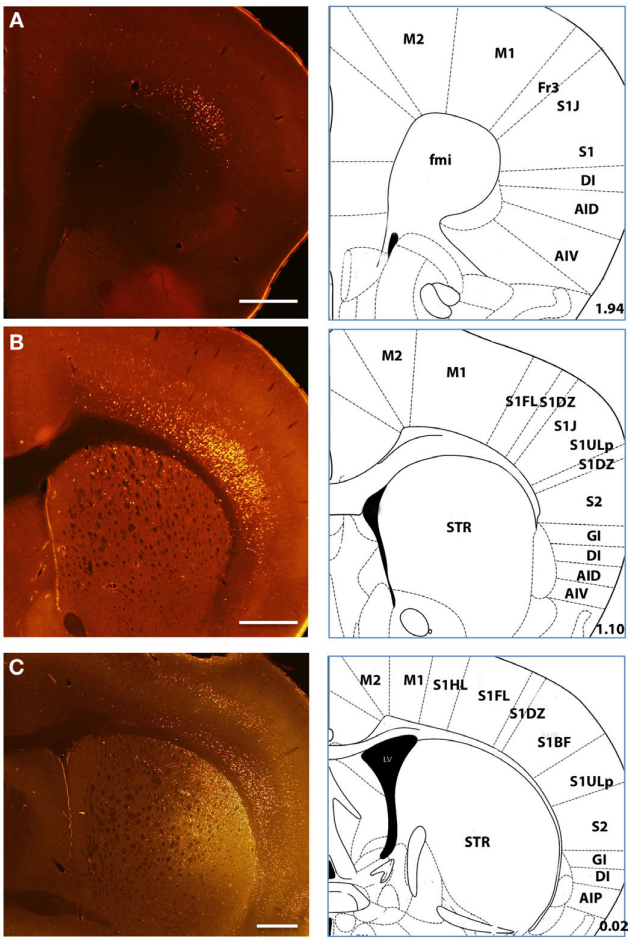

FIGURE 3 | Example labeling of deep layer cortical neurons Labeled neurons following retrograde tracer injections into the dorsal striatum showed labeling distributed over a range of sensory and motor cortical regions (A–C). Intense labeling of both superficial and deep layer V neurons was typical in many injections as shown here. **(C)** An example injection at a more posterior site with clear superficial and deep labeling of cortical neurons. Histological references are modified from Paxinos and Franklin, 2004 with coordinates relative to bregma shown (mm). Scale bars: 0.5 mm **(A–C)**.

ORGANIZATION OF STRIATAL INPUTS IDENTIFIED BY RETROGRADE LABELING

Manual inspection of the annotated database of labeled cells revealed clear tendencies in the retrograde labeling data that were partially described above. In addition, when individual injections were sorted according to the relative anterior–posterior position (blue and red coloring in **Figure 2A**, respectively) and secondarily sorted by their relative medial–lateral position (lower panel, **Figure 2A**) of injection site, clustering of the retrograde labeling was apparent. Previous work has demonstrated both a segregation of afferent inputs according to relative position along the medial–lateral neuraxis as well as a convergence of functionally related, anatomically distant brain regions. While this has most often been examined with paired injections of tracers and inspection of the resulting tissue as we have done here, we note that this model of connectivity also predicts that there should be a well-defined covariance structure in the afferent inputs to the striatum. To examine whether there was evidence of such correlations in our dataset we computed the pairwise correlations between annotated brain regions for all injection sites (**Figure 2B**).

As expected, there is a large distribution of correlation scores with the majority of correlations being weak and non-significant (**Figure A1** of Appendix). Nonetheless, comparison of the observed correlation values with the expected distribution from a random pattern reveals that our observed distribution is significantly shifted toward positive correlations (**Figure A1B** of Appendix; $p < 0.0001$).

Examination of the distribution of significant correlations (**Figure A1A** of Appendix) revealed both significant positive correlations between specific brain areas and larger trends in the overall

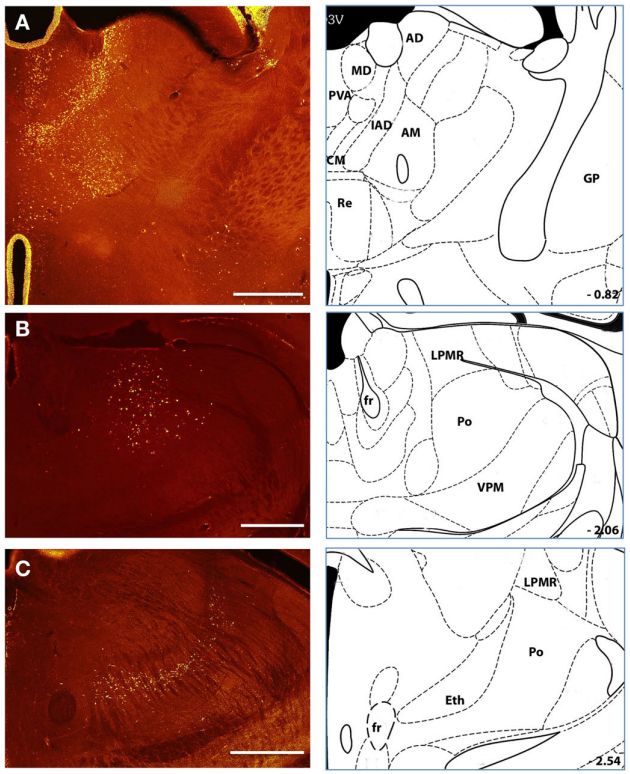

FIGURE 4 | Major sources of thalamostriatal projection Labeled neurons following retrograde tracer injections into the anterior (A) and posterior (B,C) aspect of the dorsal striatum. Anterior injection sites were associated with labeled cells throughout a wide range of thalamic nuclei **(A)** including the PAV. MD. CM, IAD. AD, Re. AM., and LPMR (see text for details). Posterior injection sites predominantly labeled PO and Eth nuclei and PF nuclei at more medial injection sites (not shown). Histological references are modified from Paxinos and Franklin, 2004 with coordinates relative to bregma shown (mm). Scale bars: 0.5 mm.

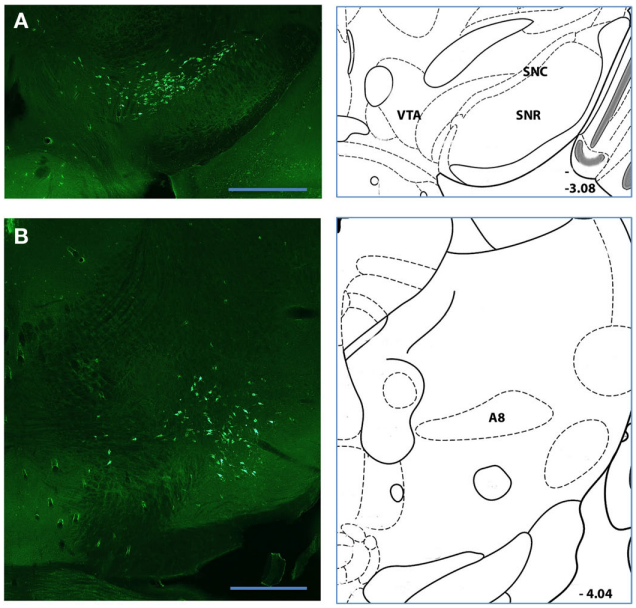

FIGURE 5 | Major sources of nigrostriatal projection (A,B) Labeled neurons following retrograde tracer injections into a posterior position of the dorsal striatum. Pronounced and intense somatic labeling was detected in putative dopaminergic neurons of the SNc **(A)** and retrorubral nucleus [A8; **(C)**]. Histological references are modified from Paxinos and Franklin. 2004 with coordinates relative to bregma shown (mm). Scale bars: 0.5 mm.

distribution of labeled areas. For example, we found that the PO nucleus of the thalamus and the border of the secondary somatosensory cortex (S2-GI) were highly correlated ($r^2 = 0.49$), whereas the PF nucleus of the thalamus was correlated with labeling in the primary somatosensory cortex (S1; $r^2 = 0.50$). The medial dorsal nucleus of the thalamus (MD) was likewise correlated with frontal and motor cortical areas (M1-Fr3; $r^2 = 0.48$). In addition to the correlations between thalamostriatal and corticostriatal projections we also found significant correlations between nigrostriatal and corticostriatal projections. For example, labeling intensity in A10 was significantly correlated with labeling of the prefrontal cortex (PFC; $r^2 = 0.46$), whereas A9 labeling was anticorrelated ($r^2 = -0.65$).

The above correlations suggested the presence of potentially important functional convergence across striatal input pathways. However, careful examination of the labeled areas and correlations indicated that the convergence of inputs is related to their position along the anterior–posterior and medial–lateral neuraxes in addition to their putative functional overlap. For example, the more medial A10 was correlated strongly with medial cortical and thalamic nuclei, whereas the more lateral A9 was anti-correlated with those structures. The more lateral PO was likewise correlated more strongly with the more lateral secondary somatosensory cortex whereas the more medial PF was more strongly correlated with the more medio-dorsal primary somatosensory cortex. In addition, the distinctions along the anterior–posterior axis were particularly profound. The more anterior thalamic nuclei (e.g., AM) were strongly correlated with more frontal cortical regions (e.g., PFC; $r^2 = 0.80$) and anti-correlated with posterior thalamic nuclei and parietal cortical regions. Further, the BLA correlated strongly with a posterior localized thalamic nucleus, PO ($r^2 = 0.57$), by contrast to the CEA which was highly correlated with a more anterior thalamic nucleus, RE ($r^2 = 0.68$).

To evaluate the relative contribution of position within the neuraxis upon the structure of the correlations between labeled brain areas we calculated the eigen decomposition of the covariance matrix (**Figure A2** of Appendix). Plotting the eigenvalues revealed, as expected, that there are many components of the covariance structure necessary to account for the dataset (**Figure A2A** of Appendix). Nonetheless, we find that the first two eigenvalues were significantly separated from the rest of the distribution and accounted for 41.6% of the variance. The corresponding eigenvectors should reflect the relative weighting given to individual brain areas necessary to generate the observed covariance. So we asked whether the weightings of the dominant eigenvectors reflected the position of labeled nuclei along the neuraxis. For clarity we used the estimated centroid position of our annotations of thalamic nuclei. We found that indeed the weightings in the first two eigenvectors are proportional to the position of the thalamic nuclei along both the anterior–posterior and medial–lateral neuraxes (**Figures A2B,C**

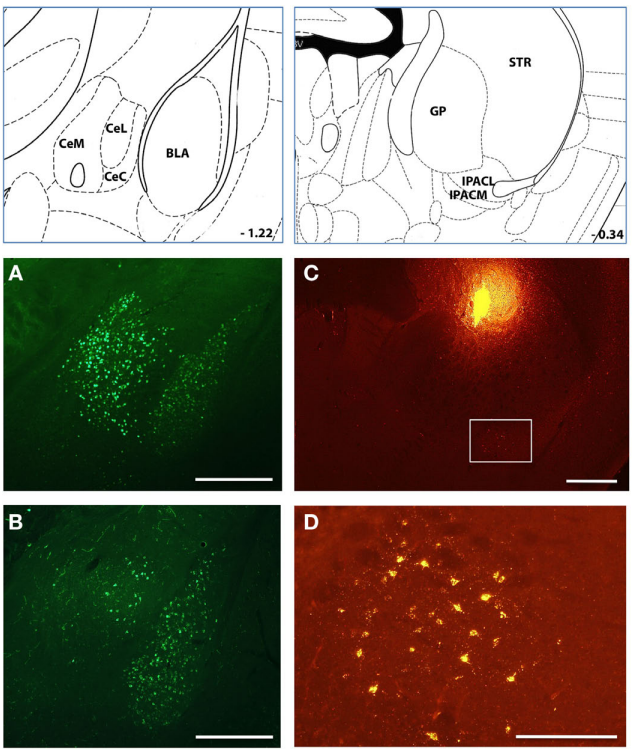

FIGURE 6 | A prominent amygdalostriatal projection in mice Injections of retrograde tracer into many sites throughout the dorsomedial striatum resulted in substantial labeling of cells in the amygdala [both central and basolateral nuclei; (A,B)] and the extended amygdala [IPAC; **(C)**]. **(D)** Inset of from c is shown in the image in **(D)**. Histological references are modified from Paxinos and Franklin, 2004 with coordinates relative to bregma shown (mm). Scale bars; 0.5 mm **(A–C)** 0,2 mm **(D)**.

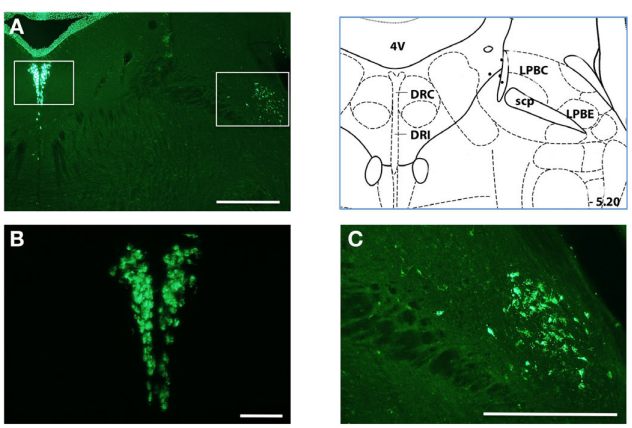

FIGURE 7 | Projections from the dorsal brainstem to the dorsal striatum (A) Injections of retrograde tracer produced less prominent but readable labeling of neurons in the dorsal brainstem. Left inset is region shown in **(B)**; Right inset is region shown in **(C)**. **(B)** inset from panel a shows putative serotonergic neurons in the dorsal raphe. Neurons of the medial raphe were also commonly labeled (not shown). More lateral nuclei were also occasionally observed. **(C)** intense labeling of the LP6E is shown in the inset from panel **(A)**. Histological references are modified from Paxinos and Franklin, 2004 with coordinates relative to bregma shown (mm). Scale bars: 0.5 mm **(A,C)** 0.2 mm **(B)**.

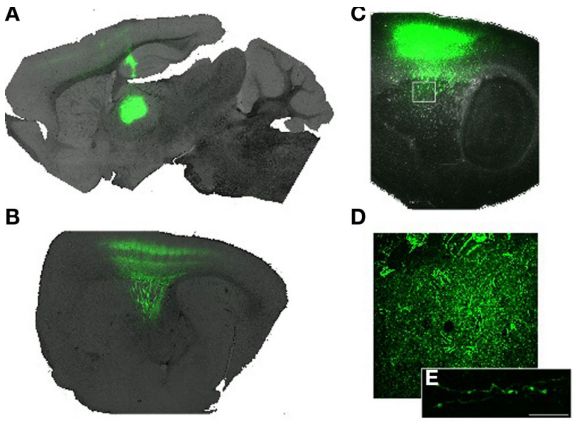

FIGURE 8 | Anterograde tracing from posterior thalamic nuclei (A) Parasagittal plane (~1.5 mm lateral of bregma) showing the infection site in the posterior and lateral region of the thalamus. **(B)** A more lateral (~2.6 mm lateral) parasagittal plane showing extensive projection of thalamocortical axons into primary somatosensory cortex. **(C)** At the lateral extreme of the striatum (~3 mm lateral) careful examination of the image reveals extensive ramification of axons in the striatum. Box indicates region magnified in **(D)**. **(D)** Magnified image showing diffuse and tortuous labeled axons. **(E)** Confocal image showing two axons studded with varicosities. Scale bar 10 m.

of Appendix), consistent with the claim that position along these axes was the most important determinant of their postsynaptic targets in the dorsal striatum.

Organization of inputs along the medial–lateral axis of the dorsal striatum

As described above, our analysis of the pairwise correlations amongst afferent input structures to the dorsal striatum indicated that position along the medial–lateral axis and the anterior–posterior axis were important determinants of their postsynaptic targets in the striatum. However, even though we selected fluorescent beads for their useful property minimal diffusion away from the injection site and uptake by actively cycling axon terminals (Vercelli et al., 2000), it is still difficult to precisely identify the site of injection and to rule out a biased diffusion or in homogeneous uptake of tracer across injections. Thus, to confirm our inferences from the correlation structure of all cases, we examined injections in which beads with distinct fluorophores were targeted to positions separated along the medial–lateral axis in the same subject.

From simultaneous injection of two tracers we found that the medial to lateral separation of inputs is maintained throughout the neuraxis. In the frontal cortex we found the infralimbic cortex was labeled clearly from the medial injection site whereas agranular motor cortex was labeled from the lateral injection site (**Figure 9A**). The separation was particularly clear in the ventral midbrain. Whereas nearly overlapping injection sites with two different fluorescent beads labeled intermingled (and largely non-overlapping) sets of dopaminergic neurons, injection sites with a significant medial to lateral separation labeled non-overlapping collections of midbrain dopaminergic neurons (**Figure 9C**). Retrograde labeling in the thalamus also showed a robust medial to lateral separation and a clear anterior to posterior separation (**Figure 9B**).

Organization of inputs along the anterior–posterior axis of the dorsal striatum

We were particularly interested in the posterior striatum because it has been relatively less studied and as we have confirmed in mice, is known to receive extensive projections from sensory regions of cortex and the sensory thalamus (**Figure 2**). As noted above, examination of the correlations between retrogradely labeled input structures suggested a surprisingly clear separation between anterior and posterior striatal injection sites. The divergence of labeling we detected between anterior and posterior injection sites was surprising based upon studies in other mammals. In the mouse as in many mammals, the sensory and motor areas of the neocortex are segregated along the anterior–posterior axis. This is also the case in the primate, but an elegant series of studies demonstrated a robust convergence of functionally related sensory and motor structures in the striatum despite this separation along the anterior–posterior axis (Ragsdale and Graybiel, 1990; Parthasarathy et al., 1992; Flaherty and Graybiel, 1995; Parthasarathy and Graybiel, 1997). In the rat sensory and motor projections to the striatum are only partially overlapping (Hoffer and Alloway, 2001; Alloway et al., 2006) even though sensory and motor areas at the same position in the anterior–posterior axis may converge more substantially (Ramanathan et al., 2002). Thus, we sought to examine the projections from the well characterized sensory and motor cortex of the vibrissa system in the mouse.

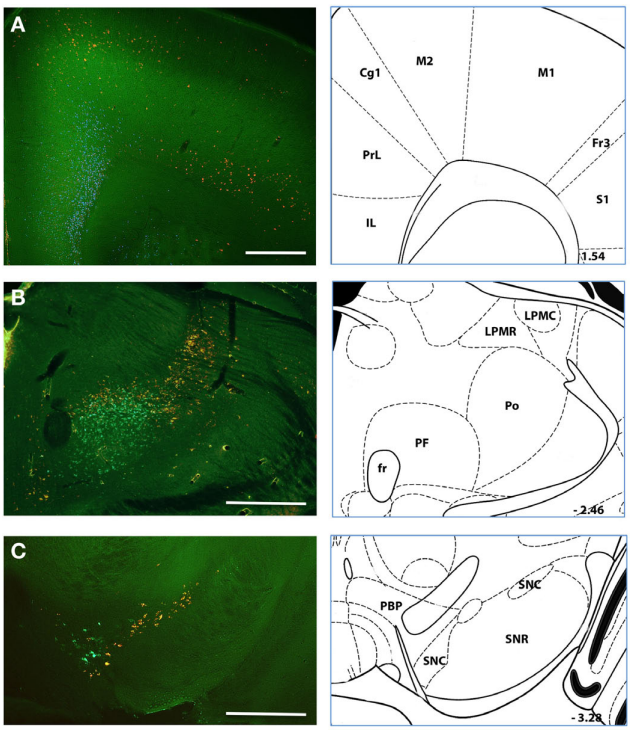

FIGURE 9 | Simultaneous injection of medial and lateral striatum Representative case in which injections of retrograde tracer were made in both a medial (green) and lateral (red) position in the dorsal striatum. Labeling in **(A–C)** shows the organization of labeled somata along the neuraxis from frontal cortices **(A)** to thalamus **(B)** to midbrain **(C)**. Histological references are modified from Paxinos and Franklin, 2004 with coordinates relative to bregma shown (mm). Scale bars: 0.5 mm.

The primary motor and primary somatosensory areas of the vibrissa system were labeled by infection with a virus driving the over expression of fluorescent protein. Injections into somatosensory cortex led to a focal projection into the dorsolateral extreme of the posterior striatum (**Figure 10B**). By contrast, injections into primary motor cortex revealed a substantial arborization of axons in the anterior striatum and an axonal projection extending along the posterior axis (**Figure 10A**). While this extension along the posterior axis indeed partially overlapped with sensory axons as seen in rat (Hoffer and Alloway, 2001), the majority of the projection was several hundred microns more anterior to the sensory axons and largely non-overlapping. This observation was consistent with our retrograde labeling (**Figure 2**). Further, we found more prominent ipsilateral projections as opposed to contralateral projections, especially following infection of the somatosensory cortex that was again consistent with retrograde tracing.

DISCUSSION

We performed a series of injections of retrograde tracer into a range of positions within the dorsal striatum of the mouse. As the mouse becomes an increasingly popular model organism for

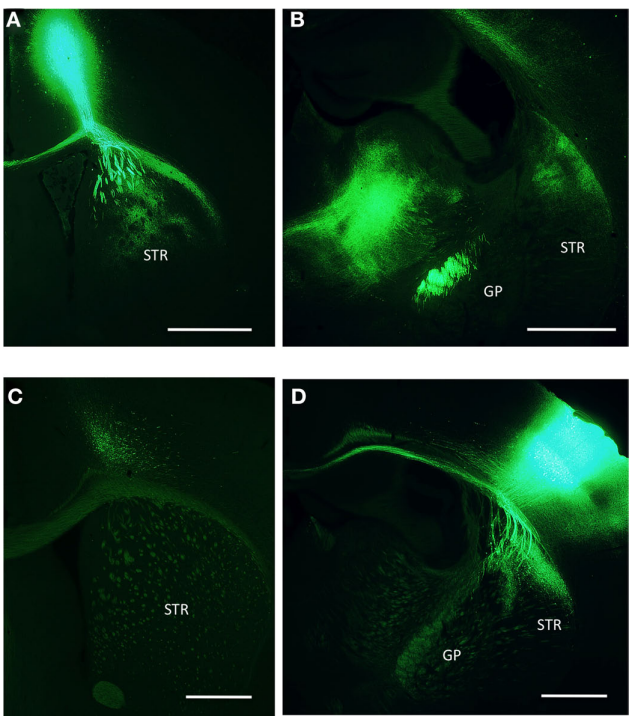

FIGURE 10 | Anterograde tracing from M1 and S1 cortices (A) Infection site in the primary motor cortex for the vibrissa. Descending axons ramify both locally within the same coronal plane of the striatum as well as fibers running caudally along the outer edge of the dorsal striatum. **(B)** Axons from the primary motor cortex are visible at a posterior coronal plane terminating densely in the thalamus and the dorsolateral striatum. **(C)** Image taken from the same anterior coronal plane as a following infection of the primary somatosensory cortex. **(D)** Posterior plane shows infection site in the primary somatosensory cortex and local projection of axons into the dorsolateral striatum. GP, globus pallidas; STR, striatum. Scale bars: 1 mm.

neuroscientists, and a tractable model for the exploration of diseases of the basal ganglia, we found it increasingly important to confirm the homology between neuroanatomy in the mouse and the wealth of anatomical data in the rat. As a preliminary move in this direction we have begun to characterize the distribution of inputs to the dorsal striatum. The dorsal striatum is a massive structure receiving converging inputs from regions distributed through the telencephalon and diencephalon. As a consequence we have necessarily provided only a partial sampling of the potential loci for injection of retrograde tracers. However, we have applied an analysis of retrograde labeling using the covariance of labeling across multiple experiments that to our knowledge had not been applied to such data previously and may prove to be a useful approach for future studies (**Figures 2, A1 and A2** of Appendix). Our analysis provided suggestive evidence for the convergence of projections from specific nuclei across the nigro-, cortico-, thalamo-, and amygdalo- striatal pathways. We also have provided some quantitative evidence that the position of an afferent structure along the anterior–posterior and medial–lateral neuraxes is the principle determinant of the termination field within the dorsal striatum, consistent with data in the rat (Paxinos, 2004). These observations from analysis of our dataset were confirmed by both dual injections of retrograde tracer (**Figure 9**) and anterograde tracing (**Figure 10**). Finally, we have provided supporting evidence for projections from the amygdala and raphe nuclei to dorsal striatum that were first identified in rat, but have received less attention in the years since their discovery.

THE THALAMOSTRIATAL PROJECTION

As expected, and described above, we find that there are deep similarities between the extensive literature on the canonical striatal inputs from the thalamus, ventral midbrain dopamine neurons and deep layer cortical neurons in the rat and our data in mice. Here we have provided some refinement of the details and interpretation of these projections when compared with the canonical descriptions. For example, in the thalamostriatal projection we found that nuclei outside of the intralaminar nuclei of the thalamus appear to make a significant contribution to the projection into the posterior striatum. This observation was discovered by retrograde labeling of PO nuclei of the thalamus, controlled for by the implantation of cannulae to prevent leakage along the injection tract, and confirmed through anterograde tracing following infection of the posterior and ventral posterior thalamic nuclei. The PO nucleus receives primary sensory afferents from brainstem nuclei (Paxinos, 2004) and the Eth nucleus additionally receives feedback from the motor cortex via zona incerta (Barthó et al., 2002). This strongly suggests that in the mouse and the rat the thalamostriatal projection, like the corticostriatal projection, carries both primary sensory and motor information in addition to associative information.

THE NIGROSTRIATAL PROJECTION

We found that the nigrostriatal projection is topographically organized along a medial–lateral axis and includes projections from throughout the anterior–posterior extent (extending from areas A8–A10) of the dopaminergic cell bodies in the ventral midbrain. This medial–lateral organization was associated with the surprising observation that a significant component of the dorsal nigrostriatal projection originated from somata located at the anteromedial surface of the SNr – a position consistent with the localization of the VTA (**Figure 5**). We also consistently observed large labeled somata in the midbrain that was dorsal and posterior to the SNc, consistent with the retrorubral field (A8; **Figure 5**). Notably absent, however, were "displaced" dopamine neurons within or along the ventral surface of the SNr (Gerfen et al., 1987; Prensa and Parent, 2001). Prior work demonstrated that displaced dopamine neurons form a specific projection to very dorsal and lateral regions of the striatum (Gerfen et al., 1987) that may have been under sampled in our injections.

Based upon retrograde and anterograde labeling in the primate it has been previously suggested that dopaminergic neurons may play a critical role in transferring information between the medial and lateral striatum (Haber et al., 2000; Haber, 2003). Although future experiments will be required to determine whether such a "spiral" structure is present in the rodent brain (Yin et al., 2008; Belin et al., 2009), our data are consistent with a topographical organization that could subserve a similar function.

THE CORTICOSTRIATAL PATHWAY

Finally, by using injection sites distributed over a range of sites along the anterior–posterior and medial–lateral axes of the dorsal striatum we were able to confirm the medial–lateral gradient of corticostriatal projections first elucidated in the rat and primate. The most parsimonious account of our data is that the topography of striatal inputs predominantly maintains the topographic organization of the neocortex and thalamus. Consistent with this interpretation we also find that there is a distinct anterior–posterior gradient present in both the thalamostriatal and corticostriatal projections. Although it has received less attention as an important axis of functional differentiation, recent results suggest that the anterior and posterior striatum have dissociable behavioral effects (Tricomi et al., 2009; Shiflett et al., 2010). Indeed, our analysis of the correlation between labeled thalamic nuclei suggests that the anterior–posterior axis may be a more significant axis of differentiation in the dorsal striatum than the medial–lateral axis (**Figure A2** of Appendix).

Whereas in the monkey there appears to be strong convergence of functionally related, but topographically distant areas in the corticostriatal projection, our results suggest that for the majority of axons in the corticostriatal projection these topographic separations are maintained in the mouse. This observation is consistent with the distribution of the projection from the vibrissa primary motor and vibrissa primary sensory cortex in the rat where less than 5% overlap in axonal projections has been observed (Hoffer and Alloway, 2001). However, even though the overlap of motor and sensory axons was an apparent minority of the labeling, the zones of overlap may be disproportionately important to the function of the dorsolateral striatum in the rat (Brown and Sharp, 1995), as has been proposed in the primate (Graybiel et al., 1994; Haber, 2003; Cohen and Frank, 2009; Haber and Calzavara, 2009). In rodents, an assessment of the functional importance of convergence and divergence in the corticostriatal and thalamostriatal projections is likely to be an important area of study for experiments in the future.

NON-CANONICAL STRIATAL PROJECTIONS

In addition to these core features of the macrocircuitry of the striatum that were common across a wide range of vertebrates (Reiner et al., 1998; Tepper et al., 2007), our injections also identified

a collection of nuclei and brain regions that have been previously observed, but are not commonly included in the canonical basal ganglia circuit. Most strikingly, we found strong labeling of the amygdala, the extended amygdala, and the raphe nuclei. The projection of serotonergic neurons to the dorsal striatum has received some attention (Di Matteo et al., 2008), especially interesting due to its proposed role in mediating opponent learning (Daw et al., 2002; Doya, 2008). However, while projections from the amygdala to the ventral striatum have indeed received quite a lot of attention (Gray, 1999; Cardinal et al., 2002; Floresco et al., 2008; Belin et al., 2009; Humphries and Prescott, 2010), the projections to the dorsal striatum that were observed with anterograde tracing in the rat (Kelley et al., 1982), have received relatively less attention. To our surprise, in the mouse we regularly detected significant labeling of the amygdala following injections across a range of different positions within the dorsal striatum – with the notable exception of the most anterior and lateral injections (**Figure 2**). A previous study in the rat also demonstrated that the only territory where amygdala fibers were absent was in the rostrolateral striatum, consistent with our results (Kelley et al., 1982). The importance of the amygdalostriatal projection into dorsal striatum was further suggested by the observation that it is an evolutionarily ancient pathway (Martínez-García et al., 2002) – present at least as far back as reptiles (Novejarque et al., 2004).

We suggest that the prominent amygdalostriatal projection to the dorsomedial striatum should be given some attention and perhaps inclusion in the canonical circuitry of the basal ganglia. Indeed, it has recently been demonstrated that activity in the amygdala is highly correlated with learning and reflects both value and error prediction signals that are tightly correlated with learning (Paton et al., 2006; Belova et al., 2008). Given the critical requirement of the dorsal striatum in the acquisition and expression of goal-directed instrumental conditioning and the central role that error prediction signals are thought to play in such learning (Schultz, 2006), the amygdalostriatal projection may be an important component of an extended learning circuit involving the hippocampus, dorsal striatum, and amygdala (McDonald et al., 2007).

LIMITATIONS OF OUR DATASET

We selected fluorescent latex microspheres because of their lack of diffusion away from the injection site and high signal-to-noise ratio compared to other approaches (Vercelli et al., 2000). Nonetheless, it is currently not possible to accurately estimate the number of beads injected or differences in the efficiency of uptake by the surrounding axons. Our analysis of the covariance of labeling is more susceptible to another problem: full sampling of a structure as large as the dorsal striatum is impractical. While we have necessarily under sampled the complete distribution of inputs to the striatum, two features of our study are important. One, there was a lack of prior datasets covering multiple striatal subregions in the mouse. Two, our analysis provides a general method that could be applied to future datasets or accommodate additional data and more complete coverage of the dorsal striatum.

Medium spiny neurons contain upwards of 10,000 synapses in the rat, with approximately a quarter of all synapses being symmetrical synapses (Wilson, 2007). Single cell labeling studies and paired recordings have indicated that many of the symmetrical synapses found on MSNs arise from local GABAergic interneurons that have extensive, locally ramifying axonal arborizations (Tepper et al., 2008). In addition, elegant single cell labeling studies have discovered a feedback pathway from the GP to the striatum (Bevan et al., 1998). Neither of these projections was reported in our summary data. For local connectivity we regularly observed labeled neurons locally within the dorsal striatum (e.g., **Figure 3C**), however, it was unclear how to quantify this labeling or if one could confidently distinguish direct labeling due to pressure injection, leakage into the underlying GP, or uptake by local axon collaterals. Thus, we have left these projections out of our analysis. By contrast, with a single exception we did not detect labeling of neurons in the GP in our injections except in cases where the injection site may have had minor overlap with the GP. It remains to be seen in future work whether the absence of a detectable pallidostriatal projection was an artifact of our technique or reflects an actual difference between rats and mice in the organization of the basal ganglia.

FUNCTIONAL ARCHITECTURE OF THE MOUSE STRIATUM

Both our study and many previous studies in the rat (Paxinos, 2004) have provided evidence for a well-defined topographic segregation along the medial–lateral and anterior–posterior axis. However, this observation of a segregation of pathways has also raised questions about how activity in anatomically distinct regions of cortex, thalamus, and striatum are coordinated during learning. Perhaps the nigrostriatal pathway mediates this integration (Haber, 2003) via thalamocortical loops (Haber and Calzavara, 2009). A complimentary possibility is that extensive intracortical projections may play an important role in rodents. A less studied possibility is that local inhibitory interactions, present throughout the basal ganglia (Tepper et al., 2007) and prominent in the striatum (Wilson, 2007), play a critical role in coordinating activity during learning. Our examination of the correlation structure of inputs to the striatum suggests a diverse collection of neuronal groups with idiosyncratic collections of afferents. These diverse target areas, as has been occasionally noted in the literature (Brown, 1992), may allow for a combinatorial map of cortical output in the striatum. Thus, local inhibitory interactions may be sufficient to mediate interactions between and across multiple sensory and motor modalities, rather than or in addition to interactions within corticothalamic loops.

Our study of the striatum in mouse has provided a helpful step in the goal of combining the power of a genetic model organism and circuit anatomy in the basal ganglia. Future work will be required to both further complete a description of the macrocircuitry of the mouse as well as to enhance the specific description of the microstructure within the corticostriatal and thalamostriatal projections. Indeed, an exciting prospect for the future is the use of tracing techniques for studies of macrocircuitry in combination with the use of optical and genetic approaches for the elucidation of microcircuitry with cellular resolution.

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APPENDIX

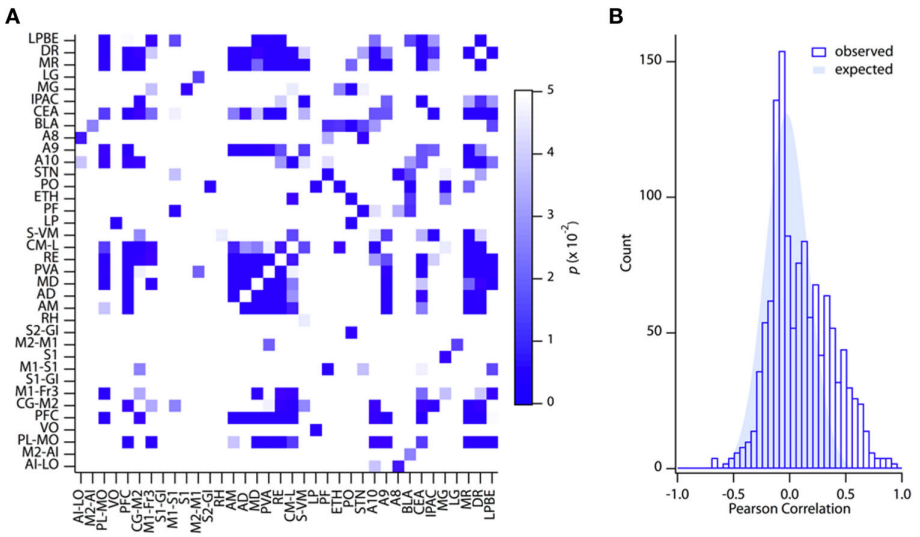

FIGURE A1 | Distribution and significance of pairwise correlations (A) Plot of estimated significance for each pairwise correlation value. For details about the plot please see corresponding plot in Figure 2 in the main text. **(B)** The observed distribution of all pairwise correlations is shown in open bars. Solid fill is a gaussian fit to the expected distribution of correlation scores for a random, equivalently scaled dataset.

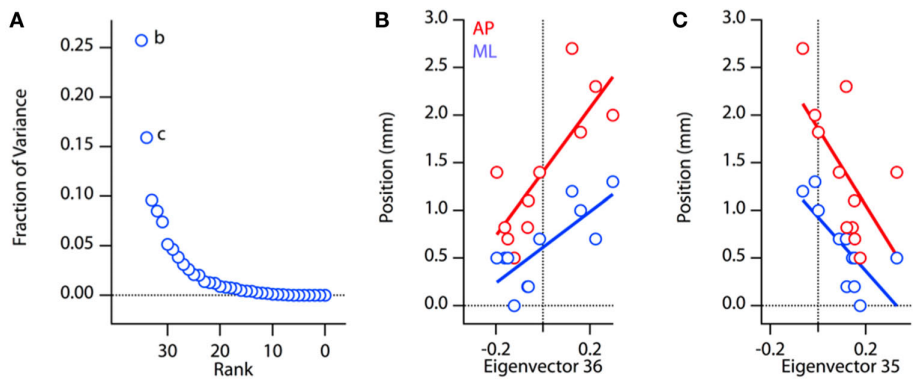

FIGURE A2 | Principal components analysis of retrograde labeling data (A) Rank ordered eigenvalues of the covariance matrix derived from retrograde labeling data in **Figures 2 and Figure A1. (B,C)** Correlation between the anterior–posterior (AP, red) and medial–lateral (ML, blue) position of annotated thalamic nuclei and the first **(B)** and second **(C)** eigenvectors (components) resulting from decomposition of the covariance matrix. Solid lines are best linear fits to the data.

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What is the degree of segregation between striatonigral and striatopallidal projections?

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In contrast to most other brain regions, in the striatum the output neurons (the medium-sized spiny neurons, MSNs) are GABAergic and act by inhibiting their targets. The standard model of the basal ganglia is built on the segregation of information processing in the direct and indirect pathways, which act in opposing directions to control movement. The MSNs participating in these two pathways can be identified according to their projection sites and the proteins they express. The differential expression of two of the five known dopamine receptor subtypes, D1 and D2, in the two populations of MSNs is of particular importance, since it confers to dopamine the ability to exert opposite functional modulation on the direct and indirect pathways. However, beyond this simple view of the striatal output organization, anatomical studies questioned the segregation of direct and indirect projections to the SNr, while other studies disclosed variable degrees of overlapping expression of dopamine receptor subtypes in striatal MSNs. New ways to address these issues have emerged recently, using mouse models in which specific populations of striatal neurons are genetically tagged. Here, we review classical and recent studies supporting the segregation of striatonigral and striatopallidal neurons. We also consider this issue at a functional level by focusing on the regulation of striatal signaling pathways in the two populations of MSNs, which clearly emphasize their profound differences. We discuss the anatomical and functional evidence challenging some aspects of this segregation and outline questions that are still to be addressed.

Keywords: striatum, medium-sized spiny neurons, dopamine receptors, signaling pathways

THE DISTINCTION BETWEEN THE TWO EFFERENT PATHWAYS OF THE STRIATUM

The basal ganglia consist of several interconnected subcortical nuclei involved in adaptive control of behavior through interactions with sensorimotor, motivational, and cognitive brain areas. The striatum is the primary input nucleus of the basal ganglia circuits and is essential for the initiation and selection of actions, as well as for the learning of habits and skills (Graybiel et al., 1994; Mink, 1996; Nicola, 2007). Its ventral extension, the nucleus accumbens (NAc), is involved in motivation and reward (see Belin et al., 2009 for a recent review). Despite the existence of different functional territories, the striatal tissue appears homogeneous and is characterized by the absence of intrinsic glutamatergic neurons. Indeed, most of the striatal neurons (~95% in rodents) are GABAergic medium-sized spiny neurons (MSNs), which coexist with GABAergic interneurons and large aspiny cholinergic interneurons (comprising altogether ~5% of the striatal neurons) (Kawaguchi, 1997; Bolam et al., 2000; Tepper and Bolam, 2004). Although they appear as a fairly uniform neuronal population, MSNs can be distinguished according to their projection targets and the selective expression of different neuropeptides and receptors. MSNs projecting monosynaptically to the medial globus pallidus (MGP) and the substantia nigra pars reticulata (SNr) form the "direct" striatonigral pathway, whereas those projecting to the LGP participate in the "indirect" pathway (Penney and Young, 1983; Alexander and Crutcher, 1990). In this latter case, the SNr is reached through successive synaptic relays in the LGP (Parent et al., 1984) and the subthalamic nucleus (Kanazawa et al., 1977; Hammond et al., 1978). Various markers were identified in one or the other of these groups of neurons. Striatonigral neurons are selectively enriched in substance P and dynorphin whereas striatopallidal neurons contain enkephalin (Chesselet and Graybiel, 1983; Beckstead and Kersey, 1985). It was also clear that dopamine (DA) D1 receptors (D1R) and D2 receptors (D2R) were preferentially distributed in striatonigral and striatopallidal neurons, respectively (Beckstead, 1988; Gerfen et al., 1990). In addition, M4 muscarinic acetycholine receptors were enriched in striatonigral neurons (Harrison et al., 1996) and A2a adenosine (A2aR) receptors in striatopallidal neurons (Schiffmann et al., 1991; Fink et al., 1992). It was later suggested that these two populations of neurons received different types of cortical inputs (Lei et al., 2004). Thus, to a large extent a dichotomy between the striatonigral and striatopallidal neurons was clearly apparent and played a major heuristic role in the understanding of basal ganglia dysfunction (Gerfen, 1992). Another fascinating level of heterogeneity of the striatum is the existence of two compartments, the striosomes or patches, and the matrix, which have specific cortical inputs and different anatomical connections (Graybiel and Ragsdale, 1978; Gerfen, 1984), although their functional role is still unclear. Remarkably, the degree of segregation of the two populations of MSNs has been continuously questioned. The variable degree of overlap between the expression

of D1R and D2R in the MSNs reported in the literature is a good example of this controversy. The anatomo-functional organization of striatal projections is a critical question, since it underlies the computing capabilities of the basal ganglia circuits and should be taken into account in any model of basal ganglia function. Here, we review classical and current neuroanatomical and functional studies in favor of the selective distribution of D1R and D2R in striatonigral and striatopallidal MSNs, respectively. We describe how the regulation of striatal signaling pathways supports a striking functional segregation between the two populations of projection neurons. We also discuss the evidence challenging some aspects of this segregation.

IS THE SEGREGATION BETWEEN STRIATONIGRAL AND STRIATOPALLIDAL MSNs MATCHED BY THE EXPRESSION OF DOPAMINE RECEPTORS?

The striatum is strongly modulated by dopaminergic afferences arising from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) (Anden et al., 1964; Beckstead et al., 1979). Within the striatum, the postsynaptic effects of dopamine (DA) are mediated mainly by stimulation of D1 and D2 types of DA receptors (D1R and D2R) (Kebabian and Calne, 1979; Stoof and Kebabian, 1984) localized in MSNs (Hersch et al., 1995; Yung et al., 1995). The other DA receptors are less abundant in the striatum: the D3 receptor is mostly expressed in the ventral striatum (Diaz et al., 1995), the D5 receptor expression is low and widespread (Rivera et al., 2002), and the D4 receptor expression is not detected (Noaín et al., 2006). The hypothesis of a segregated expression of D1R and D2R in the distinct populations of striatal projection neurons was initially suggested by indirect evidence (Beckstead, 1988) and subsequently shown by retrograde tracing (Gerfen et al., 1990). Moreover, the loss of DA innervation caused by 6-hydroxydopamine (6-OHDA) increased the expression of mRNAs encoding the D2R and enkephalin selectively in striatopallidal MSNs, whereas mRNAs encoding the D1R and substance P were only decreased in striatonigral MSNs (Gerfen et al., 1990). These results were supported by several anatomical studies using *in situ* hybridization (ISH) combined with retrograde axonal tracing or double ISH, in which the authors confirmed that D1Rs were expressed in substance P-positive striatonigral MSNs, while D2Rs were enriched in enkephalin-expressing striatopallidal neurons, with a small percentage of neurons co-expressing both receptors (Gerfen et al., 1990; Le Moine et al., 1991; Le Moine and Bloch, 1995). Studies using retrogradely transported toxins also confirmed the preferential expression of D1R in striatonigral neurons (Harrison et al., 1990; Hervé et al., 1993). A functional dichotomy between striatonigral and striatopallidal neurons was highlighted by studies of immediate early genes (IEGs) induction following pharmacological stimulation or blockade of D1Rs and D2Rs. D2R antagonists induced a rapid expression of a variety of genes selectively in striatopallidal neurons (Dragunow et al., 1990; Robertson et al., 1992), whereas administration of D1R or indirect DA receptor agonists such as cocaine or amphetamine preferentially induced IEGs in striatonigral MSNs (Cenci et al., 1992; Cole et al., 1992; Robertson et al., 1992; Steiner and Gerfen, 1993). All these studies largely validated the opposition between the striatonigral and striatopallidal pathways. This model was later supported by electrophysiological evidence (reviewed in Surmeier et al., 2007). Depending on the nature of synaptic glutamate release (sustained vs. uncoordinated or transient), stimulation of D1Rs led to a different effect, an increase or a reduction, in the responsiveness of striatonigral MSNs, respectively. On the other hand, stimulation of D2Rs reduced striatopallidal MSNs excitability and their response to glutamatergic synaptic input. However, at the same time as the evidence for the segregation of the striatopallidal and striatonigral pathways was accumulating, other results argued against this hypothesis (see **Anatomical Evidence Against the Segregation Between the Striatonigral and Striatopallidal Projections and Segregated D1R and D2R Expression in Striatonigral and Striatopallidal Neurons: Still Some Open Issues,** below for discussion of this evidence).

BAC TRANSGENIC MICE FACILITATE THE IDENTIFICATION OF DISTINCT POPULATIONS OF MSNs

The recent development of bacterial artificial chromosome (BAC) transgenic mice provides the possibility to genetically tag various populations of striatal neurons (Gong et al., 2003; Heintz et al., 2006). These new mouse lines, in which the enhanced green fluorescent protein (EGFP) is driven by D1R or D2R promoters, provide an easy way to label neurons which express high levels of D1R or D2R (reviewed in Valjent et al., 2009). In mice carrying BAC *drd1a*-EGFP or *drd1a*-dtTomato (a red fluorescent protein), striatonigral MSNs and their axonal projections to the MGP and SNr are labeled, whereas striatopallidal MSNs projecting exclusively to the LGP are stained in BAC *drd2*-EGFP mice (Gong et al., 2003; Lobo et al., 2006; Bertran-Gonzalez et al., 2008; Shuen et al., 2008; Matamales et al., 2009). The same type of approach has been applied to other proteins, including BAC-driven expression of Cre, which targets the recombinase to specific neuronal subtypes (Gong et al., 2007). The specific expression of Cre allows targeted deletion or expression of genes of interest in specific striatal neuronal populations (Monory et al., 2007; Durieux et al., 2009; Schaefer et al., 2009; Bateup et al., 2010). It should be noted that some differences may arise between populations of cells labeled with BAC-driven fluorescent proteins in the adult and those in which labeling results from Cre action occurring earlier in development. Careful comparison of the labeling patterns will be necessary to determine whether such differences can be an issue in the striatum.

Observations in BAC transgenic mice confirmed the simple model of striatal output organization initially proposed. At the striatal level, co-labeling with selective neuronal markers showed that in *drd1a*-EGFP mice fluorescence was detected in approximately half of the MSNs, whereas in *drd2*-EGFP mice approximately another half was detected, as well as large aspiny cholinergic interneurons (Bertran-Gonzalez et al., 2008; Matamales et al., 2009). Moreover, in *drd1a*-EGFP or *drd2*-EGFP mice, fluorescence was not detected in GABAergic interneurons, identified using parvalbumin, somatostatin, and calretinin antibodies (Bertran-Gonzalez et al., 2008). Future studies should precisely confirm whether the expression of dynorphin/substance P and enkephalin is also restricted to EGFP labeled neurons in *drd1a*-EGFP and *drd2*-EGFP mice, respectively. Although the levels of expression of EGFP can vary from one line to the other, these mice allowed an evaluation of the proportions of neurons expressing D1R, D2R, or both. Whereas only a small

proportion of MSNs was calculated to co-express both receptors in the dorsal striatum and the NAc core (~5–6%), D1R and D2R co-expressing neurons were predicted to be more abundant in the shell of the NAc (~17%) (Bertran-Gonzalez et al., 2008). However, direct measurement of the number of neurons that co-express the two types of receptors still needs to be carried out, in conditions in which all the neurons are detected in the same animal. At any rate, BAC transgenic mice are convenient tools for easily identifying striatonigral and striatopallidal MSNs and evaluating the functional, cellular, and molecular differences between the two subpopulations (**Figure 1**). BAC transgenic mice have already been extensively used to characterize the distinct physiological properties of striatonigral and striatopallidal MSNs, as well as the precise mechanisms involved in their plasticity (Kreitzer and Malenka, 2007; Cepeda et al., 2008; Day et al., 2008; Gertler et al., 2008; Shen et al., 2008; Taverna et al., 2008) (see **Figure 1**).

Recent studies using advanced techniques such as fluorescence-activated cell sorting (FACS) of MSNs or translating ribosome affinity purification approach (TRAP), allowed the identification and characterization of a new set of differentially expressed genes in D1R- and D2R-expressing MSNs (Lobo et al., 2006; Heiman et al., 2008). Indeed, using FACS in *drd1a*- and *drd2*-EGFP mice, Lobo et al. (2007) identified the sphingosine-1-phosphate (S1P) receptor Gpr6, an important striatopallidal regulator of instrumental conditioning. The transcription factor early B-cell factor 1 (Ebf1) identified with the same method has been shown to play a

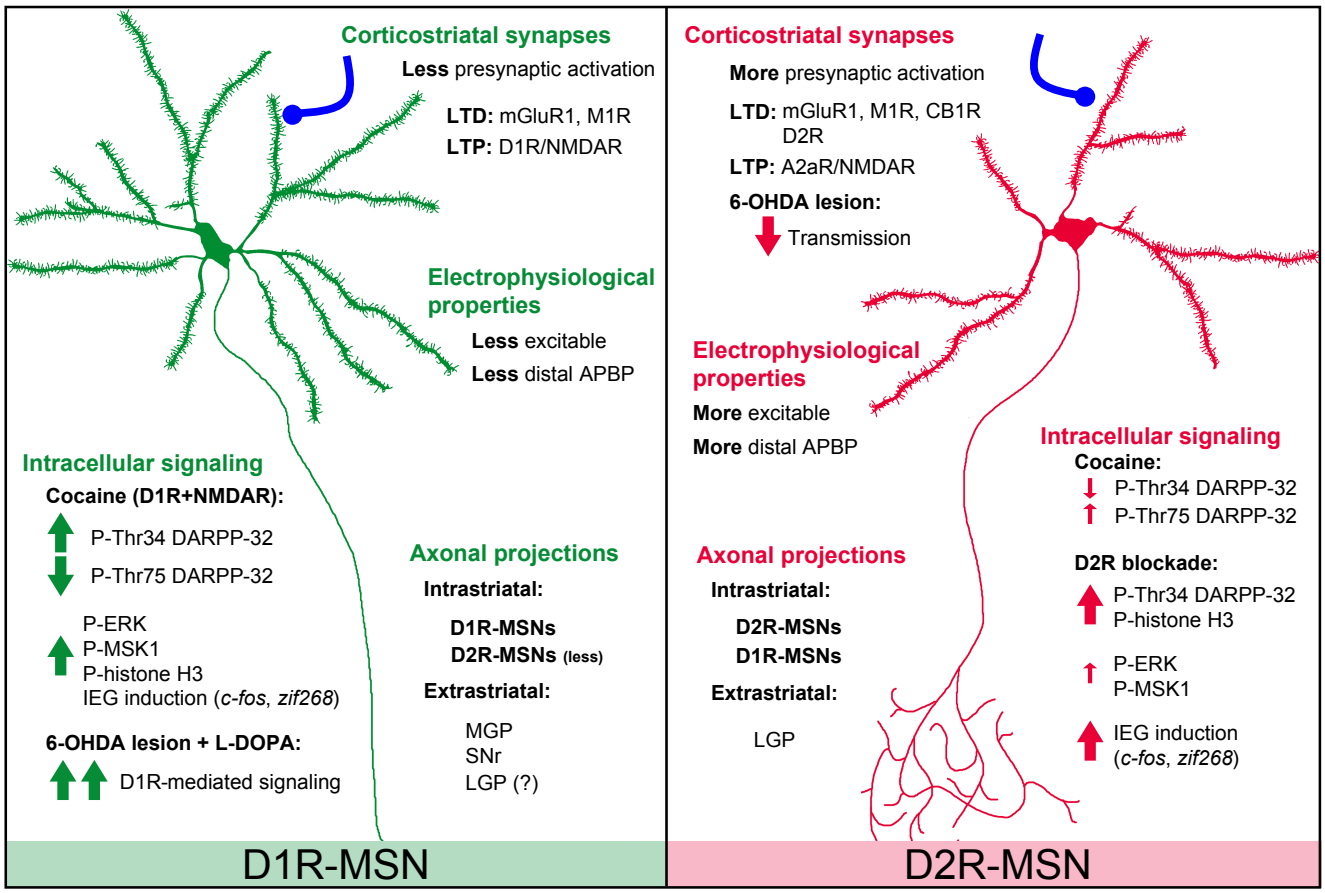

FIGURE 1 | Summary of major differences between D1R-MSNs and D2R-MSNs based on data from BAC transgenic mice. *Electrophysiological properties:* D1R-MSNs appear less excitable than D2R-MSNs probably due to the different number of primary dendrites and/or differences in presynaptic input (Kreitzer and Malenka, 2007; Cepeda et al., 2008; Gertler et al., 2008). D2R-MSNs show stronger GABAA receptor-mediated synaptic responses and tonic currents than D1R-MSNs (Ade et al., 2008; Taverna et al., 2008; Janssen et al., 2009). Single back-propagating action potentials invade more distal dendritic regions in D2R- than in D1R-MSNs, a difference involving voltage-dependent Na^+ channels and Kv4 K^+ channels (Day et al., 2008). *Corticostriatal synapses:* High-frequency stimulation-induced CB1R-mediated LTD is exclusively observed in D2R-MSNs and requires stimulation of D2Rs (Kreitzer and Malenka, 2007). D2R present in cholinergic interneurons also appear important for LTD in both D1R- and D2R-MSNs, involving M1R and mGluR1 receptors (Wang et al., 2006). NMDAR-induced LTP is increased by D1R in D1R-MSNs and by A2A-R in D2R-MSNs (Shen et al., 2008). *Axonal projections:* extrastriatal projections of D2R-MSNs are mainly detected in the LGP, whereas D1R-MSNs provide fibers to the SNr, MGP, and, possibly, some to the LGP (Gong et al., 2003; Bertran-Gonzalez et al., 2008; Matamales et al., 2009). Unidirectional intrastriatal projections (D1R–D1R and D2R–D2R) are the most common, and D2R–D1R collaterals are more abundant than D1R–D2R (Taverna et al., 2008). *Intracellular signaling:* see **BAC Transgenic Mice Facilitate the Identification of Distinct Populations of MSNs** in the text. 6-OHDA, 6-hydroxydopamine; A2aR, adenosine 2a receptor; APBP, action potential back propagation; D1R, D1-receptor; D2R, D2-receptor; CB1R, type 1 cannabinoid receptor; LGP, lateral globus pallidus; LTD, long-term depression; LTP, long-term potentiation; M1R, muscarinic acetylcholine receptor 1; mGluR1, metabotropic glutamate receptor 1; MGP, medial globus pallidus; SNr, substantia nigra pars reticulata.

pivotal role in the development of striatonigral MSNs (Lobo et al., 2008). The use of transgenic mice in which ribosomes from either population of MSNs are tagged and polyribosomes immunoprecipitated followed by RNA isolation (TRAP technique) has been recently employed to uncover selective changes in active mRNAs in D1R- and D2R-expressing MSNs following cocaine administration (Heiman et al., 2008). Moreover, other genetic approaches to specifically target and ablate striatonigral or striatopallidal neurons have clearly demonstrated the existence of distinct populations with opposing functional roles (Drago et al., 1998; Sano et al., 2003; Durieux et al., 2009). Finally, the selective expression of channelrhodopsin-2 in D1R- or D2R-expressing striatal neurons allowed the elegant manipulation of one or the other pathway with clearly distinct effects (Kravitz et al., 2010).

OPPOSING REGULATION OF SIGNALING PATHWAYS IN STRIATONIGRAL AND STRIATOPALLIDAL MSNs

ERK PATHWAY

In line with the work described above, recent studies using *drd1a*-EGFP and *drd2*-EGFP mice showed a striking degree of functional segregation between the two populations of striatal MSNs, especially at the level of signaling pathways in response to various stimuli (**Figure 1**). For instance, these two lines of BAC transgenic mice were used to study the extracellular signal-regulated kinase (ERK) and some of its downstream effectors, a signaling pathway strongly activated in striatal neurons in response to psychostimulants and other drugs of abuse (reviewed in Girault et al., 2007). These studies revealed that ERK was selectively activated in D1R-expressing MSNs of the NAc (shell/core) and dorsal striatum after acute or repeated administration of cocaine (Bertran-Gonzalez et al., 2008), amphetamine (Gerfen et al., 2008), MDMA (Doly et al., 2009) or GBR12783 – a selective DA reuptake inhibitor – (Valjent et al., 2010). These observations confirmed and largely extended previous experiments that used double staining with cell-population-specific markers (Zhang et al., 2004; Valjent et al., 2005). ERK activation induces the phosphorylation in the nucleus of mitogen- and stress-activated kinase-1 (MSK1) and histone H3, responses that are also restricted to D1R-expressing MSNs of the dorsal striatum and the NAc after cocaine (Bertran-Gonzalez et al., 2008). Similarly, in DA-depleted *drd1a*- and *drd2*-EGFP mice, L-DOPA-induced activation of the ERK pathway was exclusively observed in D1R-expressing neurons (Santini et al., 2009), confirming previous observations (Gerfen et al., 2002). Intriguingly, in all studies using *drd1a*- and *drd2*-EGFP mice, ERK activation was never observed in striatopallidal MSNs, not even in the NAc shell, where the calculated D1R/D2R co-expression levels were higher. The lack of ERK activation in neurons expressing D2R may result from a negative effect of D2R on ERK activation, since pharmacological blockade of D2Rs by haloperidol or raclopride selectively activates ERK and induces histone H3 phosphorylation in D2R-expressing striatopallidal MSNs in the dorsal striatum (Bertran-Gonzalez et al., 2008, 2009). The effects of D2R antagonists suggest that stimulation of D2Rs by basal or psychostimulant-induced DA release prevents the activation of ERK signaling events in neurons containing these receptors. Altogether, the use of BAC transgenic mice demonstrated the complete segregation of signaling responses between striatonigral and striatopallidal neurons in response to various drugs that act on DA receptors. It is important to point out that the opposing effects that D1Rs and D2Rs exert on adenylyl cyclase may contribute to the higher segregation of signaling responses in comparison with what could be expected from the distribution of the DA receptor subtypes.

DARPP-32 PHOSPHORYLATION

DARPP-32 is a dual-function protein selectively expressed in all MSNs and is therefore viewed as a critical integrator of many signaling events occurring in these neurons (Svenningsson et al., 2004). The complexity of its regulation results from the existence of several phosphorylatable residues that determine its function. On the one hand, the phosphorylation on Thr34 by protein kinase A (PKA) converts DARPP-32 into a selective inhibitor of serine/threonine protein phosphatase-1 (PP-1), thereby enhancing phosphorylation of proteins targeted by PP-1. On the other hand, DARPP-32 becomes an inhibitor of PKA when it is phosphorylated on Thr75 by cyclin-dependent kinase 5 (Cdk5). Although these two phosphorylation reactions with opposing functional consequences have been extensively studied, it had never been addressed whether or not they occurred within the same neurons or in separate populations. The generation of BAC transgenic mice expressing DARPP-32 with different tags under the control of *drd1a* and *drd2* promoters allowed the analysis of DARPP-32 phosphorylation selectively in the two populations of striatal output neurons *in vivo* (Bateup et al., 2008) (**Figure 1**). By immunoprecipitating the protein with tag-specific antibodies in one or the other subpopulation, it was shown that cocaine treatment increased Thr34 phosphorylation and decreased Thr75 phosphorylation selectively in D1R-expressing MSNs, while smaller changes in the opposite direction was observed in D2R-expressing neurons (Bateup et al., 2008). By contrast, haloperidol-induced DARPP-32 Thr34 phosphorylation was restricted to striatopallidal MSNs (Bateup et al., 2008). These data provided further experimental support for the opposing influence of DA on striatonigral and striatopallidal pathways. Surprisingly, this cell type-specific approach also revealed that selective D1R or D2R agonists were able to alter DARPP-32 phosphorylation on Thr34 and Thr75 in the same direction in both neuronal populations *in vivo* (Bateup et al., 2008). Additional experiments are necessary to determine the contribution in these effects of extrastriatal D1Rs and D2Rs, of cross-talks between the two populations, and of neurons expressing both types of receptors. It is particularly interesting that the use of indirect agonists that mimic endogenous DA (i.e., psychostimulants or L-DOPA in 6-OHDA-lesioned mice) results in a more segregated activation of D1R-expressing neurons than the use of drugs that are specific for D1Rs. This strongly suggests that the opposing regulation of signaling pathways in the two neuronal populations is a fundamental functional characteristic of striatal efferent neurons.

ANATOMICAL EVIDENCE AGAINST THE SEGREGATION BETWEEN THE STRIATONIGRAL AND STRIATOPALLIDAL PROJECTIONS

According to the classical model of the basal ganglia, about half of the MSNs would project exclusively to the SNr/MGP, and the other half to the LGP (Alexander et al., 1990; DeLong, 1990). However, in both rats and non-human primates, anatomical studies based on single axon reconstruction revealed that most (if not all) of the

striatal MSNs project to the LGP (Kawaguchi et al., 1990; Parent and Hazrati, 1995; Wu et al., 2000). The projections of half of them (Type I neurons) would terminate in the LGP, whereas the other half (Type II neurons) would send some collaterals to the LGP on their way to the MGP and SNr (Kawaguchi et al., 1990; Wu et al., 2000). This last group was divided into Type IIa (projecting to the LGP, MGP, and SNr) and Type IIb (projecting to the LGP and SNr). This anatomical evidence clearly implied a variable degree of overlap between the two pathways and questioned the simplistic view of the striatal output organization. Indeed, a pure striatonigral pathway would not exist as such, since the MSNs in this pathway appear to send projections to both LGP and SNr/MGP. It is however important to remember that single-axon reconstruction analysis is based on a limited number of neurons and the absence of MSNs projecting exclusively to the SNr/MGP still needs to be undoubtedly demonstrated. Furthermore, the axonal branching patterns of the MSNs have not been investigated with respect to their neurochemical distinction (e.g., substance P/dynorphin vs. enkephalin, D1Rs, D2Rs, or both).

Although the existence of such collaterals has not been investigated in mice, observations in *drd1a*- and *drd2*-EGFP mice suggest that they could be present. While the injection of a retrograde neuronal tracer in the SNr of *drd1a*- and *drd2*-EGFP mice showed that virtually all MSNs projecting to the SNr expressed the D1R and very few D2R (<1%) (Gertler et al., 2008; Matamales et al., 2009), sparse EGFP-positive fibers were detected in the LGP of *drd1a*-Cre and *drd1a*-EGFP mice (Gong et al., 2007; Matamales et al., 2009). These observations, which are also supported by double retrograde-tracing experiments in rats (Castle et al., 2005), suggest that either some of the D1R-striatonigral neurons give off collaterals to the LGP, or that these terminals arise from the few neurons that express both D1R and D2R. It is important to highlight that fluorescence intensity recorded in *drd1a*-EGFP mice is much higher in the SNr than in the LGP, reflecting either a higher number of D1R-MSNs projecting to the SNr or a denser terminal branching in this structure. It should be kept in mind, however, that EGFP staining does not provide information about receptor levels and their distribution, since EGFP expression only reflects the activity of D1R promoters. In conclusion, the simplest explanation to account for the remaining apparent discrepancies between the available data is that striatonigral neurons give off a small number of collaterals to the LGP. However, careful anatomical analysis in mice will be necessary to conclude, since the evidence for branching neurons was initially obtained in rats and monkeys.

SEGREGATED D1R AND D2R EXPRESSION IN STRIATONIGRAL AND STRIATOPALLIDAL NEURONS: STILL SOME OPEN ISSUES

The degree of D1R and D2R co-localization in the striatum has been a matter of intense debate. As previously mentioned, early studies put forward the concept of a differential expression of the D1R and D2R in the two MSN populations (Gerfen et al., 1990; Le Moine and Bloch, 1995). However, data supporting a strong co-localization of the two receptors in the striatum also existed. The first evidence came from some ISH studies in which co-localization of D1R and D2R mRNAs was found in 27–47% of all striatal MSNs (Meador-Woodruff et al., 1991; Weiner et al., 1991; Lester et al., 1993). These proportions were confirmed at the level of protein expression, since studies using either double immunofluorescence or retrograde labeling methods combined with immunofluorescence reported that around 20–60% of MSNs expressed both D1-like and D2-like receptors (Larson and Ariano, 1994; Shetreat et al., 1996; Deng et al., 2006). A high degree of co-localization was also supported by single cell RT-PCR studies (reviewed in Surmeier et al., 1993). Although in early studies an almost complete co-expression was detected (Surmeier et al., 1992; Surmeier and Kitai, 1993), this proportion was later re-estimated and co-expression reported to occur only in about half of the MSNs (Surmeier et al., 1996). Single cell RT-PCR, a highly sensitive technique in which it is difficult to ensure linearity of amplification for low abundance transcripts, provided higher levels of apparent co-expression of transcripts than other less sensitive techniques such as *in situ* hybridization (Gerfen et al., 1990; Le Moine and Bloch, 1995; Aubert et al., 2000).

How is the different pattern of gene expression, including that of D1R and D2R, controlled during development? The response to this important question is not known. In a schematic "instructive" model, all striatal MSNs would have a relatively undetermined phenotype until they reach their targets, which would, through a mechanism to be established, alter their patterns of gene expression. Alternatively, a "selective" model would postulate the pre-existence of two populations of MSNs with different sets of genes, which would control their projections. It is important to underline that the proportion of D1R and D2R co-localization appears much higher in cultured striatal neurons (between 60 and 100%) than in the adult striatum (Wong et al., 1999; Aizman et al., 2000; Lee et al., 2004; Hasbi et al., 2009). Thus, neurons in culture do not provide any information on the degree of co-expression of receptors or other molecules *in vivo*. Moreover, the higher degree of co-expression of markers in neurons in culture as compared to the *in vivo* situation argues in favor of the "instructive" model. Analysis of D1R and D2R mRNA expression by quantitative RT-PCR in the striatum at prenatal and early postnatal stages (embryonic day 14 to postnatal day 7) revealed that at E14, the D2R was predominant over the D1R in the striatum (Araki et al., 2007). However, dissociation between the ontogeny of DA receptor binding sites and mRNA has been reported (Jung and Bennett, 1996). It was suggested that the developmental regulation of D1R and D2R mRNAs would result from intrinsic genetic programs, while the dopaminergic innervation would control the D1R and D2R protein synthesis (Jung and Bennett, 1996). The importance of afferent fibers is well illustrated by the fact that, in cultured striatal neurons, the emergence of spontaneous and evoked excitatory synaptic currents as well as dendritic spines depends on the presence of excitatory afferents (Segal et al., 2003; Day et al., 2006). However, it is unlikely that either glutamate or DA inputs would be sufficient to induce the distinction between the two populations of MSNs, since they are, as far as we presently know, homogenously distributed. Further studies are clearly warranted to determine how the specificity of D1R and D2R expression, as well as that of other genes, is controlled in the two populations of striatal MSNs during development.

Independently of the studies on the proportion of co-localization of D1R and D2R, several lines of evidence indicate that both receptors can interact in the same cells. In addition to their ability to interact with several other G protein-coupled receptors, including CB1, A2a, and D3 receptors (see Franco et al., 2008), D1R and D2R can interact with each other and can be found in the same protein

complexes as demonstrated by co-immunoprecipitation from rat striatal extracts (Lee et al., 2004). Interestingly, evidence indicates that heteromeric DA receptor complexes are linked to calcium signaling. The pathway selectively activated through the D1R–D2R hetero-oligomer involves coupling to Gq and phospholipase C and triggers a rise in intracellular calcium, an increase in the phosphorylation of Ca^{2+}-calmodulin-dependent protein kinase II (CaMKIIα) and a subsequent production of brain-derived neurotrophic factor (BDNF) (Rashid et al., 2007; Hasbi et al., 2009). A recent study revealed that this new signaling pathway might be particularly relevant in the NAc where an interaction between co-localized receptors was supported by confocal FRET analysis using fluorophore-labeled antibodies (Hasbi et al., 2009). These results are in agreement with the anatomical observations reporting that the proportion of co-localization between D1R and D2R was higher in the NAc than in the dorsal striatum (Bertran-Gonzalez et al., 2008; Hasbi et al., 2009). Thus, it is possible that rapid calcium signaling through D1R–D2R complexes may contribute to DA-mediated striatal plasticity. A critical issue for the future will be to clearly identify in which neurons this interaction between D1R and D2R occurs *in vivo*.

CONCLUSIONS AND PERSPECTIVES

We have reviewed here some of the many studies providing evidence for a strong anatomical and functional segregation of two populations of striatal projection neurons, as well as those which challenge this view. The use of BAC transgenic mice expressing fluorescent proteins under the control of promoters of interest in the striatum provides a powerful tool to easily identify specific neuronal types *in vivo*. This allows the study of cellular functions, including signaling pathways and electrophysiological responses in identified neuronal populations. These mouse models have revealed a profound functional dichotomy of striatal neurons in physiological and pathological conditions, far beyond expectations. Thus, an overwhelming amount of data supports the anatomical and functional segregation of the striatonigral and striatopallidal MSNs. The results in rats and primates which show the existence of branched projections from striatonigral neurons to the LGP are compatible with the results in mice. Additional studies will be necessary to determine whether the degree of branching of striatofugal axons differs significantly between rats and mice, and what is its functional significance. It is possible that the functional segregation of the two types of neurons is less pronounced in rats or in primates than in the mouse. This is unlikely in our opinion and we would like to suggest that the functional segregation of the two populations of MSNs is conserved, in spite of different degrees of arborization. Other studies suggest the intriguing possibility of specific D1R/D2R coupling in neurons expressing both receptors. The extent and the functional importance of this co-expression remain to be addressed experimentally. In this respect, the comparison of the data obtained with various approaches over more than two decades, shows that many of the apparent differences between results obtained are related to the different sensitivities of the methods utilized. Methods which detect relatively high levels of expression of D1R or D2R, including BAC transgenic mice, clearly emphasize the segregation of these receptors in different neurons. Interestingly and importantly, this segregation corresponds to the clear functional dichotomy of the neurons of the direct and indirect pathway. In contrast, other methods which are more sensitive, but not necessarily linear, provide a picture in which more co-expression can be found, but with an uncertain functional signification.

Finally, the importance of other levels of heterogeneity in the striatum is still unknown. Can the MSNs be further divided into discrete subgroups depending on their location in the striosomes or matrix, on the topography of their targets, and on the genes they express? Alternatively, are all these parameters continuously and independently distributed among the MSNs? Future studies will tell whether relatively homogenous subtypes of neurons can be further identified among striatonigral and striatopallidal neurons, or whether these neurons form large populations with a continuous distribution of multiple characteristics and no rationale for distinguishing more subgroups. The use of novel types of BAC transgenic mice, combined with careful co-labeling, cell-specific transcriptional, or translational profiling, and anatomical and functional studies will undoubtedly bring further understanding… and perhaps surprises in this area.

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Neurochemical characterization of the tree shrew dorsal striatum

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The striatum is a major component of the basal ganglia and is associated with motor and cognitive functions. Striatal pathologies have been linked to several disorders, including Huntington's, Tourette's syndrome, obsessive–compulsive disorders, and schizophrenia. For the study of these striatal pathologies different animal models have been used, including rodents and non-human primates. Rodents lack on morphological complexity (for example, the lack of well defined caudate and putamen nuclei), which makes it difficult to translate data to the human paradigm. Primates, and especially higher primates, are the closest model to humans, but there are ever-increasing restrictions to the use of these animals for research. In our search for a non-primate animal model with a striatum that anatomically (and perhaps functionally) can resemble that of humans, we turned our attention to the tree shrew. Evolutionary genetic studies have provided strong data supporting that the tree shrews (Scadentia) are one of the closest groups to primates, although their brain anatomy has only been studied in detail for specific brain areas. Morphologically, the tree shrew striatum resembles the primate striatum with the presence of an internal capsule separating the caudate and putamen, but little is known about its neurochemical composition. Here we analyzed the expression of calcium-binding proteins, the presence and distribution of the striosome and matrix compartments (by the use of calbindin, tyrosine hydroxylase, and acetylcholinesterase immunohistochemistry), and the GABAergic system by immunohistochemistry against glutamic acid decarboxylase and Golgi impregnation. In summary, our results show that when compared to primates, the tree shrew dorsal striatum presents striking similarities in the distribution of most of the markers studied, while presenting some marked divergences when compared to the rodent striatum.

Keywords: caudate, putamen, GABAergic, dopaminergic, calbindin, calretinin, parvalbumin, striosomes

INTRODUCTION

The mammalian striatum is a major component of the basal ganglia and contains its major receptive nuclei, the caudate, and the putamen. These nuclei are the target of several areas of the mammalian brain including the cortex, mesencephalon, and thalamus (Kawaguchi et al., 1995; Joel and Weiner, 2000). In addition, the caudate and putamen send projections to other nuclei of the basal ganglia, such as the substantia nigra (SN) and globus pallidus (GP; Royce and Laine, 1984). There are also indirect projections to the subthalamic nucleus, thalamus, and cerebral cortex via the direct and indirect pathways (Smith et al., 1998).

The striatum receives inputs from the dopaminergic, glutamatergic, and serotonergic systems (Kubota et al., 1986; Lavoie and Parent, 1990; Fujiyama et al., 2006). In addition, some afferent projections of the striatum have been shown to synapse onto particular cytoarchitectonic components of the caudate and/or putamen (see as a review Nieuwenhuys et al., 2007). Even when there is no complete segregation of the target areas, the caudate and putamen present two distinguishable types of compartments with their own specific properties. These compartments are known as the striosomes (patch) and matrix, which receive preferentially different inputs and contain cells that express different markers (Graybiel and Ragsdale, 1983; Donoghue and Herkenham, 1986; Bolam et al., 1988; Ragsdale and Graybiel, 1988; Gerfen, 1989; Hirsch et al., 1989; Graybiel, 1990; Kubota and Kawaguchi, 1993; Holt et al., 1997). Ventral to the caudate and putamen is an area of the striatum known as the "ventral striatum." Different nuclei have been suggested to be part of the ventral striatum (Heimer and Wilson, 1975; Heimer et al., 1982; Haber and McFarland, 1999; Fudge and Haber, 2002), of which the most prominent is the accumbens nucleus.

Medium spiny projection neurons are the most numerous type of cells in the dorsal striatum, with at least 75% of neurons belonging to this type in primates (Graveland and DiFiglia, 1985; see as reviews Nieuwenhuys et al., 2007; Tepper et al., 2010), and up to 95% in rodents and in the cat (Kemp and Powell, 1971; Graveland and DiFiglia, 1985; see as a review Voogd et al., 1998). These neurons are gamma-aminobutyric acid (GABA)ergic, and receive afferent connections from different neurotransmitter systems that modulate their activity by establishing synapses with them in a hierarchically and highly organized manner (Fonnum et al., 1981; Kubota et al., 1986; Dube et al., 1988; Lavoie and Parent, 1990; Parent and Hazrati, 1995). These GABAergic neurons are responsible for striatal efferent projections and can be

subdivided into those containing enkephalin, that project to the external segment of the GP, and those containing substance P, that project to the internal segment of the GP and SN pars reticulata (Haber and Elde, 1982; Bolam and Izzo, 1988; Bolam and Smith, 1990; Reiner et al., 1999). The second class of neurons present in the dorsal striatum are interneurons (GABAergic or cholinergic), that are typically aspiny, and unlike the medium spiny neurons, do not send projections outside the striatum (Phelps et al., 1985; Cowan et al., 1990; Kawaguchi, 1993; Kawaguchi et al., 1995; Wu and Parent, 2000; see as reviews, Nieuwenhuys et al., 2007; Tepper et al., 2010). GABAergic interneurons can contain additional neurotransmitters such as parvalbumin (type I GABAergic striatal interneurons), somatostatin and neuropeptide Y (type II GABAergic striatal interneurons), or calretinin (type III GABAergic striatal interneurons; DiFiglia and Aronin, 1982; Vincent et al., 1983; Cowan et al., 1990; Kita et al., 1990; Kawaguchi, 1993; Kubota et al., 1993; Kawaguchi et al., 1995; Rushlow et al., 1995; Parent et al., 1996; Kubota and Kawaguchi, 2000; Wu and Parent, 2000; see as reviews, Nieuwenhuys et al., 2007; Tepper et al., 2010). Finally, the striatum also contains a small amount of dopaminergic intrinsic neurons. Although the number of these neurons is almost vestigial in normal rodent striatum, it is more prevalent in the primate striatum (Dubach et al., 1987; Tashiro et al., 1989; Mura et al., 1995; Ikemoto et al., 1996; Betarbet et al., 1997; Baker et al., 2003; Cossette et al., 2004, 2005; Huot and Parent, 2007).

The caudate and putamen nuclei are involved in motor and cognitive functions (DeLong and Georgopoulos, 1981; DeLong et al., 1984; Middleton and Strick, 1994). Disruptions in the afferent and efferent connections and alterations within the nuclei themselves have been linked to motor disorders such as Parkinson's and Huntington's disease (DeLong and Georgopoulos, 1981; DeLong et al., 1984; Marsden, 1986), and psychiatric disorders such as schizophrenia (Buchsbaum et al., 1992; Hietala et al., 1995; Roberts et al., 1996, 2005; Howes et al., 2009; see also as reviews Perez-Costas et al., 2010; Simpson et al., 2010), bipolar disorder (Amsterdam and Newberg, 2007; Cousins et al., 2009), and major depression (Matsuo et al., 2008; Butters et al., 2009; Khundakar et al., 2011). Although some aspects of these diseases can be studied in postmortem human tissue, the use of animal models is also necessary. Animal models allow the study of pathological states in a controlled environment, and the test of experimental treatments. The majority of animal research involves the use of rodents, and the results obtained from these studies have provided a large body of data, although their significance is limited due to the anatomical differences between the rodent and the human striatum. In the search for a striatum that anatomically (and possibly functionally) presents more similarities to that of the human, many researchers have used non-human primates for their studies. The use of non-human primates provides a more accurate model of the human basal ganglia, but these studies tend to be costly and create ethical controversy. This has moved some countries to partially or totally ban research in some groups of primates (Cohen, 2007a,b; Vogel, 2010). All of these factors raise the need for alternative models that can represent a closer frame for comparison to the human striatum than rodents, and at the same time do not incur the cost and controversy associated with primate research. One viable option that may fit this compromise is the common tree shrew (*Tupaia glis belangeri*). Tupaiids are diurnal omnivorous mammals that in their natural environment occupy arboreal, semi-arboreal, and forest floor habitats. These animals are able to use their forepaws to climb trees and handle food (see Luckett, 1980). Evolutionary genetic studies using sequencing of large DNA sets (mitochondrial and nuclear) have provided strong data supporting that Scadentia (tree shrews) and Dermoptera (flying lemurs) are the closest groups to primates, with a common ancestor far removed from other groups such as Lagomorpha (rabbits and pikas) or Chiroptera (bats; Adkins and Honeycutt, 1991; Liu et al., 2001; Murphy et al., 2001; Janecka et al., 2007). Behavioral studies have demonstrated that tree shrews have the capability to develop strategic planning (Bartolomucci et al., 2001), and when subjected to psychosocial stress they develop all the hallmarks of depression (behavioral, molecular, and anatomical), that in a similar manner to humans, are reversed with the use of antidepressants (Fuchs et al., 1995; Gould et al., 1997; Isovich et al., 2000; Czeh et al., 2001; Fuchs and Flugge, 2002; Fuchs, 2005). In addition, a recent study has shown that as it occurs in elderly humans, aged tree shrews present with accumulation of amyloid beta proteins, a pathology that has not been observed in aged wild-type mice (Dickson et al., 1992; Yamashita et al., 2010).

The tree shrew brain anatomy has only been studied in detail for pathways related to the visual system (see for example Fitzpatrick, 1996; Lyon et al., 2003; Elston et al., 2005; McCoy et al., 2008; Poveda and Kretz, 2009; Chomsung et al., 2010; Day-Brown et al., 2010). Some scarce studies have focused their interest in the general subdivisions of the neocortex (Remple et al., 2007; Wong and Kaas, 2009) as well as in punctual aspects of other brain areas including the striatum (see, e.g., Divac and Passingham, 1980; Oliver, 1982; Lin et al., 1984; Mijnster et al., 1999; Isovich et al., 2000; Keuker et al., 2003; Day-Brown et al., 2010). However, the tree shrew dorsal striatum has not been previously studied in detail, which is necessary to determine the suitability of this animal as a model for studies concerning striatal pathologies.

In this work we used histological stains as well as immunohistochemistry for markers known to be present in the dorsal striatum of humans and other mammalian species. The study of these markers allowed us the examination of chemoarchitectonic features, such as the presence of matrix and striosome compartments, and the distribution of certain neuronal cell types (for example, the presence of cells expressing different calcium-binding proteins). We also assessed the distribution of two of the main neurotransmitters in the striatum, GABA, and dopamine, by studying the expression of their synthesizing enzymes, glutamic acid decarboxylase (GAD), and tyrosine hydroxylase (TH). Finally, we used Golgi impregnation to provide a detailed description of the features of the main cell type of the striatum, the medium spiny neurons.

MATERIALS AND METHODS

ANIMALS AND TISSUE PREPARATION

A total of 10 adult (1–5 years old) common tree shrews (*T. glis belangeri*) were used in this study. Animals were kept and euthanized following protocols approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee

(IACUC). For all the experiments, tree shrews were deeply anesthetized with a ketamine/xylazine mixture (200, 0.5 mg/kg). For western-blot, animals were immediately decapitated and their brains were extracted and preserved at −80˚C. Brain homogenates were obtained by sonication in a cold lysis buffer mixture (1:5 w:v) containing 50 mM Tris pH 8.0, 5 mM EDTA, 150 mM NaCl, 1% SDS, and 10 μl/ml of a protease inhibitor cocktail (Sigma, St. Louis, MO, USA; P8340). Samples were then centrifuged at 15000 *g* for 15 min at 4˚C, the supernatant was collected, and total protein concentration was measured using a modified Lowry technique (Bio-Rad, Hercules, CA, USA; D_C Protein Assay; 500-0113, 500-0114). Aliquots of 60 μg of total protein were stored at −80˚C. For immunohistochemistry and Nissl stain, tree shrews were perfused with a 0.9% saline solution followed by a cold 4% paraformaldehyde solution in 0.1 M phosphate buffer pH 7.4 (PB). The tissue was then immersed in a 30% sucrose solution in PB for cryoprotection. Finally, six free-floating coronal parallel series of sections (50 μm thick) were obtained on a cryostat, collected in a cryoprotection solution (FD NeuroTechnologies, Ellicott City, MD, USA; PC101) and stored at −20˚C until use. For Golgi impregnation, tree shrews were perfused only with a 0.9% saline solution and their brains were removed and immediately immersed in the impregnation solution.

In addition, a stock of striatal protein extracts from adult male Sprague-Dawley rat routinely maintained in our laboratory was used for western-blot studies. Finally, 50 μm thick sections of adult male rat striatum from a stock of tissue kept in the laboratory were used as positive controls to test acetylcholinesterase antibodies.

NISSL STAIN AND GOLGI IMPREGNATION

The first whole series of each animal was stained with a standard thionin (Nissl) stain protocol, and was used as a reference series for morphology and landmark determination. To analyze in further detail the morphology of medium spiny neurons of the tree shrew striatum, Golgi-Cox impregnation was performed as described in Melendez-Ferro et al. (2009). Briefly, after perfusion with a 0.9% saline solution, hemisected brains were immersed for 2 weeks in an impregnation solution that contained mercury chloride, potassium dichromate, and potassium chromate. After impregnation, the brains were immersed in a cryoprotectant solution at 4˚C for a minimum of 1 week, frozen in dry ice, and after that, 150 μm thick sections were obtained on a sliding microtome. Sections were collected on gelatin-subbed slides and allowed to dry for 5–6 days at 35˚C on a warm plate. Development of the sections was achieved by incubation for 10 min at room temperature (RT) in a solution that contained ammonium hydroxide. Finally, sections were rinsed in distilled water, dehydrated in ethanol, cleared in xylene, and coverslipped using Eukitt (Electron Microscopy Sciences, PA, USA; 15322).

ANTIBODIES USED IN THIS STUDY

Antibodies against calbindin, parvalbumin, and calretinin were used to study the distribution of calcium-binding proteins within the caudate and putamen. The dopaminergic innervation was analyzed by the detection of tyrosine hydroxylase (TH), the rate-limiting enzyme for the production of dopamine. The striatal GABAergic system was studied using an antibody against the two isoforms of glutamic acid decarboxylase (GAD65/67), the rate-limiting enzyme for the production of GABA. In addition, anti-acetylcholinesterase antibodies were used to further analyze the striosome/matrix organization in the tree shrew striatum. For each antibody a minimum of three whole series of different animals were studied.

WESTERN-BLOT

The specificity of the antibodies used in this study has been demonstrated by the manufacturers for different vertebrate species, although none of these antibodies have been characterized in the tree shrew. To ensure that these antibodies also recognized a protein of the appropriate molecular weight in the tree shrew brain, a western-blot analysis was performed using polyacrylamide gel electrophoresis in denaturing conditions. For this, samples were diluted 1:1 in loading buffer (0.5 M Tris–HCl pH 6.8, glycerol, 10% SDS, 0.5% bromophenol blue, and β-mercaptoethanol) and were heated to 95˚C in a dry bath for 5 min before loading in a 4–20% polyacrylamide gradient gel (Lonza, Basel, Switzerland; 58505). In each lane 60 μg of tree shrew whole brain protein extract or 30 μg of rat striatum protein extract were loaded in parallel. A molecular weight marker (Lonza; 50550) was also loaded for molecular weight reference. Proteins were resolved using a constant current of 125 V, and then transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad; 162-0714) using a constant current of 30 V for 19 h at 4˚C.

The following primary antibodies were used for western-blot: mouse monoclonal anti-tyrosine hydroxylase (TH; Millipore, Billerica, MA, USA; MAB5280, diluted 1:4000), mouse monoclonal anti-Calbindin (Sigma; C9848, diluted 1:8000), mouse monoclonal anti-Parvalbumin (Millipore; MAB1572, diluted 1:4000), rabbit polyclonal anti-GAD 65/67 (Millipore; AB1511, diluted 1:32000), and rabbit polyclonal anti-Calretinin (Swant, Bellinzona, Switzerland; 7699/3H, diluted 1:32000). For all the antibodies tested, western-blot membranes were rinsed in 0.05 M Tris Buffered Saline (TBS) and blocked with a solution containing 5% non-fat dry milk (Bio-Rad; 170-6404) in TBS containing 0.1% Tween 20 (TBS-T) for 1 h at RT. The membranes were then incubated with the appropriate primary antibody at the concentrations listed above in a solution of TBS-T with 1% non-fat dry milk for 19 h at 4˚C. After that, the membranes were rinsed in TBS-T containing 1% non-fat dry milk prior to incubation with the appropriate secondary antibody, either alkaline phosphatase-conjugated goat anti-mouse (Millipore; AP124A, diluted 1:15000) or alkaline phosphatase-conjugated goat anti-rabbit (Vector Laboratories, Burlingame, CA, USA; AP-1000, diluted 1:1000) in 1% non-fat dry milk in TBS-T for 1 h at RT. The membranes were then rinsed and developed using an Immun-Star chemiluminescence kit (Bio-Rad alkaline phosphate substrate; 170-5018) and Kodak Biomax XAR films (Kodak, Rochester, NY, USA; 166-0760).

IMMUNOHISTOCHEMISTRY

In order to characterize the neurochemical composition of the tree shrew striatum, the same antibodies that were used for western-blot were also used for immunohistochemistry. The antibody concentrations used for immunohistochemistry were as follows: anti-Tyrosine Hydroxylase (diluted 1:1000), anti-Calbindin

(diluted 1:2000), anti-Parvalbumin (diluted 1:2000), anti-GAD 65/67 (diluted 1:1000), and anti-Calretinin (diluted 1:2000). In addition, two anti-AchE antibodies were tested: a mouse monoclonal antibody (Sigma; clone ZR3, A6970) that has been shown to recognize rodent, feline, and rabbit but not primate AchE (Rackonczay and Brimijoin, 1986); and a rabbit anti-AchE antibody (Sigma; HPA019704) that has been designed to specifically recognize human AchE (see as reference, the human protein atlas, www.proteinatlas.org).

For immunohistochemistry, free-floating sections were rinsed several times in 0.01 M phosphate buffered saline (PBS) and the endogenous peroxidase was blocked by incubating for 30 min at RT with a solution of 5% H202 in PBS. After this, sections were rinsed thoroughly in PBS and incubated for 1 h at RT with 10% normal serum in PBS containing 0.3% Triton X-100 (PBS-T). The sections were then incubated for 19 h at RT with the appropriate primary antibody in a solution containing 3% normal serum in PBS-T. The following day, the sections were rinsed in PBS and incubated for 45 min at RT with a secondary antibody [biotinylated horse anti-mouse (Vector Laboratories; BA2001, diluted 1:400) or biotinylated goat anti-rabbit (Vector Laboratories; BA1000, diluted 1:400)] in a solution containing 3% normal serum in PBS-T. The sections were rinsed in PBS and incubated with an avidin–biotin-complex (Vector Laboratories; PK6100, diluted 1:100) for 45 min at RT. Finally, the sections were developed using a 3,3′-diaminobenzidine peroxidase kit (Vector Laboratories; SK4100). Negative controls consisting in the omission of the primary antibody and incubation with only 3% normal serum in PBS-T were performed for all the immunohistochemistry experiments. The sections were mounted on slides, dehydrated, and coverslipped using Eukitt. In addition, GAD65/67 immunolabeled sections were counterstained using thionin prior to coverslipping.

PHOTOGRAPHY AND IMAGE PROCESSING

Single images or stacks of photographs were obtained on a Nikon Eclipse 50*i* microscope (Nikon, Tokyo, Japan) connected to a Nikon DS-Fi1 color digital camera (Nikon). Stacks were only used in the case that the depth of the element to be photographed required this technique to obtain a focused image (e.g., all the Golgi impregnated samples, and in the case of some immunolabeled sections as indicated in the figure legends). Stacks were merged into a single focused image using Heliconfocus software (Heliconsoft, Kharkov, Ukraine). Photomontage and lettering of figure plates were done using CorelDRAW-12 (Corel, Ottawa, Canada).

CELL COUNTS AND MEASUREMENTS

Cell counts were performed in entire rostrocaudal parallel series labeled for TH ($n = 2$ animals). Each of these series used for cell counts consisted of one out of every six parallel series of 50 μm sections collected from each animal. In this scheme, sections used for counts were 300 μm apart, which eliminates the possibility of counting twice the same cell. In each section analyzed, all TH labeled cells of the dorsal striatum were counted.

Measurements of cells in TH labeled sections, or cells and processes in Golgi impregnated sections, were performed using the measurement tool of CorelDRAW-12. For soma cross-section size (area) calculations, cell shape was assimilated to the geometrical figure that more closely represented its shape (for example, circle, ellipse, or polygon) and the area was calculated using the mathematical formula for the appropriate geometrical figure.

SCHEMATIC DRAWINGS FOR THE COMPARISON OF THE STRIOSOME/MATRIX ORGANIZATION

Complete series of sections stained for calbindin, TH, and AchE were used to obtain schematic drawings of the distribution of striosomes in the tree shrew dorsal striatum. The sections were photographed with a 2× lens and imported into Powerpoint software (Microsoft, Redmond, USA). Contrast was enhanced for a better identification of the striosomes. Drawings were obtained from sections from the most rostral pole of the striatum to the caudal pole of the caudate and putamen. A total of 17 sections per marker were photographed and drawn.

RESULTS

SPECIFICITY OF THE ANTIBODIES

We performed western-blots with protein extracts from whole tree shrew brain and from rat striatum to ensure specificity of the antibodies used in this study. Protein extracts from rat striatum were used as a positive control, since the specificity of the antibodies has already been verified in this species (Bender et al., 2001; Kawaguchi and Hirano, 2002; Meier and Grantyn, 2004; Nafia et al., 2008; Rostkowski et al., 2009). Western-blot experiments showed that the antibodies produced bands that were located at the same level in the gel for both rat striatum and tree shrew brain protein extracts. More importantly, the western-blots also showed that these bands had the proper molecular weight for all the antibodies examined (**Figure 1**). Acetylcholinesterase antibodies were not assessed by western-blot since this enzyme forms disulfide-linked oligomers with collagenous and lipid-containing structural subunits, which produce an array of different forms with different molecular weights (for a detailed description of acetylcholinesterase forms see www.proteinatlas.org).

NEUROANATOMICAL FEATURES OF THE TREE SHREW STRIATUM

Based on the study of several thionin-stained series the adult tree shrew striatum was approximately 7.4 mm long, from its rostral pole to its most caudal level. This represents a 23% increase in length compared to the adult rat striatum, which is approximately 6 mm long (based on Paxinos and Watson, 1998). When compared to the human striatum, the length of the tree shrew striatum was approximately 14% of that of the human [which is approximately 54.4 mm long (based on Mai et al., 2004)]. The caudate and putamen were visible from the most rostral level of the striatum (**Figure 2A**), with the putamen extending slightly more caudally than the caudate. The corpus callosum provided a clear dorsal and lateral separation between the striatum and cortex, with the anterior commissure providing a ventral delineation between the dorsal and ventral striatum (**Figures 2A,B**). The anterior commissure appeared in the most rostral areas of the striatum and continued up to the level in which the GP began to appear in the caudal striatum (**Figure 2C**). The most defining morphological feature of the tree shrew striatum was the presence of

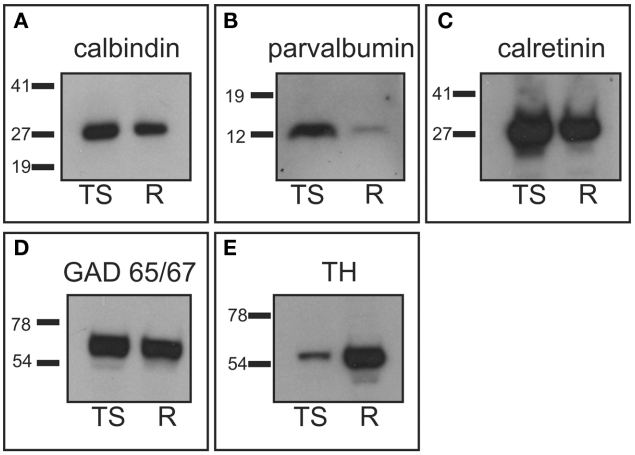

FIGURE 1 | Western-blot analysis. Tree shrew whole brain protein extracts were used to assess antibody specificity, and compared with rat striatal samples used as positive controls. **(A)** Calbindin western-blot showed a band of approximately 28 kDa in the tree shrew and rat samples. **(B)** Parvalbumin western-blot presented a single band of approximately 12 kDa in both species, which was more prominent in the tree shrew protein extract. This was likely due to the fact that the tree shrew protein extract also contained cortex tissue, which is known to have a high number of parvalbumin expressing cells (Celio, 1986). **(C)** The calretinin antibody detected a very strong band of approximately 29 kDa in both species. **(D)** GAD 65/57 appeared as a very thick band that corresponded with both isoforms of the enzyme. This is due to the fact that this antibody recognizes the two isoforms of GAD and the resolution of the gel did not allow for the separation of the two isoforms. **(E)** TH appeared as a single band of approximately 60 kDa that was stronger in the rat sample. This difference was likely due to the fact that the rat sample contained only striatum, which has a very high concentration of TH, while the tree shrew sample was a whole brain protein extract. R, rat; TS, tree shrew. Numbers indicate the molecular weight in kilodaltons.

a well defined and developed internal capsule, which created a clear separation between the caudate nucleus and the putamen (**Figures 2A–C**).

LABELING FOR PARVALBUMIN

Throughout the rostrocaudal extent of the striatum the general pattern of staining did not differ between the caudate nucleus and the putamen, although within each nucleus there were qualitative differences in the staining for parvalbumin (**Figures 3A–C**). In both nuclei, areas of higher and lower staining for parvalbumin immunoreactivity (parv-ir) were clearly observed from the most rostral to the most caudal regions (**Figures 3D–F**). This labeling pattern was due to differences in the density of parv-ir processes, with a lower density of processes present in the low parv-ir areas (**Figures 3D–F**). This pattern of expression partially matched a striosome/matrix distribution in rostral areas, with lower parv-ir in the striosomes. Parvalbumin-positive cells were loosely clustered with most of these clusters avoiding the low parv-ir areas, although some parv-ir cells were located within them (**Figures 3D–F**). These parvalbumin neurons also presented two to five labeled processes per neuron (**Figures 3D–F**). Parv-ir cells with different patterns of staining (i.e., from light to very dark cytoplasm labeling) appeared

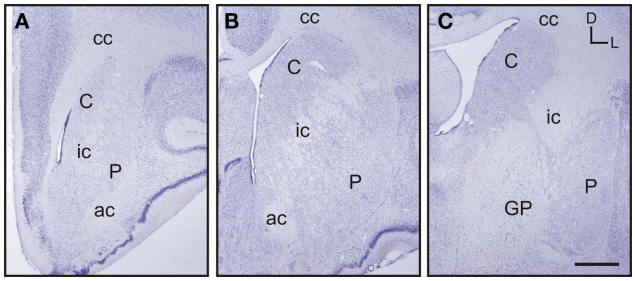

FIGURE 2 | Images of representative Nissl-stained rostrocaudal sections throughout the tree shrew striatum. (A) Rostral striatum. Note the clear separation of the caudate (C) and putamen (P) by the internal capsule (ic). **(B)** Mid striatum. The internal capsule (ic) is still present. The corpus callosum (cc) and anterior commissure (ac) are well formed and delineate the extent of the striatum. **(C)** Caudal striatum. The caudate (C) and putamen (P) are separated by the internal capsule (ic). The anterior commissure is no longer visible and the globus pallidus (GP) is now present. Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). Scale bars: 1 mm in **(A–C)**.

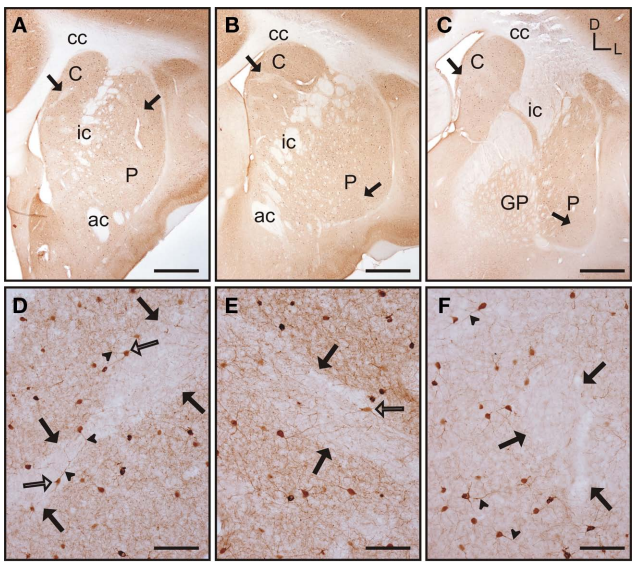

FIGURE 3 | Parvalbumin immunostaining in the tree shrew striatum. (A–C) Low magnification images of the rostral **(A)**, mid **(B)**, and caudal **(C)** striatum, showing the presence of bands of low labeling (black arrows) in these three rostrocaudal regions. **(D)** Detail of a low parv-ir band in the rostral caudate (black arrows). Several labeled neurons present multiple slender and long labeled processes (arrowheads), crossing in some cases from rich immunolabeled areas into the low parv-ir band. **(E)** Detail of parvalbumin labeling in the mid caudate showing the presence of a low immunoreactive band (black arrows) flanked by parv-ir cells distributed in clusters. Some labeled neurons are located in the low labeling band (open arrow). **(F)** Detail of parv-ir cells in the caudal putamen showing immunolabeled processes (arrowheads). A low immunolabeling band is located on the right and is devoid of labeled cells (black arrows). Note also in **(D–F)** the abundance of labeled processes in the parvalbumin rich areas, but also the presence of some processes in the areas with low parv-ir. Figures **(D–F)** are composite images of several Z-stack images. Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). ac, anterior commissure; C, caudate; cc, corpus callosum; ic, internal capsule; GP, globus pallidus; P, putamen. Scale bars: 1 mm in **(A–C)**; 100 μm in **(D–F)**.

distributed together in the same area (**Figures 3D–F**). Finally, parv-ir was absent from both the internal capsule and the corpus callosum.

LABELING FOR CALRETININ

In the tree shrew striatum there were clear differences in the general pattern of staining for calretinin between the caudate and putamen. In the caudate nucleus calretinin immunoreactivity (calret-ir) remained constant throughout its rostrocaudal extent, without any patterns or bands of different labeling (**Figures 4A–C**). Rostral areas of the putamen had a similar pattern of staining to the caudate; while in mid and caudal areas calret-ir was qualitatively lower than in the caudate (**Figures 4A–C**). This difference was mainly due to the presence of an area of low calret-ir that became progressively larger in more caudal regions. In rostral regions of the putamen the low calret-ir area occupied a small part of the nucleus and was restricted to a dorsolateral position, while in mid regions this low calret-ir area extended into ventrolateral positions (**Figure 4B**). In caudal regions the low calret-ir area encompassed the majority of the putamen, from dorsolateral to lateral and medial positions (**Figure 4C**). In rostral areas of the caudate and putamen calret-ir neurons were scarce. This pattern continued throughout the rostrocaudal extent of the caudate (**Figures 4D,F**), while in mid regions of the putamen labeled neurons were more frequently observed (**Figure 4E**) and became scarce again in caudal areas. In the caudate calret-ir neurons were mainly relegated to dorsolateral areas of the nucleus (**Figures 4D,F**). In the putamen positive neurons were mainly located in the low calret-ir area (**Figure 4E**), with neurons located primarily in dorsolateral positions in rostral regions, and progressing into more ventral and ventromedial positions caudally. Both nuclei contained moderately stained neurons, with labeling located in the cytoplasm and the initial segment of one or two processes (**Figures 4D–F**). In addition, calret-ir processes were observed crossing the internal capsule but not the anterior commissure.

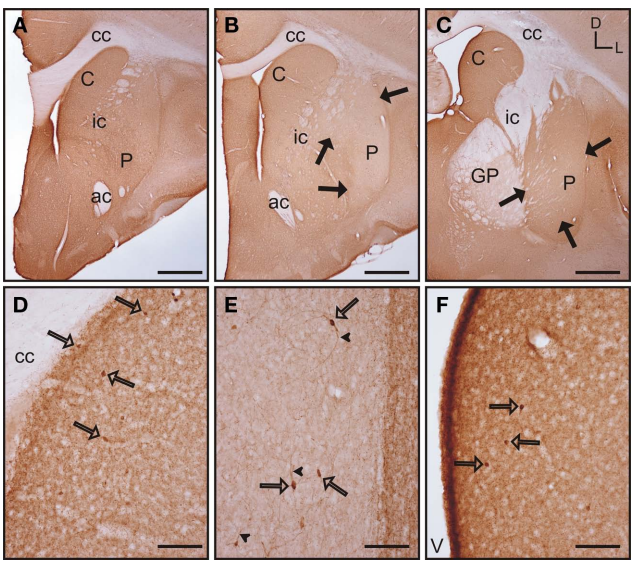

FIGURE 4 | Calretinin immunoreactivity in the tree shrew striatum. **(A–C)** Low magnification images of calretinin labeling in the rostral **(A)**, mid **(B)**, and caudal **(C)**, striatum. Note the presence of a large low calret-ir band in the mid and caudal regions of the putamen [black arrows in **(B,C)**]. **(D)** Detail of calretinin labeling in the rostral caudate showing scattered calret-ir cells (open arrows) embedded in a calretinin rich neuropil. **(E)** High magnification image of the mid putamen showing several calret-ir neurons (open arrows) located in an area of low calret-ir neuropil. Note also the presence of labeled processes in these cells (arrowheads). **(F)** Calret-ir cells in the caudal region of the caudate (open arrows). Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). ac, anterior commissure; C, caudate; cc, corpus callosum; ic, internal capsule; GP, globus pallidus; P, putamen; V, ventricle. Scale bars: 1 mm in **(A–C)**; 100 μm in **(D–F)**.

LABELING FOR CALBINDIN

In the rostral and mid areas of the striatum the caudate and putamen presented a similar pattern of calbindin immunoreactivity (calb-ir; **Figures 5A–B**), while in more caudal areas the caudate nucleus displayed more intense staining than the putamen (**Figure 5C**). Starting at the most rostral level of the striatum there were areas with qualitative differences in calbindin labeling within both the caudate and putamen. These areas followed a classical striosome/matrix organization, with stronger calb-ir present in the

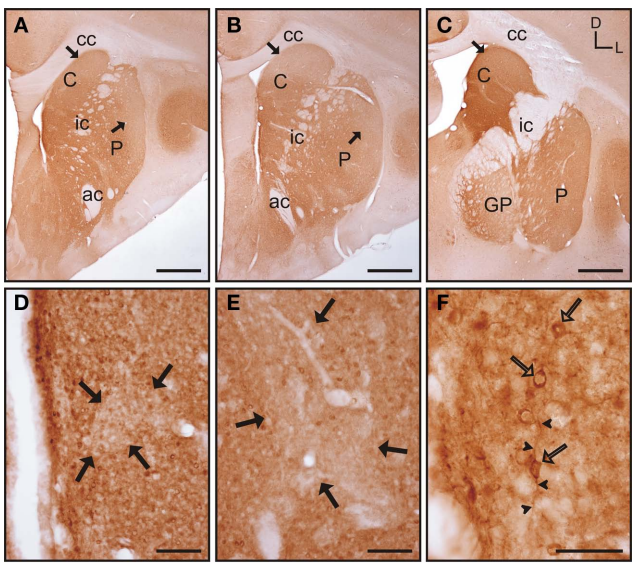

FIGURE 5 | Calbindin immunolabeling in the tree shrew striatum. **(A–C)** Low magnification images of the rostral **(A)**, mid **(B)**, and caudal **(C)** tree shrew striatum immunolabeled for calbindin. Note the presence of areas with higher and lower levels (black arrows) of calbindin immunolabeling. Also note the difference of labeling between the caudate and putamen in caudal areas **(C)**. **(D)** Detail of calbindin labeling demonstrating the presence of striosomes in the caudate (black arrows). **(E)** Detail of the putamen in which the striosome/matrix organization is also present. Note that both in the caudate **(D)** and in the putamen **(E)** the striosomes (black arrows) are not completely devoid of immunolabeling. **(F)** Detail of calbindin labeled cells (open arrows) in the caudate of the tree shrew. These cells contain cytoplasmic staining as well as labeling in the initial segment of processes (arrowheads). Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). ac, anterior commissure; C, caudate; cc, corpus callosum; ic, internal capsule; GP, globus pallidus; P, putamen. Scale bars: 1 mm in **(A–C)**; 100 μm in **(D–E)**; 50 μm in **(F)**.

matrix than in the striosomes (**Figures 5A–E**). At the most rostral level of the striatum two large striosomes with lower calb-ir were observed; one in the dorsomedial caudate, and another in the dorsolateral putamen. In addition, smaller low calb-ir striosomes were present in more ventral areas of both nuclei (**Figure 5A**). In mid areas, the large dorsolateral striosome of the putamen and the dorsomedial striosome of the caudate were still present (**Figure 5B**). In caudal areas, the dorsomedial striosome of the caudate was still visible, while in the putamen the striosome/matrix pattern was more difficult to discern due to the fact that most of the putamen was occupied by a large striosome (**Figure 5C**). A more detailed observation of the striosome and matrix compartments revealed that the matrix clearly presented a more abundant population of calb-ir cells, but cellular staining was also present in the striosomes (**Figures 5D–E**). Neurons located within the striosomes were generally lighter in their staining than the ones in the matrix. At the cellular level, calb-ir appeared as cytoplasmic labeling and in some cases the initial segment of one or two processes was also labeled (**Figure 5F**).

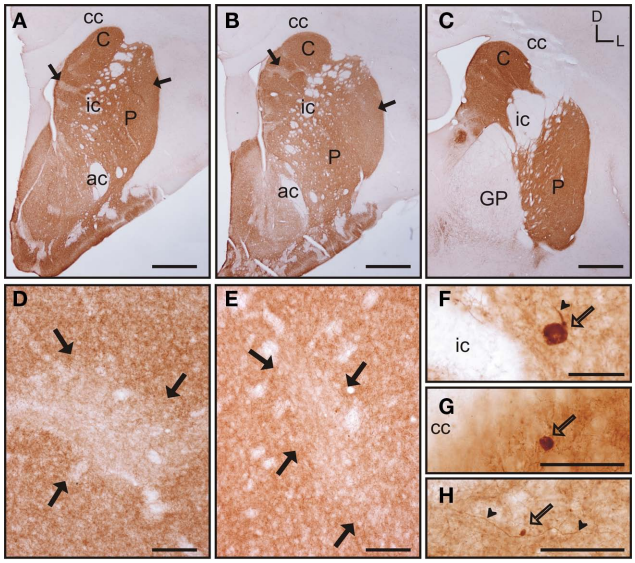

FIGURE 6 | Tyrosine hydroxylase immunostaining in the tree shrew striatum. (A–C) Low magnification images of TH labeling in the rostral **(A)**, mid **(B)**, and caudal **(C)** striatum, showing a patch-like pattern in both the caudate and the putamen. This pattern is more prominent in rostral and medial regions **(A,B)**. **(D)** Detail of a low TH-ir striosome (black arrows) in the mid caudate. **(E)** Detail of a low TH-ir striosome at the level of the mid putamen (black arrows). **(F–H)** Detail images of large **(F)**, medium-sized **(G)**, and very small **(H)** TH labeled cells in the tree shrew dorsal striatum. Some of these cells also present labeled processes [arrowheads in **(F,H)**]. Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). ac, anterior commissure; C, caudate; cc, corpus callosum; ic, internal capsule; GP, globus pallidus; P, putamen. Scale bars: 1 mm in **(A–C)**; 100 μm in **(D–E)**; 50 μm in **(F–H)**.

LABELING FOR TYROSINE HYDROXYLASE

The caudate and putamen contained abundant TH immunoreactivity (TH-ir) and the pattern of staining was similar between the two nuclei. Also, there were no differences in the pattern of staining along the rostrocaudal extent of each of these nuclei (**Figures 6A–C**). However, a patch-like pattern of labeling for TH-ir was observed in both nuclei, with the striosomes presenting lower TH-ir. Even when this pattern was present at all rostrocaudal levels of the caudate and putamen, the patch-like areas were more prominent in rostral and mid regions, especially in the caudate (**Figures 6A,B**). At high magnification this pattern of immunolabeling corresponded with areas that contained high and low density of TH-ir processes and terminals (**Figures 6D–E**). The corpus callosum, internal capsule, and the anterior commissure also contained a small number of TH-ir processes. A small number of TH-ir cells were present in the tree shrew dorsal striatum. These cells were not homogeneous in morphology, and according to their size they could be classified in three subtypes (**Figures 6F–H**). A summary of the morphological features and relative abundance of each type of TH-ir cells is shown in **Table 1**.

We found a great variability in the total number of TH-ir cells between the two animals used for neuronal counts [animal #1 had 175 TH-ir cells/series which yields a total of 1050 TH-ir cells for the whole dorsal striatum; animal #2 had 291 TH-ir cells/series which yields a total of 1746 TH-ir cells]. In contrast with the high variability observed in the total number of TH-ir cells, the percentage of cells of each subtype was very similar for both animals (see the low SD in **Table 1**).

LABELING FOR ACETYLCHOLINESTERASE

Acetylcholinesterase (AchE) histochemistry was classically used as one of the first staining techniques that revealed the striosome and matrix compartments (Graybiel and Ragsdale, 1978). This technique has as a drawback in the need for special fixatives (see Karnovsky and Roots, 1964), or very lightly fixed tissue (see, e.g., Puelles et al., 1996). This is not compatible with the immunohistochemical detection of other markers for striosome/matrix compartments (e.g., calbindin, TH) in parallel series from the same animal. To avoid this problem, instead of the histochemical procedure for the detection of AchE, we used immunohistochemistry to detect this enzyme. Two anti-acetylcholinesterase antibodies were used in our study: the first one has been shown to specifically recognize AchE in rat, feline and rabbit while not recognizing AchE in mouse, human, and chicken (Rackonczay and Brimijoin, 1986; see also Material and Methods). This antibody did not produce any labeling in the tree shrew striatum while clearly recognizing AchE in adult rat striatal sections used as a positive control (data not shown). The second antibody tested has been previously demonstrated to specifically recognize AchE in human brain (see Material and Methods). This second antibody produced positive labeling in the tree shrew striatum (**Figure 7**), while not producing any labeling in the rat striatum (data not shown).

In the tree shrew striatum AchE immunoreactivity (AchE-ir) displayed a "patchy distribution" with light striosomes and darker matrix. This pattern was evident in rostral areas of the dorsal striatum and became increasingly difficult to discern in mid and caudal regions (**Figures 7A–C**). At higher magnification it was evident that the striosomes contained a lower density of AchE-ir processes (**Figure 7D**). AchE-ir cells were present in all rostrocaudal areas of the caudate and putamen. These AchE-ir cells were moderately labeled cells and often presented labeled processes (**Figures 7E–F**).

Table 1 | Morphological features of TH labeled neurons in the tree shrew striatum.

Cell type	Average size (cross-section; μm^2)	Shape, processes, and staining	Mean percentage of total TH-ir cells (%)
Very small	20.47 ± 9.92	Oval to polygonal. Several thin processes. Staining moderate, rarely granular.	69.6 ± 3.11
Small to medium	73.12 ± 17.66	Round to polygonal with one process frequently observed. Rarely several processes. Staining from strong smooth to granular.	29.11 ± 3.27
Large to very large	259.63 ± 161.95	Round to oval with one process visible. Smooth strong labeling.	1.25 ± 0.16

TH immunoreactivity cells in the dorsal striatum were counted and classified in a total of two complete series of striatal sections from two different adult male tree shrews. Each series was composed of sections 300 μm apart and a total of 17 sections were studied per animal.

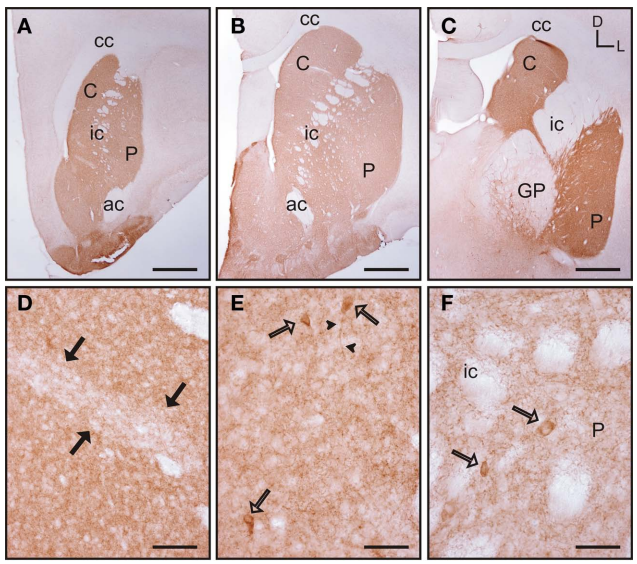

FIGURE 7 | Acetylcholinesterase immunolabeling in the tree shrew striatum. (A–C) Low magnification images of AchE labeling in the rostral **(A)**, mid **(B)**, and caudal **(C)** striatum showing a patchy distribution of immunostaining. **(D)** Detail of a patch of low AchE-ir (black arrows) in the rostral caudate. **(E,F)** Detail of AchE labeled cells (open arrows) in the caudate **(E)** and putamen **(F)**. Some of these cells present labeling in the initial segment of a process [arrowheads in **(E)**]. Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). ac, anterior commissure; C, caudate; cc, corpus callosum; ic, internal capsule; GP, globus pallidus; P, putamen. Scale bars: 1 mm in **(A–C)**; 100 μm in **(D)**; 50 μm in **(E–F)**.

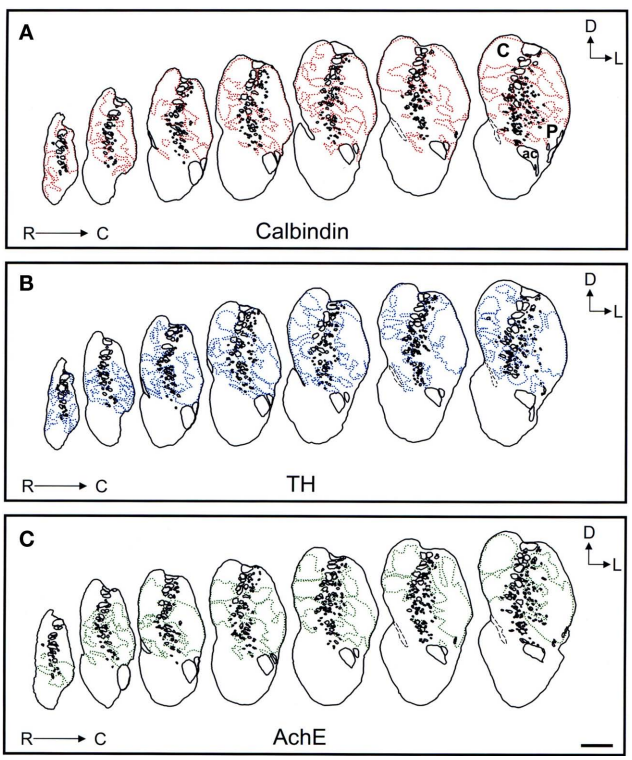

FIGURE 8 | Schematic drawings of the rostral to mid areas of the tree shrew dorsal striatum. These line drawings show the patch (striosomes) distribution for calbindin **(A)**, TH **(B)**, and AchE **(C)**. Drawings were obtained from whole series of the same animal stained for the different markers. Each of the contiguous rostrocaudal sections of the same marker are separated 300 μm. Separation between parallel sections labeled for calbindin or TH is 50 μm, while between calbindin and AchE is 100 μm. Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). ac, anterior commissure; C, caudate; P, putamen. Scale bars: 1 mm in **(A–C)**.

These AchE-ir cells appeared mostly in the matrix, sometimes surrounding the borders of the striosomes (**Figures 7E–F**).

COMPARISON OF THE STRIOSOME/MATRIX ORGANIZATION REVEALED BY CALBINDIN, TH, AND AchE IMMUNOHISTOCHEMISTRY

Schematic drawings of parallel sections were obtained for the three markers to analyze the pattern of striosome/matrix distribution (**Figures 8–10**). Calbindin and TH yielded the best definition of the striosome and matrix compartments. In rostral to mid regions calbindin demonstrated the presence of a large dorsolateral patch in the caudate that was still present in mid and caudal regions (**Figures 8A**, **9A**, and **10A**). In TH-ir and AchE-ir sections, one or several closely distributed striosomes also appeared in this dorsolateral area of the caudate across the rostrocaudal extent of this nucleus (**Figures 8B,C**,**9B,C**, and **10B,C**). However, there were some slight differences mainly in the most rostral areas of the caudate (for example, the dorsolateral striosome appeared earlier in the calb-ir sections than in the case of sections labeled for TH or AchE; **Figures 8A–C**). The three markers consistently revealed the presence of smaller striosomes in more ventral areas of the caudate, but the pattern of striosome/matrix distribution was more closely matched for calbindin and TH than for AchE (**Figures 8–10**).

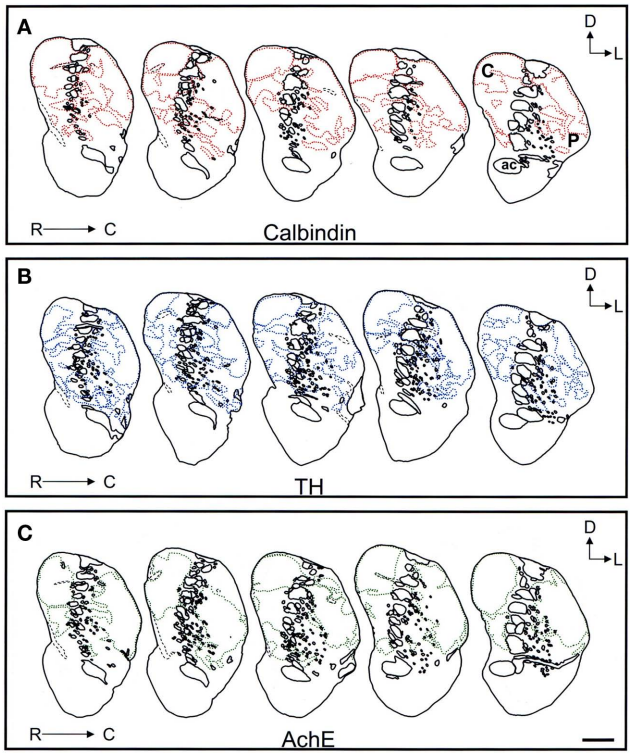

FIGURE 9 | Schematic drawings of the mid to caudal areas of the tree shrew dorsal striatum. These line drawings show the patch (striosomes) distribution for calbindin **(A)**, TH **(B)**, and AchE **(C)**. Drawings were obtained from whole series of the same animal stained for the different markers. Each of the contiguous rostrocaudal sections of the same marker are separated 300 μm. Separation between parallel sections labeled for calbindin or TH is 50 μm, while between calbindin and AchE is 100 μm. Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). ac, anterior commissure; C, caudate; P, putamen. Scale bars: 1 mm in **(A–C)**.

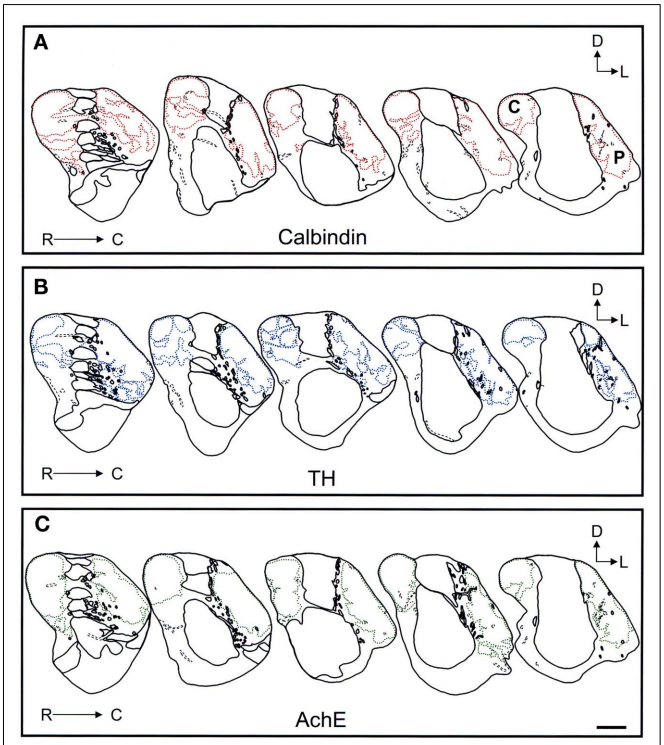

FIGURE 10 | Schematic drawings of the most caudal areas of the tree shrew dorsal striatum. These line drawings show the patch (striosomes) distribution for calbindin **(A)**, TH **(B)**, and AchE **(C)**. Drawings were obtained from whole series of the same animal stained for the different markers. Each of the contiguous rostrocaudal sections of the same marker are separated 300 μm. Separation between parallel sections labeled for calbindin or TH is 50 μm, while between calbindin and AchE is 100 μm. Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). C, caudate; P, putamen. Scale bars: 1 mm in **(A–C)**.

In the putamen the three markers showed a very consistent pattern with a large striosome (sometimes observed as several closely attached striosomes in rostral regions) that occupied most of the nucleus. This large striosome was especially consistent in mid and caudal regions (**Figures 8–10**).

LABELING FOR GAD65/67 AND MORPHOLOGY OF MEDIUM SPINY NEURONS

The caudate and putamen presented a similar pattern of immunolabeling for GAD 65/67, and this remained constant throughout the rostrocaudal extent of both nuclei (**Figures 11A–C**). GAD 65/67 staining within the caudate and putamen was observed as strongly labeled puncta surrounding unstained neurons. The corpus callosum and the anterior commissure also had GAD 65/67 labeling. Staining within these fiber bundles was observed as scattered immunoreactive processes, running in parallel to the fiber bundles (**Figure 11D**). In addition, the corpus callosum also contained a scarce number of GAD 65/67 positive neurons (**Figure 11D**). In the internal capsule immunoreactive processes were observed crossing perpendicular to the fiber bundles (**Figure 11E**). Due to the strong labeling of processes and terminals, the detection of immunopositive neurons in the caudate and putamen was difficult. However, the use of Nissl counterstained sections allowed discernment of the presence of some GAD65/67 labeled neurons that mostly contained cytoplasmic granular labeling (**Figure 11F**).

Golgi impregnation was used to study in more detail the morphological features of the medium spiny neurons of the tree shrew striatum. A summary of their morphological features is shown in **Table 2**. This type of GABAergic neurons were easily distinguished from other cell types in Golgi impregnated tissue (**Figures 12A–C**). A qualitative observation revealed that these cells presented a lower density of dendritic spines in their primary dendrites than in their secondary and higher order dendrites (**Figures 12B,C**), which were densely covered with dendritic spines of diverse morphologies (**Figures 12D,E**).

DISCUSSION

Recent advances in molecular phylogenetics have allowed the study of large DNA data sets and reach the current consensus that Dermoptera (flying lemurs) and *Scandentia* (tree shrews) are the closest living relatives to Primates (Adkins and Honeycutt, 1991;

Table 2 | Morphological features of the medium spiny neurons of the tree shrew striatum.

Shape of soma	Number of primary dendrites	Mean soma cross-section (area; μm^2)	Mean distance soma to secondary dendrites (μm)	Mean distance soma to first dendritic spine (μm)
Round to polygonal	5–6	273.26 ± 49.86	15.86 ± 6.19	10.36 ± 6.69

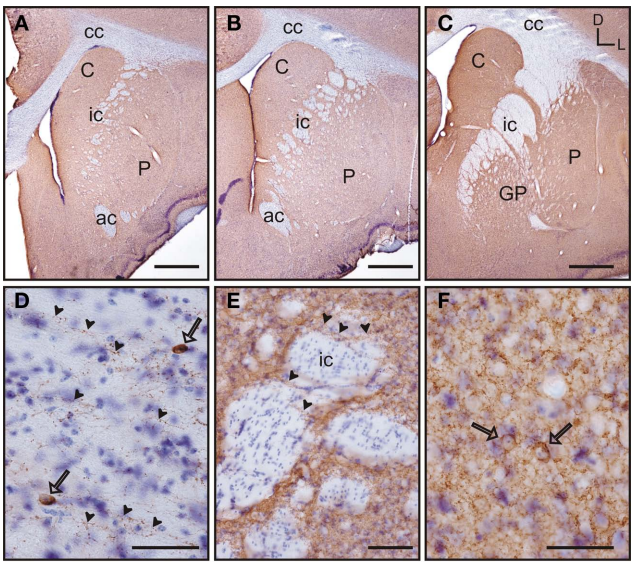

FIGURE 11 | GAD 65/67 immunolabeling in the tree shrew striatum. GAD65/67 stained sections were counterstained with thionin (Nissl stain) to improve the visualization of labeled neurons. **(A–C)** Low magnification images of GAD 65/67 labeling in the rostral **(A)**, mid **(B)**, and caudal **(C)** striatum. GAD 65/67 appears as a rather homogeneous labeling. **(D)** Detail of GAD 65/67 immunoreactive fibers (arrowheads) running in parallel to the fiber bundles of the corpus callosum. Some GAD 65/67 positive neurons are also observed (open arrows). **(E)** Detail of GAD 65/67 immunolabeled fibers (arrowheads) crossing perpendicularly to the fiber bundles of the internal capsule (ic). **(F)** Detail of two GAD 65/67 immunoreactive neurons (open arrows) embedded in a rich GAD 65/67-ir neuropil in the caudate nucleus. Coordinates indicate the orientation of the sections in the figure (D, dorsal; L, lateral). ac, anterior commissure; C, caudate; cc, corpus callosum; ic, internal capsule; GP, globus pallidus; P, putamen. Scale bars: 1 mm in **(A–C)**; 100 μm in **(E)**; 50 μm in **(D,F)**.

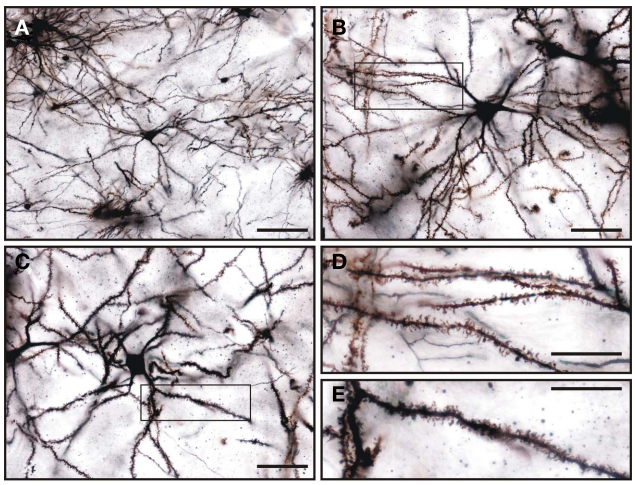

FIGURE 12 | Golgi impregnated medium spiny neurons in the tree shrew dorsal striatum. (A–C) General view of medium spiny neurons of the tree shrew striatum showing the classical morphology of this type of neuron. Details of dendrites and dendritic spines are shown in **(D)** for the boxed area shown in **(B)**, and in **(E)** for the boxed area shown in **(C)**. Note the presence of a high density of dendritic spines of different morphologies in these dendrites. Scale bars: 100 μm in **(A)**; 50 μm in **(B–C)**; 25 μm in **(D–E)**.

Liu et al., 2001; Murphy et al., 2001; Janecka et al., 2007). Even when these molecular studies have advanced our knowledge on tree shrew phylogenetics, data in other related fields such as comparative neuroanatomy is fragmented and largely focused on the study of the visual system. Comprehensive studies on the visual system together with studies of cortical territories have already provided hints that indicate the tree shrew brain may share many common features with primates (see, e.g., Lyon et al., 2003; Remple et al., 2007; Chomsung et al., 2010). A second important set of data comes from behavioral and physiological studies that provide compelling evidence that tree shrews are closer to primates in their responses than to rodents (Bartolomucci et al., 2001; see as reviews Fuchs and Flugge, 2002; Fuchs, 2005). All this body of data strongly supports that tree shrews are closely related to primates in brain anatomy and function, and can be a suitable model for neuropathology studies (see as a review Cao et al., 2003).

The tree shrew striatum has not been previously studied in detail. Although some research has provided punctual data concerning this brain region (Lin et al., 1984; Mijnster et al., 1999; Isovich et al., 2000; Day-Brown et al., 2010), as far as we are aware this is the first study that aims to characterize this brain area in detail. The tree shrew striatum presents most of the main morphological features observed in primates and other higher mammals, such as the presence of a well-developed internal capsule that clearly separates the caudate and the putamen nuclei. These features place the tree shrew striatum, at least from a morphological perspective, closer to primates than to rodents. Based on these morphological similarities, we undertook the study of several well-known striatal markers in order to further characterize this brain region.

The pattern of expression of some of the major calcium-binding proteins has been thoroughly studied in the striatum of mammals including primates and rodents (Cote et al., 1991; Hiroi, 1995; Figueredo-Cardenas et al., 1996; Parent et al., 1996; Holt et al., 1997; Prensa et al., 1998; Wu and Parent, 2000; Morel et al., 2002). Some of these proteins differ considerably in their pattern of distribution in the striatum when primates and rodents are compared (De las Heras et al., 1994; Wu and Parent, 2000). Among these calcium-binding proteins, calbindin has been proven to reveal the striosome/matrix cytoarchitecture of the dorsal striatum

(see, e.g., Liu and Graybiel, 1992; Kubota and Kawaguchi, 1993; Rajakumar et al., 1993; Parent et al., 1996; Holt et al., 1997; Morel et al., 2002).

Labeling for parvalbumin in the tree shrew striatum has been recently reported by Day-Brown et al. (2010) as a marker for the striosome/matrix compartments. These authors showed that parvalbumin labeling was low in the striosomes and high in the matrix, in a similar manner to what we found in our study for rostral areas of the caudate and putamen (present results). However, our detailed study of all the rostrocaudal extent of the striatum did not support that parvalbumin aligns with a striosome/matrix organization in caudal areas. This discrepancy may be due to the fact that these authors studied the expression of parvalbumin in specific areas of the striatum, while our study encompasses the analysis of the entire rostrocaudal extent of this region. In accordance with our data, in primates including humans, bands of higher and lower staining have also been found in the caudate and putamen (Waldvogel and Faull, 1993; Parent et al., 1996; Holt et al., 1997; Prensa et al., 1999), but these bands did not completely align with the striosome/matrix organization revealed by calbindin staining (Waldvogel and Faull, 1993; Parent et al., 1996). Apart from the partial recognition of the striosome/matrix pattern some authors have reported a rostrocaudal gradient of labeling for parv-ir in the squirrel monkey and in human striatum (Parent et al., 1996; Prensa et al., 1999). However, Holt et al. (1997) and Bernacer et al. (2008) did not report the existence of a rostrocaudal gradient for parvalbumin in the human striatum. In our study we did not find any rostrocaudal gradient in the tree shrew caudate and putamen. An additional increasing dorsoventral gradient has been reported in human and non-human primates (Parent et al., 1996; Holt et al., 1997; Prensa et al., 1999; Morel et al., 2002; Bernacer et al., 2008). This gradient was not observed in the tree shrew (present results).

Parv-ir neurons in the tree shrew striatum appeared mostly distributed in the matrix and were loosely arranged in clusters that may correspond with the so-called matrisomes (clusters of projection neurons) found in the striatal matrix in felines and primates (Gimenez-Amaya and Graybiel, 1990; Selemon and Goldman-Rakic, 1990; Graybiel et al., 1991; Desban et al., 1995). Parv-ir cells in the tree shrew dorsal striatum were morphologically very similar to these cells in human and non-human primates, presenting cytoplasmic labeling with several processes emanating from the stained cell (Holt et al., 1997; Prensa et al., 1999; Wu and Parent, 2000; Kataoka et al., 2010). In addition, as reported in humans (Prensa et al., 1999), parv-ir cells were distributed both in areas of high and low parv-ir. In summary, despite of the lack of a dorsoventral gradient, parvalbumin expression in the tree shrew dorsal striatum presents many of the same core features of parv-ir in human and non-human primates.

Immunolabeling for calretinin in the tree shrew striatum presented both similarities and differences when compared to primates. In the tree shrew putamen there was a decreasing rostrocaudal gradient of calretinin labeling, largely due to the presence of a band of low immunoreactivity in the dorsal region. This low immunoreactivity band increased in size in more caudal regions of the striatum, occupying more ventrolateral and medial positions within the putamen. A previous study reported a similar pattern of low calret-ir in dorsal areas of the squirrel monkey striatum, although in that case both the putamen and caudate presented this gradient (Parent et al., 1996). Calretinin positive neurons in the tree shrew dorsal striatum were characterized by the presence of one to two labeled processes, and were medium-sized. These findings were similar to what has been reported in the caudate and putamen of human and non-human primates, as well as in rodents (Bennett and Bolam, 1993; Fortin and Parent, 1994; Parent et al., 1995, 1996; Cicchetti et al., 1998; Mura et al., 2000). In the human, an additional population of large-sized calretinin positive neurons has been observed in the dorsal striatum, even when these neurons represented only a small fraction of the calret-ir cell population (Parent et al., 1995; Cicchetti et al., 1998). In the squirrel monkey, this population of large-sized calretinin immunolabeled neurons has only been reported in the limbic striatum, outside of the caudate and putamen nuclei (Parent et al., 1996). Large-sized calret-ir neurons have been tentatively identified as large cholinergic striatal interneurons (Parent et al., 1995; Cicchetti et al., 1998) and may be restricted to the ventral striatum of the tree shrew, similar to what has been reported in the squirrel monkey (Parent et al., 1996). Even when calretinin expression in the tree shrew striatum presented some similarities with primates, there were also specific features, such as the presence of a clear difference in the distribution of calretinin labeling between the caudate and putamen, that have not been reported in primates (Fortin and Parent, 1994; Parent et al., 1995, 1996).

STRIOSOME/MATRIX PATTERN IN THE TREE SHREW

The striosome and matrix are two distinct cytoarchitectonic compartments consistently present in the striatum of many mammals including primates, felines, and rodents (see, e.g., Graybiel and Ragsdale, 1978; Graybiel et al., 1981; Gerfen, 1984; Donoghue and Herkenham, 1986; Desban et al., 1993; Parent et al., 1996; Holt et al., 1997; Prensa et al., 1999). The striosome/matrix organization was first visualized by Graybiel and Ragsdale (1978) using acetylcholinesterase histochemistry. In this work we showed that this organization is also present in the tree shrew striatum using *calbindin immunolabeling*, another well-established marker for these cytoarchitectonic compartments (see, e.g., Gerfen et al., 1985; Gerfen, 1992; Parent et al., 1996; Holt et al., 1997; Prensa et al., 1999). One prominent feature of the striosome/matrix organization in the tree shrew is the existence of two large striosomes in the dorsomedial caudate and dorsolateral putamen. This pattern may correspond with the increasing dorsoventral gradient that has been observed in human and non-human primates (Parent et al., 1996; Holt et al., 1997). On the contrary, in the rat, instead of a gradient, the dorsolateral caudate/putamen is free of calbindin neuropil, while calb-ir neurons are still present (see as a review Hontanilla et al., 1998). In agreement with our findings in the tree shrew, Prensa et al. (1999) reported that these large striosomes that occupy the dorsal half of the caudate nucleus were also present in humans. Also, in agreement with our results, Parent et al. (1996) reported that this striosome/matrix pattern became less apparent in more caudal sections. In fact, in our study we have observed that calb-ir decreased, but was still present in the striosomes to a certain degree. This observation is similar to what has been found in both squirrel monkey (Parent et al., 1996), and

rat (Hontanilla et al., 1998). An extra layer of complexity in the striosome/matrix organization has been described using calbindin in the human striatum. In the posterior human striatum it has been reported that calbindin labeling produced a ring-like structure within the striosomes, and this could be interpreted as the presence of a "core" and a "shell" within the striosomes (Bernacer et al., 2008). We did not observe any ring-like structure in the tree shrew striatum, although there was a gradient of calbindin labeling toward the center of the striosomes (see **Figures 5D,E**). This gradient has also been observed in non-human primates such as the squirrel monkey (Levesque et al., 2004). Calbindin neuronal labeling in the tree shrew was similar to that found in non-human primates, with calbindin positive neurons displaying labeling in the cytoplasm and in the initial segments of one or more processes (Parent et al., 1996). Overall, the pattern of calbindin expression in the tree shrew striatum was remarkably similar to the expression of this marker in human and non-human primates. This supports that the striosome/matrix distribution observed in the tree shrew dorsal striatum is very similar to what has been reported in primates including humans.

Studies on *tyrosine hydroxylase* expression in the dorsal striatum have shown that this enzyme also reveals the striosome/matrix compartments in primates (Graybiel et al., 1987; Prensa et al., 1999; Stephenson et al., 2005; Bernacer et al., 2008), with the striosomes appearing as low TH-ir areas. In the tree shrew striatum TH-ir also revealed the striosome/matrix organization, and our detailed mapping showed that the distribution of these compartments matched almost exactly the organization recognized by calbindin labeling (see **Figures 8–10**). Similar results have been reported in the striatum of primates including humans (Graybiel et al., 1987; Prensa et al., 1999; Stephenson et al., 2005; Bernacer et al., 2008) but not in adult rodents (see, e.g., Hedou et al., 2000; Sutoo et al., 2002). On the other hand, it has been reported by Sutoo et al. (2002) that a gradient of TH staining was present in the striatum of the rodent, although this gradient did not correspond with a striosome/matrix organization.

Another salient feature of TH labeling in the tree shrew striatum was the presence of a discrete number of TH-ir cells that can be morphologically classified in three subtypes. The presence of discrete numbers of dopaminergic cells in primates including humans has been consistently reported (Dubach et al., 1987; Ikemoto et al., 1996; Betarbet et al., 1997; Cossette et al., 1999, 2004, 2005; Huot and Parent, 2007), while in rodents TH-ir cells are almost anecdotal in the normal striatum (Tashiro et al., 1989; Mura et al., 1995; Baker et al., 2003; Huot and Parent, 2007). In addition, as we observed in the tree shrew (present results), dopaminergic cells in the primate striatum presented a variety of subtypes readily distinguishable by their size and other morphological features (Dubach et al., 1987; Betarbet et al., 1997; Cossette et al., 2005; Huot and Parent, 2007), and it has been postulated that most of these TH-ir neurons are GABAergic interneurons (Betarbet et al., 1997). In summary, TH labeling in the tree shrew striatum strikingly resembled the pattern observed in primates and clearly diverged with the expression of TH in rodents.

Acetylcholinesterase was the first enzyme found to reveal the striosome/matrix organization in the striatum. Histochemistry and immunohistochemistry against this enzyme, or immunohistochemistry against choline acetyltransferase (the rate-limiting enzyme for the production of acetylcholine), have been used to reveal these cytoarchitectonic compartments in primates as well as in several non-primate species (see, e.g., Graybiel and Ragsdale, 1978; Natuk and Graybiel, 1985; Graybiel et al., 1986; Loopuijt et al., 1987; Rhodes et al., 1987; Hirsch et al., 1989; Holt et al., 1996, 1997; Prensa et al., 1999; Bernacer et al., 2008).

In this study we used two different antibodies against acetylcholinesterase. One of the antibodies was designed to specifically recognize human AchE (see Materials and Methods). The second antibody has been reported to recognize AchE in rodents, felines, and rabbits, but not in primates (Rackonczay and Brimijoin, 1986). In our work we found that the antibody that recognizes AchE only in non-primates did not produce any positive results in the tree shrew tissue, while producing positive results in rat striatal sections incubated together. On the contrary, the antibody specifically produced to recognize human AchE yielded positive results in the tree shrew. This finding may indicate that tree shrew acetylcholinesterase is closer to the primate forms of this enzyme than to the forms present in rodents and other non-primate species. Acetylcholinesterase immunolabeling in the tree shrew presented a similar pattern to what has been shown in primates including humans (see, e.g., Graybiel and Ragsdale, 1978; Natuk and Graybiel, 1985; Hirsch et al., 1989; Holt et al., 1996, 1997; Prensa et al., 1999; Bernacer et al., 2008). Our results also showed that the striosomes identified by AchE immunolabeling aligned in a great manner with the striosomal organization revealed by calbindin and TH (see **Figures 8–10**, present results). Comparison of the three striosome/matrix markers used in the present study clearly demonstrated that the tree shrew also possesses these cytoarchitectonic compartments. Our results also showed that calbindin and tyrosine hydroxylase were the most effective markers for the delineation of these compartments in the tree shrew, although acetylcholinesterase antibodies also revealed a very similar striosome/matrix organization.

LABELING FOR GAD 65/67 AND MORPHOLOGY OF MEDIUM SPINY NEURONS

The major cell type of the mammalian dorsal striatum is the medium spiny GABAergic neuron. This type of projection neurons constitute up to 95% of the total number of neurons in the striatum of felines and rodents (Kemp and Powell, 1971; Graveland and DiFiglia, 1985; see as a review Voogd et al., 1998) and approximately 75% in the primate striatum (Graveland and DiFiglia, 1985; see as reviews Nieuwenhuys et al., 2007; Tepper et al., 2010). GABA in the striatum is expressed in the medium spiny projection neurons as well as in local interneurons (Cowan et al., 1990; Kita et al., 1990; Kawaguchi, 1993; Kubota et al., 1993; Kubota and Kawaguchi, 2000; see as reviews, Nieuwenhuys et al., 2007; Tepper et al., 2010), producing a very rich and intricate network of GABAergic cell bodies and processes.

In the tree shrew dorsal striatum *GAD 65/67 labeling* consisted of densely packed and strongly labeled puncta. Even when they were rarely observed, GAD 65/67 positive neurons were also present and displayed cytoplasmic granular staining. Cellular immunolabeling is difficult to detect with anti-GAD 65/67 antibodies in the striatum under normal conditions, due to the

rapid transportation of GAD from the soma to processes and terminal buttons (Ribak et al., 1978, 1979; Kita and Kitai, 1988). The difficulty associated with the observation of GAD neuronal staining has also been reported in primates and rodents (see, e.g., Levesque et al., 2004; Stephenson et al., 2005). GAD soma staining can only be clearly observed in a high number of neurons after using colchicine, a chemical treatment that prevents the rapid transport of GAD from the soma (Ribak et al., 1978, 1979; Kita and Kitai, 1988; Vuillet et al., 1990; Kubota et al., 1993). GAD 65/67 labeling in the dorsal striatum of the tree shrew appeared as strong puncta immunolabeling that was homogeneously distributed and did not present a patch-like pattern or clear gradient. Levesque et al. (2004) reported that the expression of GAD resembled a patch-like pattern in primates, but not in rodents. However, studies using quantitative immunohistochemistry for GAD in the human striatum did not replicate these findings of a patch-like distribution (Sutoo et al., 2000). In this quantitative study by Sutoo et al. (2000), the only reported change in the intensity of GAD involved the presence of a dorsoventral decreasing gradient in the caudate, and a slight dorsoventral increasing gradient in the putamen. In support of this, Stephenson et al. (2005) neither reported nor showed any evidence of a patch-like distribution for GAD 65 or GAD 67 in the striatum of untreated monkeys. In view of these findings, a patch-like pattern of GAD immunolabeling in primates as a group appears to be rather controversial, with quantitative work in the human supporting a non-patch-like distribution of GAD staining (Sutoo et al., 2000). Although a qualitative observation of GAD labeling in the tree shrew striatum did not reveal any gradient of staining in the caudate or putamen, we can not discard the possibility that a quantitative analysis may reveal some slight differences between dorsal and ventral regions of these nuclei.

To further characterize the medium spiny GABAergic neurons in the tree shrew we used *Golgi impregnation* techniques. Our results showed that medium spiny cells in the tree shrew presented a round to polygonal soma and 5–6 primary dendrites that emanated from the cell body. Similar cell shapes and numbers of primary dendrites have been reported in medium spiny neurons of primates, felines, and rodents (Kemp and Powell, 1971; Mensah and Deadwyler, 1974; DiFiglia et al., 1976; Braak and Braak, 1982; Graveland et al., 1985; Yelnik et al., 1991). In our study we also showed that the average cross-section of the soma was 273.26 μm^2, and that the mean distance from the soma to secondary dendrites was 15.86 μm. These measurements were in the same range that has been observed in primates (DiFiglia et al., 1976; Braak and Braak, 1982; Graveland et al., 1985; Yelnik et al., 1991), while in rodents and in the cat, the soma size for this type of cells varies in a larger manner (10–20 μm of diameter in the rat, and 12–18 μm in the cat, which assuming an spheroid shape for the soma is equivalent to cross-sections of 78–314 and 113–254 μm^2, in the rat and cat respectively; Kemp and Powell, 1971; Mensah and Deadwyler, 1974). In addition, as we report here for the tree shrew, in some rare cases dendritic spines were observed in the soma of medium spiny neurons in primates (DiFiglia et al., 1976; Graveland et al., 1985).

Overall, the neurochemical characterization of the tree shrew dorsal striatum revealed striking similarities in the distribution of most of the markers studied when compared to primates, while presenting more marked divergences compared to the rodent striatum. Our results suggest that the tree shrew striatum could represent a good animal model for studies of the basal ganglia aimed at the analysis of the expression of neurotransmitters or other molecules in normal or diseased brain.

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Role of basal ganglia in sleep–wake regulation: neural circuitry and clinical significance

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Researchers over the last decade have made substantial progress toward understanding the roles of dopamine and the basal ganglia (BG) in the control of sleep–wake behavior. In this review, we outline recent advancements regarding dopaminergic modulation of sleep through the BG and extra-BG sites. Our main hypothesis is that dopamine promotes sleep by its action on the D2 receptors in the BG and promotes wakefulness by its action on D1 and D2 receptors in the extra-BG sites. This hypothesis implicates dopamine depletion in the BG (such as in Parkinson's disease) in causing frequent nighttime arousal and overall insomnia. Furthermore, the arousal effects of psychostimulants (methamphetamine, cocaine, and modafinil) may be linked to the ventral periaquductal gray (vPAG) dopaminergic circuitry targeting the extra-BG sleep–wake network.

Keywords: dopamine, Parkinson's disease, insomnia, ventral periaquductal gray, globus pallidus, psychostimulants, D2 receptors

NEUROCHEMISTRY OF THE BASAL GANGLIA

The BG consists of four major nuclei, including the striatum (caudate and putamen), globus pallidus (GP), subthalamic nucleus (STN), and substantia nigra. Each of these nuclei differs in their neurochemistry, projection pattern, and function (see following sections). More than 95% of neurons in the striatum are projection neurons while the remaining 5% are interneurons (Wichmann and DeLong, 2006; Kreitzer, 2009). The projection neurons, which are medium spiny neurons (MSN), contain gamma amino butyric acid (GABA) as their neurotransmitter and hence are inhibitory in nature (Wichmann and DeLong, 2006). Some of the interneurons in the striatum, on the other hand, are excitatory and contain acetylcholine as their neurotransmitter (Lobo, 2009; Nambu, 2009). These cholinergic interneurons express vesicular glutamate transporter 3 (VGLUT3), and thus may also contain glutamate. The rest of the interneurons in the striatum are mostly GABAergic.

Although traditionally the GP is considered as a single nucleus, it comprises two neurochemically and functionally distinct subnuclei – the globus pallidus external (GPe) and globus pallidus internal (GPi). All the neurons in the GPe are GABAergic whereas the GPi contains both glutamatergic and GABAergic neurons (Barroso-Chinea et al., 2008). It is likely that both GABA and glutamate are present in the same neurons in the GPi, but it has never been confirmed. STN neurons are also glutamatergic, while the substantia nigra pars compacta (SNc) and pars reticulata (SNr) comprise mostly dopaminergic and GABAergic neurons, respectively (Wichmann and DeLong, 2006). In addition to these major neurotransmitters, BG neurons also contain other transmitters or neuropeptides, including enkephalin (ENK) and dynorphin in striatal MSNs; substance P, neuropeptide Y and nitric oxide in the striatal interneurons; and ENK in the GPe neurons.

IMPLICATIONS OF THE BG IN SLEEP–WAKE CONTROL

The BG has been implicated in a variety of motor and cognitive behaviors, including planning and other executive functions (Nambu, 2008). While these "higher level" cognitive processes are associated with the vigilance state of wakefulness, the involvement of the BG in the control of wakefulness *per se* has received very little attention. The BG is very likely involved in sleep regulation, considering the occurrence and high prevalence of sleep-related issues in patients with BG-related neurogenerative disorders, such as Parkinson's disease (PD) and Huntington's disease (HD). Disturbances of sleep are highly prevalent in PD, affecting up to 88% of patients in a community-based study (Factor et al., 1990), although a range of 55–98% has been reported across various studies (Larsen and Tandberg, 2001; Kaynak et al., 2005; Gjerstad et al., 2007). However, the interaction between PD and sleep is complex because PD patients may also have primary sleep disturbances such as rapid eye movement (REM) sleep behavior disorder, periodic limb movements during sleep and restless legs syndrome (Askenasy, 2001). Other factors such as depression, reactions to medications, nocturia, akinesia, pain, and dystonia may also underlie their sleep disturbances (Dhawan et al., 2006; Chaudhuri and Schapira, 2009). Nevertheless, insomnia and daytime sleepiness are the most frequent sleep-related complaints of PD patients. Although both sleep-onset insomnia and sleep-maintenance insomnia are reported in PD, sleep-maintenance insomnia is more common, affecting 74–88% of PD patients (Factor et al., 1990). Moreover, polysomnographic studies have demonstrated that these patients experience light and fragmented sleep. Similar to PD patients, HD patients have also been found to spend less total time in slow wave sleep (SWS) and REM sleep and have fragmentation in all states of sleep–wakefulness (S–W). In the most severe cases of HD, REM sleep has been reported to be completely absent (Arnulf et al., 2008). In addition to these clinical findings suggesting the involvement of the BG in S–W, many preclinical studies in animal

models have shown that the BG may play an important role in S–W behavior and cortical activation, which will be the main focus of this review.

NEURONAL ACTIVITY OF THE BG ACROSS S–W BEHAVIOR

Although the activity pattern of BG neurons has been studied extensively, only a few studies have investigated BG unit activity in relation to cortical EEG or the sleep–wake states. Nevertheless, the available studies indicate that most of the BG neurons are wake-REM active, meaning they fire faster during wake and REM sleep (desynchronized EEG) than during SWS. For example, Mahon and colleagues performed intracellular recordings from striatal MSNs and showed that MSN activity critically depended upon the vigilance state. MSNs exhibited rhythmic action potential fluctuations between a DOWN state (hyperpolarized quiescent state) and an UP state (depolarized state) during SWS, coincident with cortical activity, but during wake and REM sleep they switched into high rates of discharge with "random" patterns that were not synchronized with cortical EEG UP and DOWN states (Detari et al., 1987; Mahon et al., 2003; Mahon et al., 2006). Similar to striatal MSNs, GPe neurons are more active during wake and REM sleep (Urbain et al., 2000). These findings from head-restrained animals were recently confirmed in our laboratory using freely-moving animals (S. Thankachan and J. Lu, unpublished data). This study demonstrated that ca. 50% of GPe neurons increased their firing during REM sleep and during active wakefulness. In addition, GPe neurons tend to display burst-firing regardless of S–W state. Moreover, it has been shown that GPe burst-firing is reduced following dopamine depletion in the BG in experimental animals and in PD patients (Pan and Walters, 1988; Filion and Tremblay, 1991; Filion et al., 1991; Hutchison et al., 1994; Bouali-Benazzouz et al., 2009). Finally, while SNc dopaminergic neurons do not particularly change their firing rates across S–W behavior, they switch to burst-firing mode during wake and REM sleep (Dahan et al., 2007; Monti and Monti, 2007). Burst-firing in the SNc may trigger more dopamine release in the BG during wake and REM sleep, and thus may have a stronger influence on striatal and GPe activity during wake and REM sleep than during SWS. Taken together, it appears that BG neuronal activity is lower during SWS than during wake or REM sleep, and depends upon cortical activity. In accordance with these conclusions, cortical stimulation triggered a sequence of postsynaptic responses including initially a short EPSP, then a short IPSP, and then a late EPSP with multiple spikes in all the BG nuclei including the striatum, GPe, GPi, STN, and SNr (Wilson et al., 1983; Kita, 1992; Fujimoto and Kita, 1993; Nambu et al., 2000). However, considering the numerous excitatory and inhibitory connections between the BG nuclei, it is not clear how cortical stimulations elicit the same sequence of postsynaptic events in all these nuclei. It is possible that powerful cortical drives can overpower other within-BG synaptic events. It is also possible that the latency of these events could be different in different nuclei following cortical stimulation, as none of these studies recorded neuronal activity from the different BG nuclei simultaneously following cortical stimulation. Nevertheless, it is known that neuronal activity in all the BG nuclei is strongly synchronized with cortical activity and is modulated by dopamine, serotonin and thalamic inputs during wakefulness and REM sleep.

To supplement the electrophysiological data, we used cFos as a marker for neuronal activity and studied cFos expression in the various nuclei of the BG during wake and sleep in rats. Animals were sacrificed either after spontaneous sleep (sleep group, $n = 5$), after 2 h of sleep deprivation (wake group, $n = 5$) or 2 h after intraperitoneal injection of methamphetamine (drug-induced wake group, $n = 5$) at a dose of 1 mg/kg body weight. The animals were sacrificed during the same circadian time to avoid any circadian influence in cFos expression. After perfusions, rat brains were sectioned at 40 μm into four series, and one series was immunohistochemically dual-labeled for tyrosine hydroxylase (TH, a marker for dopaminergic neurons) and cFos. Our data (**Figure 1**) showed that cFos expression in the dorsal striatum and STN was elevated during wakefulness (immediately after sleep deprivation) and during active wakefulness following administration of methamphetamine (1.0 mg/kg), compared to during sleep. The medial part of the GPe also showed a moderate increase in cFos expression during both types of wakefulness than during sleep. On the other hand, cFos expression in the ventral striatum (i.e., the nucleus of the accumbens), GPi and SNr was considerably lower during both wakefulness and sleep but slightly higher in the GPi and SNr during wakefulness (of both types) than during sleep. Finally, cFos expression was not observed in the dopaminergic neurons in the VTA, SNc and retrorubal area in any of these conditions. Collectively, these results suggest that cFos activity and neuronal firing patterns are distinct in different nuclei of the BG, and that they may play specific independent roles in S–W regulation. These differences in activity pattern can be partially explained by their differences in neurochemistry, and thus juxtacellular and dual immunolabeling studies are ultimately required for a better understanding of BG neuronal activity across S–W stages.

THE EFFECTS OF NEUROTOXIC LESIONS IN THE BG ON S–W

While the abovementioned electrophysiological and cFos studies provide evidence for the association of BG neuronal activity with the sleep–wake cycle, the lesion studies conducted in our lab and others' established that the BG plays a significant causal role in S–W regulation. In order to study the specific roles played by different nuclei of the BG in S–W regulation, we placed small cell-specific lesions in the BG of rats using ibotenic acid (Qiu et al., 2010). As expected, we found dramatic changes in S–W amounts and architecture following discrete lesions of the various BG nuclei. Bilateral striatal lesions resulted in a significant reduction (ca. 15%) of wakefulness, and in fragmentation of both sleep and wakefulness. Sleep and wake bouts were shorter in general, but there were more frequent transitions, especially during the dark period in the animals with striatal lesions. Interestingly, this reduction in wakefulness after striatal lesions was attenuated when the lesions included the nucleus of accumbens core (NAc) (Qiu et al., 2010), suggesting that the dorsal and ventral striatum may play opposing roles in S–W regulation. Consistent with this, lesions restricted to the NAc transiently produced an increase in wakefulness (Qiu et al., 2010). Thus, these results indicate an important role for the striatum in the maintenance of wakefulness, as the dorsal striatum is one of very few structures that produces an increase in sleep and fragmentation of wakefulness after cell-specific lesions. For example, lesions of many previously-established wake-promoting cell groups,

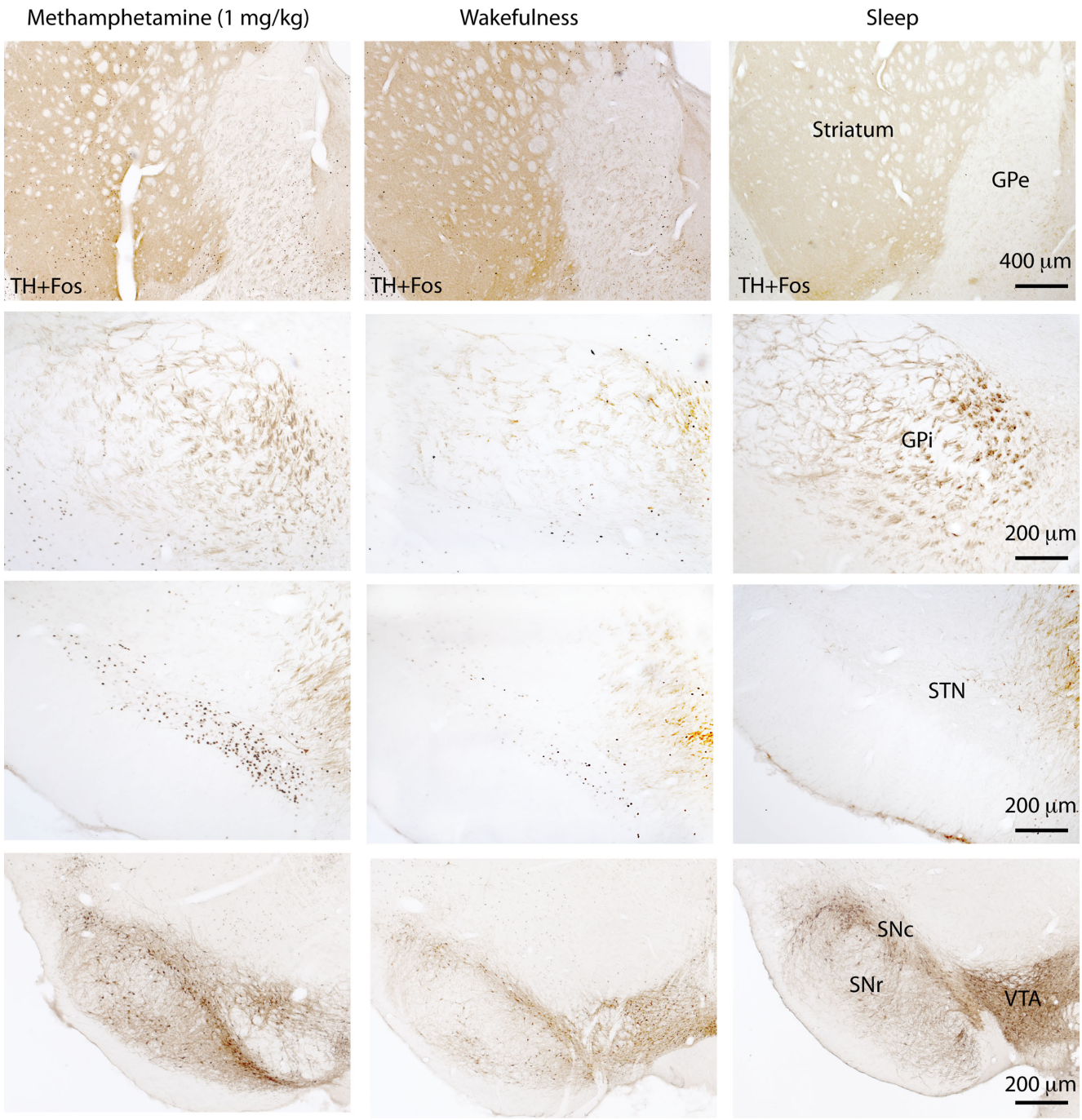

FIGURE 1 | cFos expression in the basal ganglia 2 h after methamphetamine (1.0 mg/kg) administration, after 2 h of wakefulness or during spontaneous sleep. Both waking states are associated with a higher number of cFos-labeled cells in the dorsal striatum, external globus pallidus (GPe), and subthalamic nucleus (STN) than the sleep state cFos expression in the internal globus pallidus (GPi) and substantia nigra pars reticulata (SNr) is low in all three conditions and indistinguishable between wakefulness and sleep. On the other hand, dopamine neurons in the ventral tegmental area (VTA), substantia nigra pars compacta (SNc) and the retrorubal area do not express cFos in any of these states.

including the cholinergic basal forebrain (BF) neurons, serotonergic dorsal raphe (DR) neurons, histaminergic tuberomammillary nucleus (Parmentier et al., 2002; Gerashchenko et al., 2004; Lu et al., 2006a; Blanco-Centurion et al., 2007), noradrenergic locus coeruleus (Blanco-Centurion et al., 2007), cholinergic pedunculopontine and laterodorsal tegmental nuclei (Lu et al., 2006b) and orexin neurons in the lateral hypothalamus (Hara et al., 2001) did not produce an increase in sleep, although S–W fragmentation was observed after these lesions. Other nuclei that have been found to increase sleep following cell-specific lesions, similar to the dorsal

striatum, are the medial parabrachial nucleus, the lateral hypothalamus and the dopaminergic ventral periaquaductal gray matter (vPAG) (Gerashchenko et al., 2003; Lu et al., 2006a,b).

Conversely, lesions of the GPe resulted in profound insomnia in rats with a striking 45.5% increase in total wakefulness and pronounced fragmentation of sleep and wakefulness. These S–W changes are comparable to the lesion effects produced by the only established sleep-promoting cell group, the ventrolateral preoptic area (VLPO), and thus suggest that the GPe may be another key player in the S–W control mechanism. In addition to the S–W changes, GPe lesions also produced hyperactivity and weight loss in rats. Although we were the first to report sleep changes after GPe lesions, hyperactivity and weight loss have been observed previously. We hypothesize that the weight loss following GPe lesions may be due to an oral motor disorder, based on our observations during the initial stimulatory phase of ibotenic acid injections. Since ibotenic acid is a glutamate agonist, it has a strong excitatory effect immediately following the injection (acute phase) before causing neuronal cell death. We observed capricious chewing behavior lasting over 24 h following ibotenic acid injections, and this chewing behavior did not result in food intake even in the presence of food. Following this acute phase, animals stopped eating and drinking and started losing weight abruptly. Similar effects were observed after electrolytic lesions of the lateral hypothalamus, which damages both the cell bodies and the fibers of passage, and were collectively recognized as lateral hypothalamic syndrome (Rowland and Stricker, 1982). 6-hydroxydopamine (6-OHDA) injections into the lateral hypothalamus that eliminate dopaminergic inputs to the striatum and GPe produced a similar reduction in body weight (Lenard et al., 1988). Finally, aphagia, adipsia and weight loss were observed in mice with dopamine deficiency, and these symptoms were corrected by daily administration of L-DOPA (Zhou and Palmiter, 1995). Altogether, these results strongly suggest that dopaminergic inputs to the GPe may play a key role in controlling oral motor behavior, thus affecting food/water intake. In light of these findings, it is interesting to note that PD patients, particularly in the advanced stages, also have difficulty controlling their oral motor muscles and thus have difficulty swallowing, drinking and displaying normal facial expressions. In addition to these oral motor problems, rats with GPe lesions showed other abnormal motor behaviors such as long bouts of standing and a scuffling locomotion. However, unlike S–W alterations, these motor disorders seem compensated for within 2 weeks.

In addition, we speculate that the effects of GPe lesions may mimic the symptoms of fatal familial insomnia (FFI), an autosomal dominant inherited neurodegenerative disease caused by a prions (PrC, a disease-associated prion protein) (Montagna et al., 2003). Patients with FFI exhibit total insomnia, autonomic dysregulation, rapid weight loss, abnormal motor behaviors and dementia, and die within months of disease onset. Brain autopsies showed extensive neuronal lesions, particularly in the thalamus and brainstem, and it is widely believed that the thalamic lesions underlie the insomnia in FFI (Perani et al., 1993). However, discrete neurotoxic lesions in the thalamus do not produce significant changes in the S–W cycle, motor behavior or body weight in rats (Vanderwolf and Stewart, 1988). Furthermore, infarctions in FFI patients are rarely confined to the bilateral thalamus, and thalamic strokes often include the midbrain and/or caudal hypothalamus, which may cause hypersomnia but not insomnia (Castaigne et al., 1981; Hermann et al., 2008). As mentioned earlier, rats with GPe lesions show behavioral changes that match the symptoms of FFI including insomnia, weight loss and abnormal motor behavior and these rats often die within 3–4 weeks. So, we hypothesize that pathology in the GPe may be the underlying cause of FFI. Consistent with this, hypometabolism in the BG has been reported in FFI patients (Cortelli et al., 1997).

In contrast to striatal and GPe lesions, ibotenic acid lesions in the GPi, STN or SNr did not significantly alter S–W, despite their close interactions with the striatum and GPe (Qiu et al., 2010). Furthermore, extensive lesions of the thalamus do not significantly affect S–W and motor behavior (Vanderwolf and Stewart, 1988). These results suggest that the integrity of the GPi, STN, SNr, and even thalamus may not be critical for the generation and maintenance of S–W states, although they may modulate S–W. These findings provide a basis on which a putative network for the BG control of S–W may be framed.

PROPOSED BG CIRCUITRY CONTROLLING S–W BEHAVIOR

Neuroanatomical connections between the BG and cerebral cortex are complex and characterized by many recurrent circuits (**Figure 2A**). On the basis of our lesion and cFos studies, we hypothesize that the recurrent interactions between the BG and cerebral cortex may influence cortical arousal and S–W behavior. Overall, there are five potential neural pathways that connect the BG and cortex: cortex-striatum-GPe-cortex; cortex-STN-GPe-cortex; cortex-STN-GPe-thalamus-cortex; cortex-striatum-GPe-GPi/SNr-thalamus-cortex; and cortex-striatum-GPe-thalamus-cortex (**Figure 2A**). The GPi and SNr have descending projections to the intermediate layer of the superior colliculus and midbrain extrapyramidal area (MEA), but not to the cholinergic neurons in the pedunculopontine tegmentum nucleus (PPT) (Rye et al., 1987; Steininger et al., 1992). The MEA targets the spinal-projecting neurons of the midbrain locomotor region (MLR) involved in the regulation of motor behavior (D. Sherman and J. Lu, unpublished data). Thus, this circuit may be involved in the BG control of posture and locomotion, but not of S–W regulation. The lack of sleep changes following lesions of the GPi and SNr further confirms this hypothesis. On the other hand, there are two structures within the BG-cortex pathways that contain direct cortical-projecting neurons: the GPe and the thalamus. As it has been shown that thalamic lesions do not have any significant effect on S–W, we can confine the key S–W control circuit of the BG to the striato-pallido-cortical loop (highlighted by black lines in **Figure 2A**). In this framework, the striatum-GPe-cortex loop may relay and enhance cortical arousal signals and thereby regulate wakefulness. This loop may also enhance and stabilize the excitatory state of the pyramidal neurons, i.e., cortical activation, which is consistent with the spectral changes in EEG recordings following lesions of the striatum and GPe. Lesions in both the striatum and GPe caused a significant shift in EEG power from the theta to the delta range during wake, NREM sleep and REM sleep, indicating that lesions in the BG resulted in a slowing down of the cortical EEG. Thus, we can conclude that the BG are involved in regulating the level of electrocortical arousal as well as behavioral arousal.

As mentioned previously, the GPe contains direct cortical-projecting neurons (Gritti et al., 1997; Sarter and Bruno, 2002; Furuta et al., 2004; Hur and Zaborszky, 2005). These pallidocortical projections have not been well-recognized mostly because they were previously assumed to be the part of the BF cortical projections. However, the BF cholinergic neurons (Nucleus Basalis of Mynert) located along the medial and ventral borders of the GPe seem to be distinct from the GABAergic corticopetal neurons within the GPe. This was supported by the finding that non-cholinergic, GABAergic neurons in the GPe (well within the boundaries) send ascending projections to the cortex (Gritti et al., 1997; Sarter and Bruno, 2002; Furuta et al., 2004). Interestingly, similar to the BF cortical projections, pallidocortical projections may be topographical. For example, the medial part of the GPe projects to the mPFC (**Figure 3**), while other parts of the GPe project to the insular, motor, and sensory cortices (Saper, 1984). In order to identify the GPe target neurons in the cortex, we injected a cocktail of tracers containing a retrograde tracer, cholera toxin subunit B (CTB), and an anterograde tracer, biotinylated dextranamide (BD), into the GPe into two rats. One week after the injections, these rats were sacrificed and the brains were harvested and processed for immunohistochemical identification of BD, CTB, and GAD 67. We found that most of the BD-labeled axon terminals (anterogradely labeled from the GPe) were in cortical layers IV-V (**Figure 4C**), where they formed appositions with CTB-immunoreactive (CTB-ir) neurons (**Figure 4**) and GAD 67-ir neurons. These CTB-ir neurons were pyramidal neurons and are presumably glutamatergic. Thus, these results suggest that GPe neurons target both GABAergic interneurons and glutamatergic pyramidal neurons in the cortex, and that these two regions are reciprocally connected. Projections to layer V are particularly important, as this layer is critical for generating EEG waves (Sanchez-Vives and McCormick, 2000).

On the basis of these results, we hypothesized the possible mechanism by which the GPe contributes to S–W behavior and cortical activity. Our view is that corticopetal GABAergic GPe neurons act to suppress cortical activity regardless of S–W state and synchronize the fast cortical oscillations by targeting layer V pyramidal neurons and GABAergic interneurons (**Figure 4**). Once this inhibition is reduced (such as by the loss of dopamine inputs) or removed (such as in cell-body lesions), the layer V pyramidal neurons in the cerebral cortex, including the motor cortex, may switch to a more depolarized state as occurs during the wake state. This model is consistent with the observation that both lesions in the GPe and SNc (the source of dopaminergic neurons to the BG) increase wakefulness. This hypothesis is further supported by the finding that EEG delta power is increased and theta power is reduced after GPe lesions (Qiu et al., 2010), and that the EEG waves are slower in PD patients where dopamine inputs to the BG are lost (Soikkeli et al., 1991; Serizawa et al., 2008; Morita et al., 2009). Hence, it can be concluded that the GPe may be one of the many cell groups, including the basal forebrain, thalamus, lateral hypothalamus, LC, and VTA, that directly modulate cortical activity.

HOW DOES MIDBRAIN DOPAMINE MODULATE THIS CIRCUIT?

It is well-established that the loss of dopamine inputs to the BG produces PD, and insomnia is highly prevalent among PD patients. The major source of dopamine in the BG is the midbrain dopaminergic neurons, particularly the SNc (A9 group), and these neurons primarily target the striatum, GPe and GPi. Neurotoxic lesions of the SNc in animals using 6-OHDA or NMDA agonists produced an increase in wakefulness (Lai et al., 1999; Sakata et al., 2002; Gerashchenko et al., 2006), suggesting that nigral dopaminergic inputs to the BG may promote sleep and the loss of dopaminergic inputs to the BG may underlie insomnia in PD patients. Moreover,

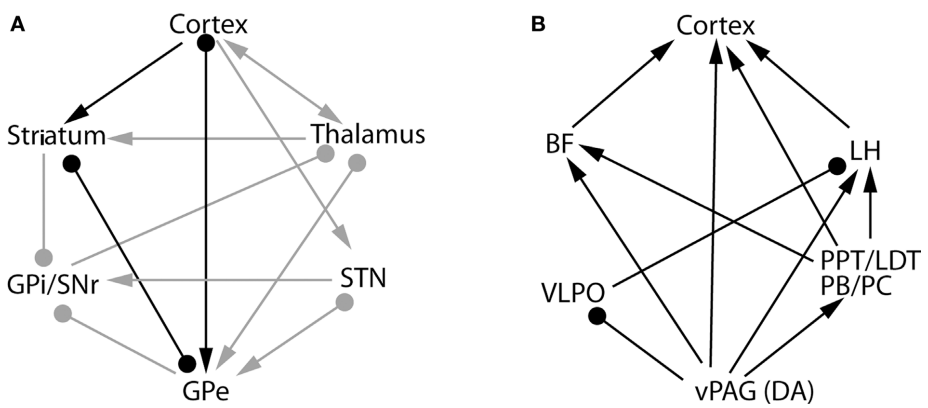

FIGURE 2 | The basal ganglia network (A) and extra-basal ganglia network (B) hypothesized to be involved in sleep–wake control mechanisms. Several pathways connect the basal ganglia with the cerebral cortex, including the cortex-thalamus-striatum-cortex pathway, the cortex-striatum-GPe-cortex pathway, the cortex-STN-GPe-cortex and others. Of these pathways, our data indicate that the cortex-striatum-GPe-cortex network, marked by the black lines [in **(A)**], is involved in the control of sleep–wake behavior and cortical activation. By activating the D2 receptors in this network, dopamine from the midbrain disinhibits the GPe and promotes sleep. On the other hand, wake-active dopaminergic neurons in the vPAG promote wakefulness by inhibiting the sleep-promoting neurons in the VLPO and stimulating the wake-promoting cell groups, including the LH orexin neurons, PPT/LDT cholinergic neurons, PB/PC glutamatergic neurons and BF cholinergic neurons **(B)**. Thus, the collective actions of dopamine in the extra-basal ganglia circuitry promote wakefulness. This network may also be responsible for the arousal effects of psychostimulants. BF, basal forebrain; vPAG, ventral periaquductal gray; PPT, pedunculopontine tegmental nucleus; LH, lateral hypothalamus; LDT, laterodorsal tegmental nucleus; PB, parabrachial nucleus; PC, precoeruleus nucleus; DA, dopamine; VLPO, ventrolateral preoptic nucleus. Arrows represents excitatory synapses; roundheads represent inhibitory synapses. The bars with both symbols represent the reciprocal connection between these nuclei with excitatory and/or inhibitory synapses on the target structure.

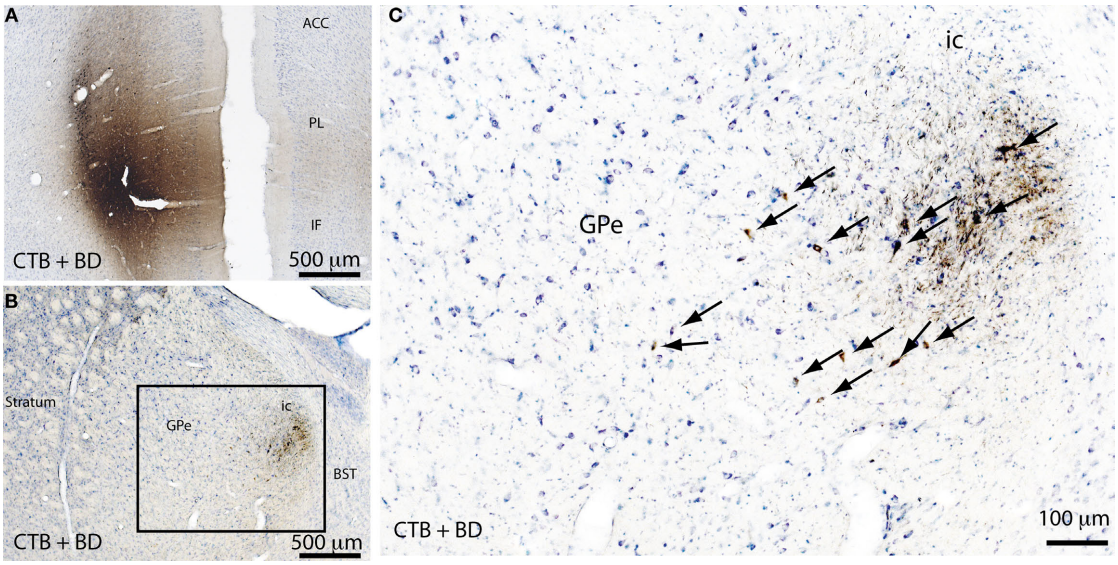

FIGURE 3 | Pallidocortical projections. A mixture of a retrograde tracer (cholera toxin subunit B, CTB) and an anterograde tracer (biotin dextranamide, BD) were injected into the medial prefrontal cortex (mPFC) of rats to assess the pallidocortical projections. The injection site in the mPFC **(A)** was identified by CTB + BD immunolabeling. A large number of retrogradely-labeled (CTB-immunoreactive) neurons were found in the medial part of the GPe **(B)** and the same region also contained anterogradely labeled (BD-immunoreactive) neuron terminals **(B,C)**, indicating that the GPe and mPFC are reciprocally connected. The boxed region in **(B)** is enlarged in **(C)**, and arrows in **(C)** indicate the CTB-ir neurons in the GPe. ACC, anterior cingulate cortex; PL, prelimbic cortex; IF, infralimbic cortex; ic, internal capsule; BST, bed nucleus of stria terminalis.

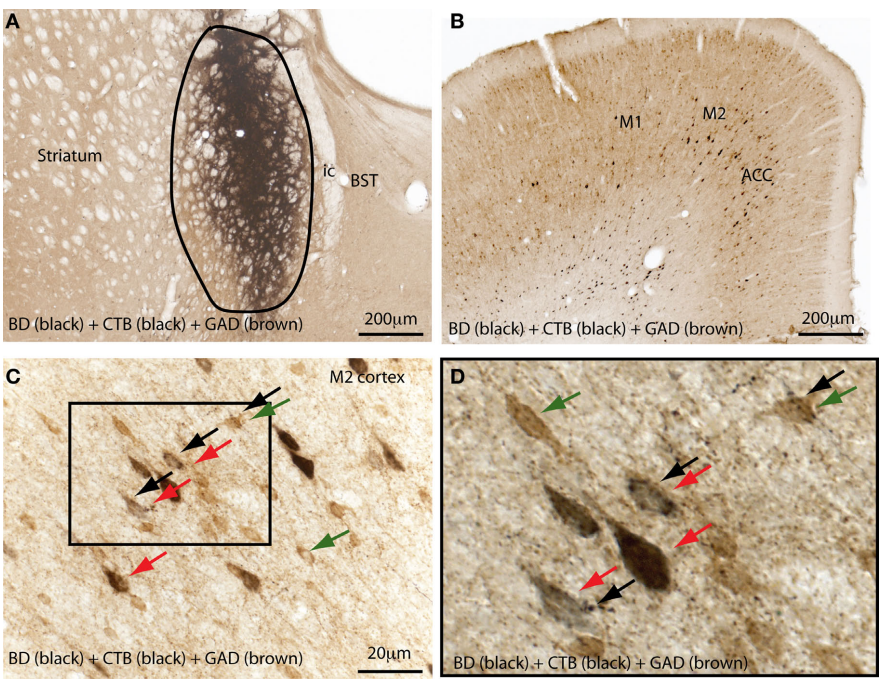

FIGURE 4 | The external globus pallidus (GPe) targets both GABAergic and non-GABAergic neurons in the cortex. A mixture of a retrograde tracer (cholera toxin subunit B, CTB) and an anterograde tracer (biotin dextranamide, BD) was injected into the GPe **(A)** in a rat to characterize the target neurons of pallidal projections to the cortex. Brain sections were immunolabelled for BD, CTB, and glutamic acid decarboxylase (GAD 67). **(A)** Shows the injection site in the GPe. Anterogradely-labeled (BD-ir) axon terminals were mostly present in layers IV and V of the M2 and anterior cingulate cortices **(B,C)**, where a high number of retrogradely-labeled (CTB-ir) cells were present **(B)**. The boxed region in **(C)** is enlarged in **(D)**. The BD-labeled boutons appose both the GABAergic neurons (green and black arrows) and glutamatergic pyramidal neurons (red and black arrows) in the cortex **(D)**. Black arrows mark the axon terminal boutons; CTB-labeled neurons are stained in black color (red arrows) and GABAergic neurons are stained in brown color (green arrows). M1, primary motor cortex; M2, secondary motor cortex.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated animals, one of the best-known animal models of PD, displayed a significant reduction in the amount of SWS (Almirall et al., 1999; Monaca et al., 2004). Although it is believed that MPTP may damage noradrenergic and serotonergic cells in addition to dopaminergic neurons, sleep loss in the MPTP-treated animals seems to be primarily caused by the loss of nigrostriatal dopaminergic neurons. The reasons for this claim are several fold: (1) noradrenergic LC and serotonergic DR neurons have been implicated in promoting wakefulness, hence damage to these neurons would be expected to produce an increase in sleep rather than wakefulness. However, specific neurotoxic lesions of the LC or DR did not affect spontaneous sleep in rats (Lu et al., 2006b). (2) MPTP-induced insomnia cannot be attributed to extra-BG dopaminergic systems, including the VTA and vPAG, because VTA neurons have been shown to be protective against MPTP toxicity and VTA lesions do not alter S–W. On the other hand, vPAG lesions produced an increase in sleep, although vPAG neurons' vulnerability to MPTP is not known. These findings clearly suggest that sleep loss observed after MPTP-lesions in animals may due to the loss of nigrostriatal dopaminergic neurons, and thus indicate that dopamine signaling in the BG may be involved in promoting sleep.

The view that endogenous dopamine in the BG promotes sleep appears contradictory to the popular view that dopamine may be important for wakefulness and arousal. However, it is well-known that low doses of apomorphine (100 μg/kg), a mixed D1/D2 dopamine receptor agonist, and L-DOPA induce sleep in animals and humans and trigger sleep attacks in PD patients, even during high arousal states such as when driving. However, high doses of apomorphine and L-DOPA induce arousal (Schafer and Greulich, 2000; Bonuccelli et al., 2002; Dimpfel, 2008; Hirayama et al., 2008; Garcia Ruiz, 2009). A D2 receptor antagonist has been shown to block the sleep-inducing effects of apomorphine, while a D1 receptor antagonist blocked the arousal effects (Gessa et al., 1985). In addition, systemic injections of a selective D1 agonist resulted in desynchronization of the EEG and an increase in wakefulness, whereas D1 antagonists produced a dose-dependent increase in sleep in mammals. Even in humans, a novel D1 receptor antagonist, NNC-687, increased the length of the first NREM period up to 47% over baseline and enhanced the total number, incidence, and burst-duration of sleep spindles. These evidences collectively indicate that activation of the D1 receptors may promote wakefulness and activation of the D2 receptors may promote sleep, although their specific site of action is not clear. On the basis of the current literature and our results, we hypothesize that activation of the D2 receptors in the BG promote sleep while activation of the D1 receptors in the extra-BG sites promote wakefulness, though these actions may not be exclusive.

Dopamine may act on presynaptic or postsynaptic dopaminergic receptors to bring about its effect on the target tissues. D1 and D2 dopaminergic receptors are abundantly expressed in the cerebral cortex while D1, D2, D3, and D5 receptors are expressed in the striatum and D2, D3, and D5 receptors are expressed in the GPe. Within the striatum, dopamine may exert its actions on the striatonigral neurons via D1 receptors, on striatopallidal neurons via D2 receptors, and on cholinergic interneurons via D5 receptors (Rivera et al., 2002). The D2 receptors are found to be located presynaptically on striatopallidal GABAergic terminals in the GPe, although dopamine may also exert its actions on GPe and GPi neurons via postsynaptic D2 receptors. Most GPe neurons have been shown to fire more slowly and in a bursting pattern following the loss of dopamine inputs, suggesting that the loss of dopamine inputs results in hypoactivity of the GPe neurons. Hence, it is likely that dopamine primarily acts on postsynaptic D2 receptors in the striatum and presynaptic D2 receptors in the GPe to bring about its effects on GPe neurons (Cooper and Stanford, 2001; Querejeta et al., 2001). It can therefore be inferred that the loss of D2 postsynaptic and presynaptic inhibition in the striatum and GPe would result in hyperactivity of the striatopallidal neurons, resulting in inhibition (presumably GABA-mediated) of the GPe neurons, which would then result in increased arousal. We thus hypothesized that endogenous dopamine in the BG promotes sleep by acting on the D2 receptors in the striatopallidal neurons. However, it was found that D2 receptor global knockout mice are hyperactive but show a significant reduction in wakefulness (Qu et al., 2010). Due to the wide distribution of D2 receptors in the brain, it has not been possible to identify the site where D2 receptor activation may promote wakefulness and where their absence in the D2 knockout mice may reduce wakefulness. Since our model predicts that the loss of D2 receptors in the BG may result in an increase in wakefulness, the reduced wakefulness in D2 knockout mice may be due to the absence of functional D2 receptors in the extra-BG sites, such as the cerebral cortex, BF, LH, and other wake-promoting cell groups in the brainstem and caudal hypothalamus (**Figure 2B**).

The VTA was thought to be such an extra-BG dopaminergic system involved in promoting wakefulness because it has direct projections to the prefrontal cortex. In line with this hypothesis, microinjection of apomorphine (D2 agonist) into the VTA produced a dose-dependent increase in behavioral and EEG sleep in rats, suggesting that VTA dopaminergic neurons may promote wakefulness, and D2-mediated inhibition of these neurons may promote sleep. However, these results are hard to reconcile with the findings that NMDA-induced VTA lesions in cats produced an increase in wakefulness rather than an increase in sleep, and orexin-saporin lesions of the VTA in rats did not produce any change in S–W. Although the loss of non-dopaminergic neurons in the VTA (which may play an opposing role in sleep regulation) cannot be overruled in these studies, we recently found that selective lesions of VTA dopaminergic neurons using 6-OHDA also did not produce an increase in sleep in rats (R. Vetrivelan and J. Lu, unpublished observations). Moreover, VTA neuronal firing rate and cFos expression (**Figure 1**) did not differ significantly across S–W states. Hence, the role of VTA dopaminergic neurons in promoting wakefulness is inconclusive.

However, we recently identified a novel dopaminergic cell group, the vPAG (Lu et al., 2006a), which may be critically involved in the promotion and maintenance of wakefulness. Unlike the VTA, a variety of experimental approaches confirmed the vPAG neurons as being a wake-promoting cell group. For example, vPAG neurons express cFos during wakefulness, and selective cell-specific lesions of the vPAG produced a significant (ca. 20%) reduction in wakefulness (Lu et al., 2006a). In addition, the vPAG is reciprocally connected with all the major components of the sleep–wake circuitry, including the sleep-promoting cell group, the VLPO, and

with the wake-promoting cell groups, including the BF, orexinergic neurons in the lateral hypothalamus, and brainstem cholinergic (LDT) and monoaminergic (LC, DR, and median raphe) nuclei. As the above-mentioned wake-promoting cell groups express D1 and D2 receptors extensively, the vPAG may activate (via D1) or disinhibit (via D2) these groups to promote wakefulness. On the other hand, the vPAG dopaminergic neurons may inhibit the sleep-promoting VLPO neurons and, interestingly, this inhibition is mediated by alpha2 adrenergic receptors (Cornil et al., 2002; Gallopin et al., 2004). Finally, the vPAG has direct projections to the majority of the cortex (whereas the VTA projects primarily to mPFC), and thus may also directly influence cortical activity. As in the wake-promoting cell groups, this influence may be mediated by both D1 and D2 receptors. In support of this, a recent study indicated that D1 and D2 receptors act synergistically to activate cortical pyramidal neurons; that is, D1 receptors mediate direct excitation and D2 receptors mediate indirect excitation (via disinhibition of the GABAergic interneurons) of the pyramidal neurons (Xu and Yao, 2010). Thus, the vPAG may promote wakefulness by activating the above-mentioned wake-promoting cell groups and cortex via D1 and/or D2 receptors, and by simultaneously inhibiting the sleep-promoting VLPO neurons via alpha2 receptors. Furthermore, low doses of apomorphine and other dopaminergic agonists may induce its sedative effects via D2-mediated inhibition of the vPAG neurons, although evidence supporting this assertion is currently lacking.

Another important and relevant aspect of the vPAG dopaminergic system is that these neurons express dopamine transporters (DAT), which may partially explain the arousal mechanism of many psychostimulants such as methamphetamine and cocaine. These drugs may inhibit DAT-mediated reuptake of dopamine in the terminals of the vPAG neurons, increasing the postsynaptic availability of dopamine in the wake-promoting cell groups (**Fig 2B**) and thus increasing wakefulness. Consistent with this hypothesis, DAT knockout mice did not display hyperactivity and arousal after administration of methamphetamine, although their spontaneous wakefulness was higher than that of intact mice (Wisor et al., 2001). In contrast, animals with SNc lesions are paradoxically supersensitive to the arousal and motor effects of methamphetamine or amphetamine (Mandel and Randall, 1985; Schroeder et al., 1997, M. Qiu and J. Lu, unpublished observations). Previously, up-regulation of dopamine receptors in the BG had been proposed to be a potential mechanism of methamphetamine supersensitivity. However, this explanation is hard to reconcile with the fact that there is a very low level of dopamine in the BG after elimination of the SNc – the main dopamine source of the BG. Therefore, we hypothesize that methamphetamine supersensitivity may be due to the additive effects of (1) increased wakefulness following the loss of dopamine inputs to the BG and (2) increased wakefulness due to excess dopamine in the extra-BG sites following methamphetamine administration.

CONCLUSIONS

Research during the last decade has provided important insights into the BG circuitry controlling S–W behavior, and how dopamine may modulate this circuit. Electrophysiological, cFos and lesion studies point to a distinct role for the individual BG nuclei in the regulation of S–W, and also suggest that dopamine plays opposing roles in the BG and extra-BG networks. We hypothesize that dopamine in the BG may promote sleep via D2 receptors, while the extra-BG vPAG dopaminergic system may promote wakefulness via D1 and D2 receptors. Further studies using novel genetic methods are required to confirm this hypothesis. Developing a better understanding of the role of dopamine in S–W may shed light on the neurochemical mechanisms underlying sleep disturbances observed in patients with PD or HD, which in turn will aid in the development of novel treatment options.

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High field fMRI reveals thalamocortical integration of segregated cognitive and emotional processing in mediodorsal and intralaminar thalamic nuclei

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Thalamocortical loops, connecting functionally segregated, higher order cortical regions, and basal ganglia, have been proposed not only for well described motor and sensory regions, but also for limbic and prefrontal areas relevant for affective and cognitive processes. These functions are, however, more specific to humans, rendering most invasive neuroanatomical approaches impossible and interspecies translations difficult. In contrast, non-invasive imaging of functional neuroanatomy using fMRI allows for the development of elaborate task paradigms capable of testing the specific functionalities proposed for these circuits. Until recently, spatial resolution largely limited the anatomical definition of functional clusters at the level of distinct thalamic nuclei. Since their anatomical distinction seems crucial not only for the segregation of cognitive and limbic loops but also for the detection of their functional interaction during cognitive–emotional integration, we applied high resolution fMRI on 7 Tesla. Using an event-related design, we could isolate thalamic effects for preceding attention as well as experience of erotic stimuli. We could demonstrate specific thalamic effects of general emotional arousal in mediodorsal nucleus and effects specific to preceding attention and expectancy in intralaminar centromedian/parafascicular complex. These thalamic effects were paralleled by specific coactivations in the head of caudate nucleus as well as segregated portions of rostral or caudal cingulate cortex and anterior insula supporting distinct thalamo–striato–cortical loops. In addition to predescribed effects of sexual arousal in hypothalamus and ventral striatum, high resolution fMRI could extent this network to paraventricular thalamus encompassing laterodorsal and parataenial nuclei. We could lend evidence to segregated subcortical loops which integrate cognitive and emotional aspects of basic human behavior such as sexual processing.

Keywords: salience processing, centromedian/parafascicular thalamus, mediodorsal thalamus, basal ganglia, cognition, emotion, high field fMRI, sexual processing

INTRODUCTION

Functional imaging studies of the last decade have vastly illustrated the importance of cortical networks for the highly differentiated subfunctions of human behavior, while the role of subcortical structures, foremost the basal ganglia, in guiding human behavior has not been investigated to an equal extent. This is mainly due to the fact that these comparably small structures remained difficult to characterize given the limited spatial resolutions of non-invasive imaging techniques such as fMRI. However, a huge body of literature from animal studies or clinical insights from brain lesions exists, which suggests that the elaborate set of cortical functional networks may be orchestrated by anatomically well-defined subcortical structures. Since subcortical components of cortico–subcortical networks could not be sufficiently characterized so far, their functional segregation based on non-invasive imaging studies seems crucial to understanding the brain's functional architecture as a whole. Existing concepts, as suggested by Alexander and others, rely on segregated but integrating circuits, some of them forming functional subsystems or cortico–striato–thalamic "loops" (Alexander et al., 1986).

These integrated loops may also control basic processes such as sexual arousal which has been previously characterized as multidimensional, spanning various functional systems and brain areas in humans. During processing of sexually salient information, cognitive, motivational, emotional, and autonomic processes can be discerned which contribute to different aspects of the subjective experience and which are known to interact heavily (Redouté et al., 2000). Recent studies could differentiate subsystems involved in processing of either specific sexual arousal or general emotional intensity or valence (Walter et al., 2008a). While the ventral striatum and hypothalamus were identified as core structures of sexual arousal, the pregenual anterior cingulate cortex

(pgACC) was found to integrate information of sexual intensity and emotional valence. The role of the thalamus, however, was defined as mediating emotional intensity, mainly via activations in its mediodorsal compartment. Insufficient spatial resolution in prior studies limited interpretation of thalamic activations despite its well described functional parcellation according to a number of intriguing studies in animals. In humans, recent investigations confirmed functional and structural specificity of thalamocortical connectivities (Johansen-Berg et al., 2005; Klein et al., 2010; Zhang et al., 2010). Given the functional heterogeneity of the cortical regions, an involvement of the thalamus as a whole in one subcomponent of sexual arousal could at least be questioned, and in light of its considerably diverse connections to a number of subcortical and cortical "hubs", its role may have been underestimated or oversimplified.

In the same direction, the functional integration of distinct thalamocortical loops, and thus thalamic nuclei, into cortical and basal ganglia networks could not be considered for clinical concepts based on human imaging findings. Accordingly, controversial findings of increased or decreased functional connectivity between anterior cingulate cortex (ACC) and "the thalamus" as a whole could not be satisfactorily traced back to different thalamic target structures, but were related to differences in patient populations or methodologies (Anand et al., 2005; Greicius et al., 2007; Walter et al., 2009).

Thalamocortical loops, connecting functionally segregated, higher order cortical regions, and basal ganglia, have been proposed not only for well described motor and sensory regions, but also for limbic and prefrontal areas relevant for affective and cognitive processes (Alexander et al., 1986). The high specificity of these latter functions to humans, however, renders most invasive neuroanatomical approaches impossible and interspecies translations difficult.

Mirroring one major distinction of cortical functional networks, a thalamic set of regions mediating either cognitive attentional or affective interoceptive processing may be hypothesized. Since both of these functional networks, namely the default mode network and the task positive network, comprise of characteristic nodes in the prefrontal cortex (PFC), current parcellations of the thalamus into components with preferential connectivity to PFC, or other large cortical lobes (Zhang et al., 2010), may not adequately address this functional segregation within the thalamus. There is however strong evidence, that anatomical parcellations of the thalamus may in fact serve the purpose of functional segregation both in animals and humans.

The involvement of the mediodorsal thalamus (MD) in emotional processing and its distinct detectability by high resolution fMRI has been shown even on a single-subject level (Walter et al., 2008b). Coactivation with rostral ACC, previously coined the "affective division" of the cingulate cortex, has been reported in the context of increased emotional salience during erotic processing (Walter et al., 2008a).

The intralaminar thalamic nuclei, particularly the centromedian/parafascicular thalamic complex (CM/PF) are involved in attention processing and general arousal including the control of the level of cortical activity (Haber and Calzavara, 2009). They provide strong projections to the dorsal anterior cingulate cortex (dACC) (van der Werf et al., 2002). The dorsal, "cognitive division" of the ACC (Devinsky et al., 1995) together with the anterior insula form the core components of both the salience network (Seeley et al., 2007) and the cingulo–opercular attention network (Dosenbach et al., 2008) which is crucial for maintaining attention to previously selected tasks or targets.

In addition to a functional segregation, the inclusion of basal ganglia, functional cortical divisions and thalamic subregions into the distinct loops has to be shown to support functional integration. The MD is one major relay nucleus in the thalamus and its putative role in connecting basal ganglia and cortex has been widely addressed invasively, however, it has been poorly substantiated using direct support from non-invasive imaging data in humans. As part of the salience network, MD was proposed to connect anterior insula and dACC, two regions which are themselves characterized by specific neuronal setup of large bipolar neurons. Besides its relation to salience that closely links MD to the processing of stimuli capable of drawing and binding our attention, this nucleus has also been related to the processing of the emotional experience that is often associated with salient stimuli, but processed in an affective network encompassing more rostral portions of ACC (Devinsky et al., 1995).

In this context, the processing of sexually salient and emotionally relevant stimuli, in combination with an attentional task, seems a perfect model to investigate the functional segregation and integration of thalamocortical networks. These networks are set up by specific thalamic hubs, residing in anatomically predefined nuclei and specifically process e.g., preceding attention, which can be used to discern cognitive and stimulus-driven components of attention to salient material (Corbetta and Shulman, 2002).

Direct comparison of functional connectivity of MD and CM/PF, with its putative targets in affective and cognitive divisions of the ACC and insula, has not been attempted. Therefore, our study aimed to reveal this relationship by using the high resolution of a 7 Tesla functional imaging setup, able to specify small structures on subcortical level, that are not detectable on lower fields. In addition, it was tested, if exceeding these two components of the multiple dimensions of sexual arousal, specific hubs of the other two dimensions, namely the motivational and autonomous components, could be found at an adequate spatial resolution.

MATERIALS AND METHODS

SUBJECTS

We scanned 10 healthy, heterosexual male right-handed subjects (mean age: 25.6 years SD: 1.51). All subjects had a partner at the time of scanning, were sexually active and recent or previous sexual dysfunction was excluded in a standard clinical interview. Prior to the fMRI experiment, all subjects were further examined by an experienced neurologist. No subject had to be excluded for history of neurological or psychiatric disorders and all subjects performed within the normal range during neuropsychological assessment of individual attention and concentration performance using the d2 test of attention (Brickenkamp and Zillmer, 1998). The study was approved by the local IRB. Research subjects participated after giving informed written consent.

PARADIGM

We adopted the stimulation paradigm described in Heinzel et al. (2006) and Walter et al. (2007, 2008b), which has been reported to reliably induce sexual and emotional arousal by means of subjective self-assessment and which was found to effectively elicit neural responses in key structures relevant for sexual and emotional arousal (Walter et al., 2007). To gain sufficient power for a single-subject single-run paradigm, the number of stimulus repetitions was increased, extending the total duration to 13.6 min. Picture sets consisted of 20 erotic and 20 non-erotic emotional pictures of humans, taken from the international affective picture system (IAPS) (Lang et al., 2005). Picture sets were counterbalanced for standard values of arousal, pleasantness, and dominance as provided by the IAPS. Furthermore, the categories were balanced for mean ratings of perceived emotional intensity as well as for perceived saliency, defined as the degree to which a stimulus captures a subject's attention. These were rated separately to account for possible interactions between both aspects of general arousal. Together with a measure of pleasantness and sexual intensity, the ratings were previously obtained from 22 healthy male subjects (mean age: 26.4 years SD: 4.1). The values were 14 (SD 6) for sexual intensity in emotional, and 61 (SD 13) for specific sexual intensity (SSI) in sexual pictures. There was no significant difference between erotic and non-erotic emotional pictures (mean values ± SD) regarding the values of emotional intensity, which is a marker of general emotional arousal (GEA) (55 ± 13, 54 ± 12), salience (54 ± 14, 57 ± 10), or valence (62 ± 10, 66 ± 9).

After the scanning session, our 10 subjects were asked to rate erotic and non-erotic stimuli for induced sexual arousal, emotional intensity, salience, and perceived feeling of pleasure. This was done to assure that the stimuli induced sexual arousal in our subjects and that they were matched for all categories except sexual intensity, as emotional pictures were taken to be non-erotic.

Pictures were presented for 4 s and were projected to a screen mounted to the head coil via a LCD projector. After each picture presentation, a white fixation cross appeared for a variable duration of 7.5–10.5 s and served as an experimental resting period. Stimuli were preceded by short presentations of arrows at durations of 3–5 s. The subjects were instructed to actively anticipate the upcoming picture category as sexual or emotional intense indicated by special types of white arrows on a black screen: Arrows either indicated the type of the subsequent picture when they were presented with an exclamation mark (erotic picture: upward, non-erotic picture: downward arrows) or provided information about the number of people on the subsequent picture (upward with one dot: one person; upward with two dots: two people). Arrows indicating numbers of subjects did not indicate whether the subsequent picture was an erotic or non-erotic emotional picture and both picture categories were equal in regards of number of displayed persons. Both erotic and emotional pictures were explicitly cued in 50% of the cases and a total of 12 arrows were presented without subsequent picture conditions. Active anticipation of the upcoming picture category was explicitly required from the subjects and was explained to be necessary for the experiment. Subjects were asked to passively view the upcoming pictures during the subsequent picture condition and let it act on them. No active response was requested to avoid confound on our experiment e.g., by motor preparation. The instruction was given that during each fixation period (indicated by a fixation cross, also shown to the subject prior to the experiment), subjects should disengage themselves from the last condition, just fixating the cross. They were explained, that pictures randomly appear, being cued or not cued by a preceding arrow and that in very rare cases, arrows could appear without subsequent picture, just being followed by a fixation cross. They were told, that this was necessary for the experimental design, but very rarely the case, so active anticipation should be performed any time. No misleading cues were used by our design, to assure subjects' compliance and all pictures were only shown once. After subjects have entered the scanner, prior to experiment, indication of arrows as well as a very short repetition of the instruction was given to the subjects and they were asked for open questions.

In our analysis, we focus on the erotic and emotional anticipation periods as well as the picture perception phase.

IMAGE ACQUISITION

All experiments were performed on a 7 Tesla whole body MR system (Siemens, Erlangen, Germany). An eight-element phased array coil (Rapid Biomedical, Germany) was used for signal transmission (RF power distributed to result in a pseudo CP excitation) and reception (eight independent receive channels). Anatomical reference data were acquired with 3D-MPRAGE (1 mm isotropic resolution, TI 1050, TR 2300 ms, flip angle 5°). For high resolution functional imaging, single-shot EPI was optimized for 7 Tesla (see **Figure 1**). SAR was reduced by decreasing the nominal fat saturation flip angle. Imaging parameters were FOV 220 × 220 mm, matrix size 128 × 128, 16 slices, 3-mm slice thickness, 0.6-mm gap, TR 1000, TE 24 ms, 6/8 partial Fourier, GRAPPA factor 2, sinusoidal readout gradient. The small voxel volumina of 12 µl result in reduced

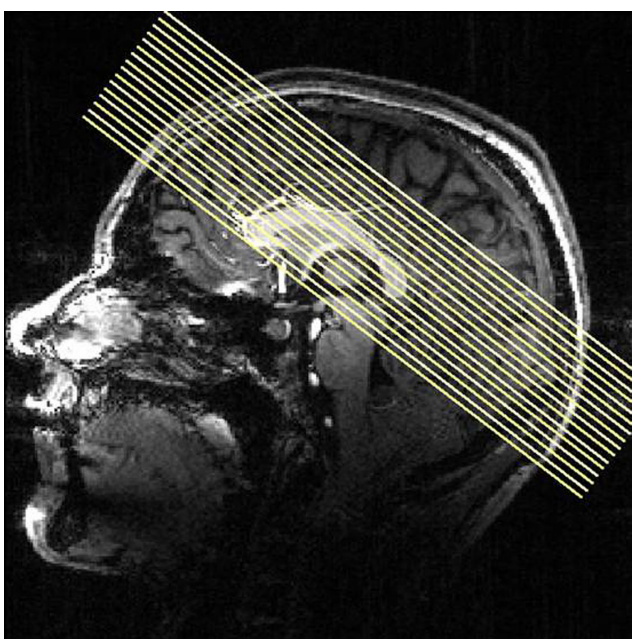

FIGURE 1 | Orientation of functional slices during fMRI session. Sixteen slices were acquired in an interleaved order.

dephasing across the voxel. Therefore, high spatial resolution allows a minimization of signal dropouts. During the online reconstruction, all data were motion and distortion corrected based on a reference measurement of the local point spread function (Zaitsev et al., 2004), which was optimized for use in high fields (Speck et al., 2008).

DATA PREPROCESSING AND ANALYSIS

Preprocessing and statistical analysis was performed using BrainVoyager QX 1.9 (Brain Innovation, Maastricht, The Netherlands) (Goebel et al., 2006). Preprocessing of the functional scans included a more accurate offline correction of residual head motion, slice scan time correction and removal of linear trends. A high pass filter of 0.0037 Hz was applied, corresponding to three replication cycles or less over the whole session, to remove low frequency noise that could not be explained by our design.

Functional images were co-registered with anatomical images and resliced to 3D data sets using a trilinear interpolation algorithm. This transformation resulted in isotropic voxels of $2 \times 2 \times 2$ mm, which was found to be a reasonable trade off between spatial resolution and the number of voxelwise comparisons to correct for. Anatomical and functional data were transferred into Talairach space as implemented by the software used. Statistical analysis was performed creating individual three-dimensional statistical maps for each subject. Smoothing of 4 mm was applied to all data. Parameter estimates for our experimental conditions were calculated using a general linear model (GLM) (Friston et al., 1995) on 3D volume time courses.

The fixation period was entered as a regressor of no interest in our design matrix and was not further analyzed for the purpose of this study. The design matrix included regressors of interest for the different types of picture presentation and for the anticipation periods. Group analysis was performed using a random effects model.

Conditions and contrasts were tested separately. To control for multiple comparisons, the standard false discovery rate (FDR) (Benjamini and Hochberg, 1995; Genovese et al., 2002) method implemented in BrainVoyager QX was used for orienting contrasts. This thresholding method computes a single voxel threshold for the desired level of false positives according to the number of detected suprathreshold voxels. The FDR was set to $q < 0.05$, which means that less than 5% of all voxels were accepted to be false positives.

Subsequent statistical analyses for thalamic subregions MD and CM are reported on an (uncorrected) p-threshold of 0.001. Complex conjunction analyses were calculated for a $p < 0.05$, using the conjunction null approach. This method tests for voxels in which both contrasts included in the conjunction yield significant results reflecting the assumptions of a logical conjunction. This is preferred over the global null conjunction testing against the case that both contrasts are non-significant (Nichols et al., 2005).

Resulting 3D statistical maps were then overlaid on subjects' anatomical high resolution images to relate activations to underlying structures which were identified using a standard anatomical atlas (Mai et al., 2004). Group results are displayed on a high resolution template provided by the MRIcron software (Rorden et al., 2007), which was transferred into Talairach space. Effects were further validated by direct overlays on the original EPI data to account for possible susceptibility artifacts from neighboring structures. Following a standard protocol in BrainVoyager QX for anatomical overlays, statistical maps were interpolated to correspond to the underlying anatomical resolution of 1 mm³.

Subjective ratings of sexual intensity, emotional intensity, and arousal were assessed on a visual analog scale from 10 to 90 (Walter et al., 2008a). Differences between emotional and erotic picture conditions in all three subjective dimensions were compared using a two-sided paired t-tests with a significance level of $p < 0.05$.

RESULTS

BEHAVIORAL ASSESSMENT

To test the matching of emotional dimensions in our subjects, we directly compared their ratings of GEA, SSI, and emotional valence for both erotic and emotional stimuli. Comparing emotional/erotic stimuli, the mean values ± SD were 60 ± 13/56 ± 12 for GEA, 56 ± 13/58 ± 9 for salience, 14 ± 5/62 ± 16 for SSI and 69 ± 11/65 ± 9 for valence. While erotic and non-erotic emotional stimuli did not differ in subjective ratings of GEA, salience and valence ratings ($p > 0.2$), there was a significant difference of their SSI ratings ($t = -9.319$, $p < 0.0001$).

SEGREGATION OF GENERAL EFFECTS OF PICTURE VIEWING AND PICTURE EXPECTANCY

To differentiate between networks related to attentional and perceptive task components, anticipation (representing a rather attentional task), and picture viewing (representing a more perceptive task) were compared: Anticipation of both erotic and emotional pictures revealed overlapping thalamic activations in bilateral CM/PF, superior colliculus and pulvinar by the conjunction analysis [expectancy of erotic × expectancy of emotional]. The effects of anticipation were restricted to intralaminar portions of the thalamus even at the uncorrected conjoint threshold of $p < 0.05$ (see **Figure 2**, blue voxels).

In contrast, the conjunction of main effects of picture viewing [erotic pictures × emotional pictures] revealed common effects in the bilateral MD formation. However, these effects did not extend into the intralaminar portions, covered by the first conjunction (see **Figure 2**, red voxels). Similar to the expectancy condition, additional subcortical activations for main effects of picture viewing were found in the bilateral pulvinar, superior colliculus and in the right putamen ($p < 0.05$ for conjunction).

This distinct assignment of CM/PF to anticipation and MD to visual perception of erotic and emotional stimuli was also reflected by coactivation of characteristic cortical networks of the respective main effects of expectancy or picture viewing: Anticipation was related to bilateral activations in the dorsal ACC, supracallosal midcingulate cortex (MCC), posterior cingulate cortex (PCC), and anterior insula (see **Figure 3**) as well as (bi-)lateral supramarginal gyrus (SMG), medial temporal complex (MT+), superior temporal sulcus (STS), visual cortex and activation in right medial frontal gyrus (MFG), superior frontal sulcus (SFS), inferior frontal sulcus (IFS), and superior temporal gyrus (STG). Picture viewing instead was found to show common activations in the visual cortex and supracallosal MCC as well as left insular cortex, bilateral inferior frontal gyrus (IFG), MFG, and precentral gyrus (PrecG, see **Table 4**).

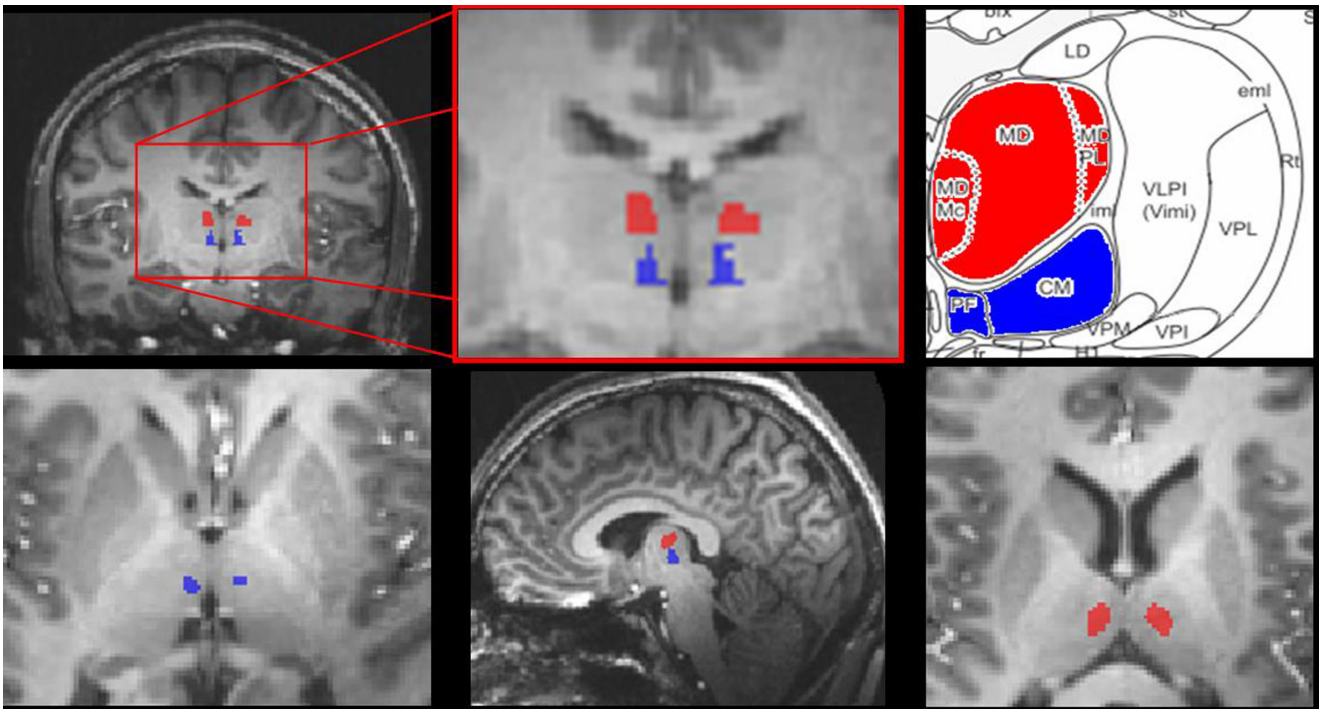

FIGURE 2 | Thalamic activation during anticipation and picture period. Thalamic activation for the conjunction [erotic pictures × emotional pictures] revealed the bilateral mediodorsal thalamus (red voxels) while the anticipation period, examined by the conjunction [erotic anticipation × emotional anticipation], activated the intralaminar centromedian/parafascicular thalamic nucleus (blue voxels) (conj. $p < 0.05$, x: –5, y: –13, z: 10, right; z: 3, left). Right upper figure adapted from Mai, J., Assheuer, J., and Paxinos, G. (2004). Atlas of the Human Brain. San Diego: Academic Press/Elsevier.

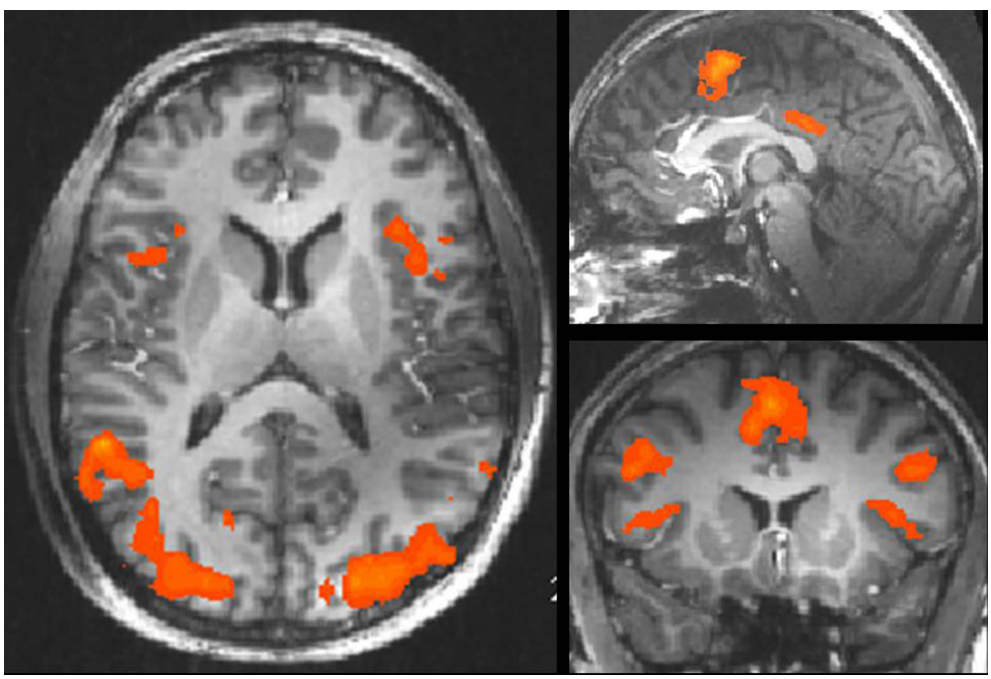

FIGURE 3 | Cortical activation during anticipation period. Regions with significant signal increases during anticipation period [erotic anticipation × emotional anticipation] (conj. $p < 0.05$ uncorrected, x: –1, y: 1, z: –10; see also Table 2).

MAIN EFFECT OF SEXUAL AROUSAL IN SUBCORTICAL STRUCTURES

Sex-specific effects were defined as those effects that were task independent and irrespective of GEA. They were revealed by the conjunction of contrasts between sexual and emotional conditions of both tasks [anticipation of sexual picture > anticipation of emotional picture] × [sexual picture > emotional picture] (at a conjoint threshold of $p < 0.05$ uncorrected). Main effects of sex were found on subcortical level in the right paraventricular portion of the thalamus (**Figure 4**), reflecting the overlapping effect of sex on anticipation and picture period (including the paraventricular mediodorsal, laterodorsal (LD), and parataenial (PT) thalamic nuclei), and the right head of caudate nucleus.

Cortical activation affected the bilateral inferior parietal sulcus (IPS), medial temporal gyrus (MTG), right superior parietal lobule (SPL) as well as left SMG, postcentral gyrus, and occipital gyrus (**Table 3**). It is noteworthy that we did not find general effects in the main regions of specific effects of either expectancy or picture conditions with the exception of the paraventricular thalamus. Medial prefrontal and cingulate cortex did not show overlapping effects of sex neither in expectancy nor in picture condition. We also did not find general effects of sexual intensity in insular cortex as far as it was covered by our investigation.

SPECIFIC EFFECTS OF SEX WITHIN EXPECTANCY AND PICTURE PERIODS

Erotic versus emotional anticipation

To analyze the sex-specific, task dependent effect within the anticipation period, anticipation of erotic and emotional pictures was analyzed by direct comparison: The contrast between anticipation of erotic versus anticipation of emotional stimuli (see **Table 2**) elicited significantly greater thalamic activation for the erotic condition ($p < 0.001$) in the dorsal intralaminar portion with peak activations in the lateral habenula and medial centromedian complex, posterior to the PF.

Further significant activations of specific thalamic nuclei were found in the bilateral ventral anterior (VA) thalamic nucleus (**Figure 5**) as well as in the paraventricular portion of the thalamus, corresponding to paraventricular mediodorsal, LD, and PT thalamic nuclei. In addition, we found increased activation in the right head of caudate nucleus, left pallidum, and right putamen upon anticipation of erotic stimuli.

On the cortical level, increased activations during expectancy of erotic stimuli were found in the right anterior insular cortex and right dACC. In addition to the regions that were already revealed by the conjunction analysis of general effects of expectancy, increased activations for only sexual expectancy were found in the right frontal eye field (FEF), the bilateral IPS, STS, MT+, and left SPL (see **Table 2**).

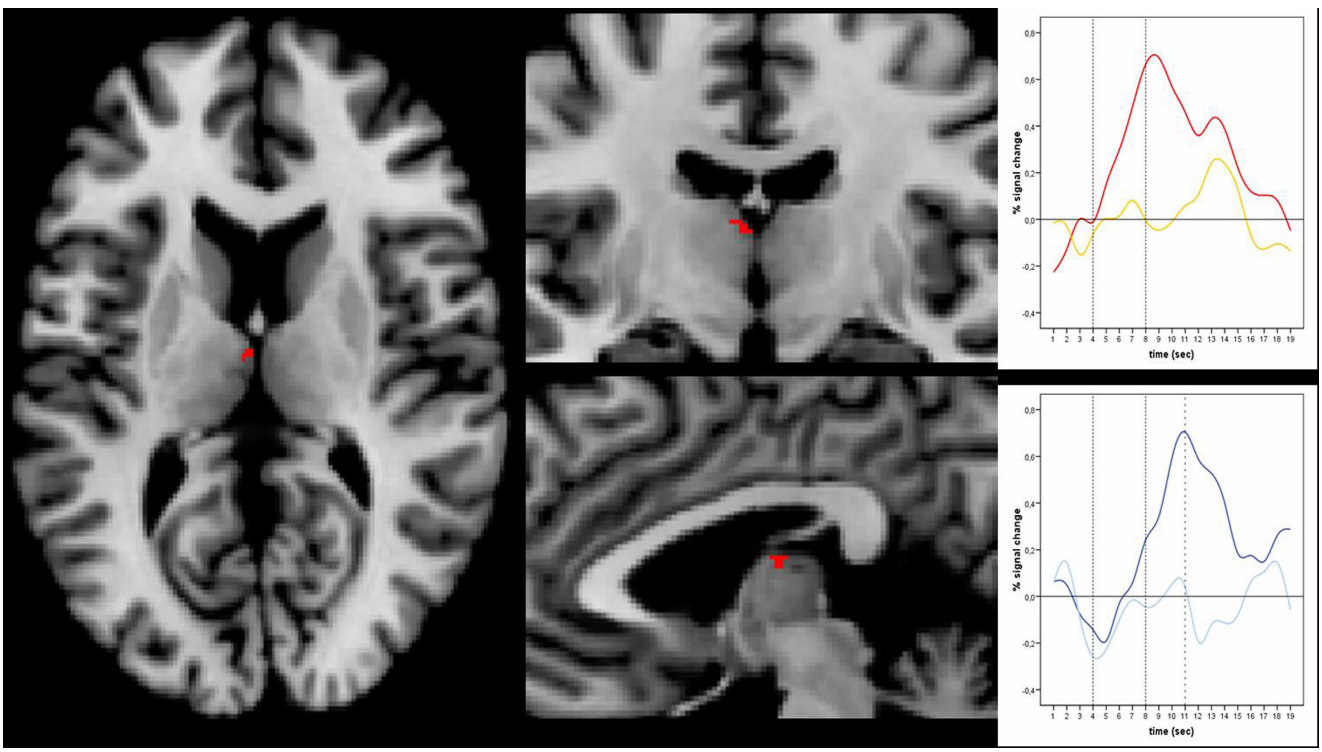

FIGURE 4 | Main effects of sex. Left and middle: Main effects of sex in the right paraventricular thalamus including laterodorsal, parataenial, and paraventricular mediodorsal thalamic nucleus was shown by the conjunction [anticipation of erotic pictures > anticipation of emotional pictures] × [erotic pictures > emotional pictures] at an uncorrected threshold of conj. $p < 0.05$ (x: 3; y: −13; z: 12). Right: Timecourses in the paraventricular thalamus shown separately in the upper picture for erotic (red) and emotional picture perception (yellow) and in the lower picture for erotic (dark blue) and emotional anticipation (light blue). Peaks for erotic expectancy conditions lagged those of the erotic picture conditions by 3 s, which was the actual lag of expectancy and subsequent picture conditions. This indicates at least a confound on the expectancy effects by their subsequent picture conditions.

Table 1 | Table of abbreviations.

ACC	anterior cingulate cortex
aIfO	anterior insula, frontal operculum
Cd_head	head of caudate nucleus
CM/PF	centromedian/parafascicular thalamic complex
dACC	dorsal anterior cingulate cortex
FEF	frontal eye field
IFG	inferior frontal gyrus
IFS	inferior frontal sulcus
Insula	insular cortex
IPS	inferior parietal sulcus
LD	laterodorsal thalamic nucleus
LHb	lateral habenula
MCC	midcingulate cortex
MD	mediodorsal thalamic nucleus
MFG	medial frontal gyrus
MT+	medial temporal complex
MTG	medial temporal gyrus
NclCaud	caudate nucleus
OcG	occipital gyrus
Pall	globus pallidus
parav. Thal	paraventricular thalamus
PCC	posterior cingulate cortex
pgACC	pregenual anterior cingulate cortex
PoCG	postcentral gyrus
PrecG	precentral gyrus
Precun	precuneus
PT	parataenial thalamic nucleus
Put	putamen
SFS	superior frontal gyrus
SMA_lat	supplementary motor area (lateral)
SMG	supramarginal gyrus
SPL	superior parietal lobule
STG	superior temporal gyrus
Str. term.	Stria terminalis
STS	superior temporal sulcus
VA	ventral anterior thalamic nucleus

Picture period

To identify the effects of sex and emotion on the perception phase, activity during erotic, and emotional picture viewing were analyzed separately in orienting contrasts for erotic and emotional picture periods:

We found distinct significant effects of erotic picture viewing and emotional picture viewing in the mediodorsal thalamic nucleus ($q < 0.05$, FDR corrected).

However the peak activations differed between picture conditions with sexual picture periods leading to peaks which were located more mediodorsally, while emotional pictures led to activations in the (bi-)lateral portions of MD (see **Figure 6**).

Additional activations for erotic picture presentation were located in the right anterior thalamus and pulvinar, the bilateral head of caudate nucleus, tectum, right putamen, and left claustrum ($q < 0.05$, FDR corrected), while emotional picture viewing only showed effects in bilateral pulvinar, tectum and putamen, but not in claustrum and caudate nucleus.

The conjunction analysis of general effects of picture viewing further revealed a cortical effect in the mid-insular cortex. However, this effect was left lateralized for erotic and more dorsally and right lateralized for emotional picture conditions.

Additionally, both erotic and emotional picture conditions elicited significant signal increases in the supracallosal anterior and MCC.

Other effects were found in the MFG, IFS, precentral gyrus (PrecG), postcentral gyrus, precuneus (Precun), SFS, SMG, STG, STS, MTG as well as primary, and secondary visually areas (FDR, $q < 0.05$, see also **Table 4**).

Erotic versus non-erotic emotional picture perception

The effect of sex on the perception phase was analyzed by direct comparison of erotic and emotional picture viewing, the latter matched for emotional intensity and number of displayed people. We thus extracted the sex-specific effect independent of emotional intensity or general processing of human figures.

The contrast revealing greater activations during sexual conditions than during emotional picture periods showed main subcortical effects in the right paraventricular portion of the thalamus (corresponding to paraventricular mediodorsal thalamus, LD, and PT thalamic nucleus), the bilateral head of caudate nucleus and the stria terminalis ($p < 0.001$ uncorrected). Cortical effects were located in the right PCC, left precuneus, bilateral IPS, and MTG as well as left precentral gyrus ($p < 0.001$, see also **Table 4**).

GREATER EFFECTS OF SEX DURING ANTICIPATION OR PICTURE PERIODS

We compared the sex effects during anticipation and picture period to identify those sex-specific effects that were task dependent. A task-by-stimulus interaction was found with greater effects of sex during expectancy conditions than during picture periods: The contrast [anticipation of erotic pictures > anticipation of emotional pictures] > [erotic pictures > emotional pictures] revealed subcortical effects only in the right dorsal intralaminar portion with peak activations in the lateral habenula and medial centromedian thalamic complex, posterior to the PF – the same region that was also shown for the effects of sex on the anticipation period itself ($p < 0.001$ uncorrected). The only cortical structures that showed greater effect of sex on the expectancy condition were the bilateral anterior insular cortex, dACC, SMG, STS, and left precentral gyrus (see **Table 5**).

Except one cluster in right IPS, we did not find regions that showed greater influence of sex on the picture conditions: No other significant effect was found for the inverse comparison of erotic versus emotional effects in both conditions by the contrast [erotic pictures > emotional pictures] > [anticipation of erotic pictures > anticipation of emotional pictures] for an uncorrected threshold of $p < 0.001$.

DISCUSSION

Extending prior results in single subjects (Walter et al., 2008b) on a group level, we were able to detect subcortical thalamic activation restricted to single nuclei using a single-run design with an optimized high resolution single-shot EPI acquisition method at 7 Tesla.

SUBCORTICAL FINDINGS

Our data confirm, for the first time, activations of distinct thalamic nuclei that are restricted to their respective anatomical boundaries and specific to their proposed function. Therefore,

Table 2 | Effects of anticipation.

	[anticipation of erotic × anticipation of emotional pictures]					[anticipation of erotic > anticipation of emotional pictures]				
	x	*y*	*z*	*t*	*p*	*x*	*y*	*z*	*t*	*p*
SUBCORTICAL REGIONS										
CM/PF	3	–17	0	3.7	0.01	5	–19	4	6.4	0.001
	–7	–13	6	2.9	0.05					
Parav. Thal						1	–9	12	5.3	0.001
Tectum	–7	–27	–4	4.0	0.01					
Pulvinar	17	–25	0	4.5	0.01					
	–17	–27	0	4.2	0.01					
NclCaud						7	–3	10	7.8	0.001
VA						–9	–3	4	8.4	0.001
						5	–7	4	7.6	0.001
Pall						–15	–9	2	6.7	0.001
Put						25	–15	4	5.2	0.001
CORTICAL REGIONS										
aIfO	35	13	12	3.9	0.01	41	23	12	5.2	0.001
	–35	19	10	3.7	0.01					
dACC	5	7	46	6.4	0.001	5	3	50	6.8	0.001
	1	11	48	6.8	0.001					
FEF						39	1	36	5.6	0.001
IFS	41	29	20	3.6	0.01					
IPS						31	–55	34	7.7	0.001
						–49	–35	40	10.1	0.001
MCC	3	3	30	3.6	0.01					
	–5	–3	34	3.6	0.01					
MFG	31	45	30	3.0	0.05					
MT +	41	–63	4	6.0	0.001	47	–59	6	6.6	0.001
	–39	–66	4	7.2	0.001	–53	–65	16	6.8	0.001
PCC	–1	–31	22	3.9	0.01					
	–7	–31	28	3.8	0.01					
SFS	35	37	32	3.4	0.01					
SMA_lat	41	–7	44	6.9	0.001					
	–35	–5	44	6.2	0.001					
SPL						–31	–43	40	6.0	0.001
STG	41	–23	–2	3.7	0.01					
STS	53	–43	12	9.3	0.001	47	–45	16	8.2	0.001
	–49	–45	16	4.7	0.01					

Effects of anticipation revealed by the conjunction [anticipation of erotic pictures × anticipation of emotional pictures] (left) (conj. $p < 0.05$, uncorrected) and effects of sexual arousal during anticipation period revealed by the contrast [anticipation of erotic pictures > anticipation of emotional pictures] (right) ($p < 0.001$, uncorrected, x,y,z coordinates in Talairach space). For abbreviations see Table 1.

our findings lend support to the theory of functional subdivisions within the thalamus coactivating with distinct basal ganglia and cingular as well as insular subregions and thus support the distinction of affective and cognitive cortical subdivisions, based on segregated thalamocortical loops (Devinsky et al., 1995; Bush et al., 2000).

According to our findings, activations in the mediodorsal thalamic nucleus can be primarily related to the emotional intensity of a stimulus while the centromedian/parafascicular complex is rather affected by general attentional processing, irrespective of the emotional context. In contrast, the paraventricular mediodorsal complex, including parts of the LD and PT thalamic nucleus can be related to stimulus-specific sexual content independent of the current task, i.e., expectation or picture perception. These sex-specific activations were mirrored correspondingly by a main effect of sexual intensity in the head of caudate nucleus. Interactions in terms of task dependent effects of sex that appeared specifically during anticipation periods were limited to posterior intralaminar and habenula portions of medial thalamic regions. This suggested functional distinction is well in line with a number of invasive studies in animals and serves as an indirect evidence for non-invasive studies in humans.

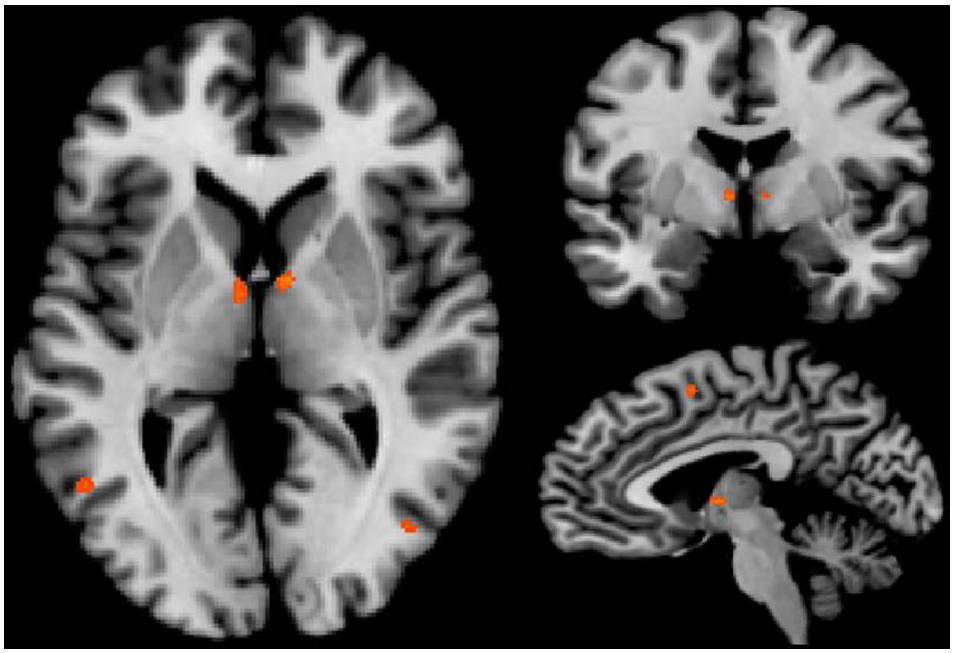

FIGURE 5 | Bilateral activation in the ventral anterior thalamic nucleus was revealed by the contrast [anticipation of erotic pictures > anticipation of emotional pictures] at an uncorrected threshold of $p < 0.001$, *x*: 4; *y*: −6; *z*: 5.

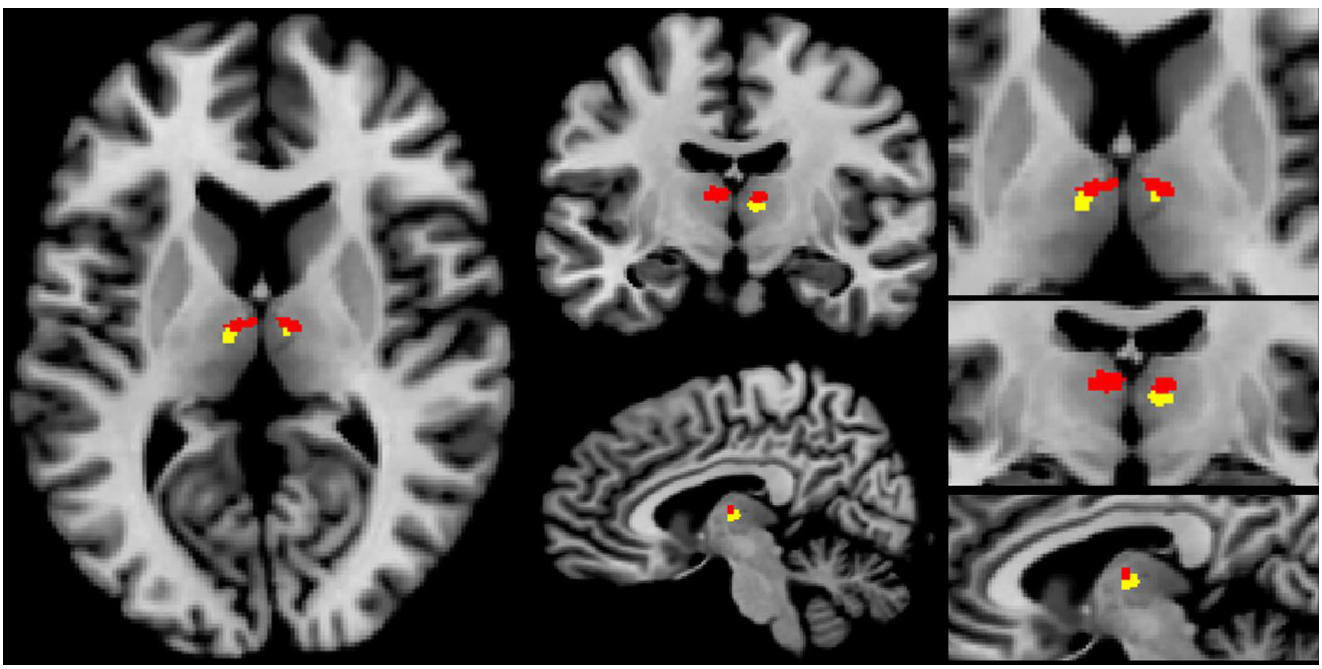

FIGURE 6 | Thalamic activation for erotic and emotional picture perception in the bilateral mediodorsal thalamic nucleus. Erotic picture perception showed a more mediodorsal activation pattern (red voxels), while activation for emotional picture perception was located more laterally (yellow voxels) ($p < 0.001$, uncorrected, *x*: −7, *y*: −13, *z*: 10).

Intralaminar activations: the centromedian/parafascicular complex is involved in attention processing

The centromedian/parafascicular thalamic complex was previously found in animals to be related to attentional processing (Kinomura et al., 1996; Haber and Calzavara, 2009) and attentional shift to salient stimuli (van der Werf et al., 2002). It was shown to be part of the ascending reticulo–thalamo–cortical activating system (Moruzzi and Magoun, 1949; Isaacson and Tanaka, 1986; Cornwall

and Phillipson, 1988b) and to be involved in sexual arousal and erection in rats and gerbils (Heeb and Yahr, 1996, 2001; Coolen et al., 1997, 2003a,b; Veening and Coolen, 1998; Temel et al., 2004).

As shown by Matsumoto and colleagues, activations in macaque CM/PF neurons were not responsive to reward but to unexpected stimuli when investigated with electrophysiological recordings. Their pharmacological inactivation via muscimole, however, also abolished reward related processing which was specifically related to tonic activations in striatum (Matsumoto et al., 2001). Similarly, our results suggest the involvement of these intralaminar nuclei in general attentional processes, which are not specific to sexual processing but may be regarded necessary to reorient attention toward these specifically salient stimuli.

Activations in the CM/PF portions were paralleled by coactivations in anterior insula cortex and dorsal ACC in our study, and in these regions activations were greatest for erotic anticipation. For MD, on the other hand, no such indication of functional connectivity, in terms of coactivation during a certain condition (Friston, 1994), with the cingulo–opercular network could be found. In the attentional task, CM/PF, on the contrary, was coactive with those cortical regions with largest evidence for involvement in attentional processes: The dorsal ACC as part of the cognitive division of ACC (Devinsky et al., 1995; Bush et al., 2000) has been found in a number of studies including executive control and direction of attention. Together with the anterior insula, it has thus been proposed to form the so called attention set network (Dosenbach et al., 2008). In our study, the expectancy condition requires subjects to direct their attention to the content of the subsequent picture period. This task is likely to set attention to the upcoming event. The activation of the anterior insula during this task supports the

Table 3 | Main effects of sexual arousal.

	x	*y*	*z*	*t*	*p*
Cd_head	11	1	14	3.9	0.01
IPS	31	–53	34	3.4	0.01
	–21	–61	34	3.4	0.01
PoCG	–43	–33	42	4.2	0.01
SMG	–51	–33	40	3.4	0.01
SPL	25	–73	28	3.8	0.01
MTG	39	–71	4	3.0	0.05
	–51	–67	4	3.6	0.001
OcG	–39	–65	–12	3.2	0.05
Parav. Thal	3	–13	12	3.0	0.05

Main effects of sexual arousal revealed by the conjunction [(anticipation of erotic picture > anticipation of emotional picture) × (erotic picture > emotional picture)] at a conjoint threshold of p < 0.05 (x, y, z coordinates in Talairach space). For abbreviations see Table 1.

Table 4 | Effects of picture viewing.

	[erotic pictures × emotional pictures]					[erotic pictures > emotional pictures]				
	x	*y*	*z*	*t*	*p*	*x*	*y*	*z*	*t*	*p*
SUBCORTICAL REGIONS										
Cd_head						11	3	16	6.0	0.001
						–9	–5	14	6.6	0.001
MD	9	–17	14	4.3	0.01	3	–11	16	5.9	0.001
	–7	–13	8	4.2	0.01					
Pulvinar	17	–27	2	11.5	0.001					
	–23	–29	2	11.8	0.001					
Putamen	19	–1	10	3.2	0.05					
Str. term.						1	–3	4	7.9	0.001
Tectum	–3	–25	0	4.3	0.01					
CORTICAL REGIONS										
IFG	45	31	16	3.5	0.01					
	–43	27	20	2.8	0.05					
Insula	–37	–7	18	3.2	0.05					
IPS						37	–33	38	12.7	0.001
						–41	–35	40	9.5	0.001
MCC	–3	1	36	3.2	0.05					
MFG	37	17	26	4.6	0.01					
	–53	9	28	3.2	0.05					
MTG						51	–61	0	5.8	0.001
						–49	–63	0	6.4	0.001
PCC						11	–41	20	5.9	0.001
PrecG	41	–1	32	5.0	0.001					
	–35	–7	44	5.9	0.001	–25	–11	46	5.9	0.001
Precun						–13	–57	34	9.3	0.001

Effects of picture viewing revealed by the conjunction [erotic pictures × emotional pictures] (left) (conj. p < 0.05, uncorrected) and the effect of sex on the picture period shown by the contrast [erotic pictures > emotional pictures] (right) (p < 0.001, uncorrected, x, y, z coordinates in Talairach space). For abbreviations see Table 1.

Table 5 | Comparison of effects of sexual arousal during anticipation and picture period.

	x	*y*	*z*	*t*	*p*
aIfO	39	9	14	5.2	0.001
	–31	19	14	5.5	0.001
CM/PF	9	–21	4	5.5	0.001
dACC	9	19	36	5.7	0.001
	–3	15	30	5.3	0.001
SMG	49	–39	38	7.1	0.001
	–51	–43	34	5.0	0.001
STS	49	–41	14	5.9	0.001
	–53	–45	22	6.5	0.001
PraecG	–39	–7	42	5.9	0.001

Comparison of effects of sexual arousal during anticipation and picture period shown by the contrast [(anticipation of erotic pictures > anticipation of emotional pictures) > (erotic pictures > emotional pictures)] for an uncorrected p < 0.001. The inverse contrast [(erotic pictures > emotional pictures) > (anticipation of erotic pictures > anticipation of emotional pictures)] did not show any significant clusters up to an uncorrected level of p < 0.001 (x, y, z coordinates in Talairach space). For abbreviations see Table 1.

hypothesis of its involvement in attention set. Since no direct motor task is required in our design, the hypothesized role of CM/PF for mere motor adjustment, as suggested by studies in non-human primates and rodents (van der Werf et al., 2002), may need adaptation in humans. While van der Werf and colleagues proposed a differentiation of PF and CM functions based on their different projections to medial and lateral striatum, with a specific role of CM during sensory motor and PF during associative-limbic motor functions, our study cannot contribute to such a distinction in humans mainly due to insufficient resolution. We can, however, confirm their clear distinction from MD regarding its function as well as its functional connectivity.

The CM/PF has been described to be strongly connected to the basal ganglia (Royce and Mourey, 1985; Cornwall and Phillipson, 1988b; Berendse and Groenewegen, 1990; Nakano et al., 1990; Fenelon et al., 1991; Sadikot et al., 1992; Haber and Calzavara, 2009) and the motor-, premotor, and primary somatosensory cortex (Berendse and Groenewegen, 1991; François et al., 1991). These connections have been found especially for CM, while PF seems to be connected to the dACC and there especially to BA24 (Vogt et al., 1987a,b; van der Werf et al., 2002). Only few studies reported connections of CM/PF with the anterior insula in hamsters (Reep and Winans, 1982a,b) and in rhesus monkeys (Mufson and Mesulam, 1984). In humans, anatomical connections especially from CM/PF to dACC, anterior insula and to a majority of subcortical structures are supported by diffusion tensor imaging (DTI) results (Eckert et al., 2009). Our findings show a coactivation of CM/PF with dACC and the anterior insula indicating a functional connectivity of these regions under the task condition. These findings support the existence of at least a functional connectivity between CM/PF, dACC and the anterior insula also in humans.

The effect of greater activation during erotic as compared to emotional anticipation periods as found for the anterior insula, in addition to the main effect of attention, parallels results of the interaction analysis in CM/PF. While this activation was located more dorsally and may thus represent rather CM than PF activations, due to limited spatial resolution, also contributions from lateral habenular complex would be possible. In case of interpretation of the structure as lateral habenular complex, however, a negative effect of sex would need to be discussed. Neurons in the lateral habenula (LHb) inter alia respond to negative prediction errors, e.g., when an outcome of any kind is worse than expected, when positive reward is not delivered or when punishment is received. In that context, it has been strongly related to disappointment in primates and in humans (Matsumoto and Hikosaka, 2007, 2009; Salas et al., 2010). The LHb has strong connections to the ventral tegmental area (via the rostromedial tegmental nucleus) and inhibits dopamine release in this structure when activated. Besides reward modulation, the habenula has been suggested to modulate sexual, maternal and feeding behavior as well as pain (Felton et al., 1999; Klemm, 2004; Hikosaka et al., 2008; Hikosaka, 2010). On a transmitter level, it also influences the other two monoaminergic systems via direct projections to the locus coeruleus and dorsal raphe nuclei and has thus been claimed to be causally involved in depression (Sartorius and Henn, 2007; Sartorius and Meyer-Lindenberg, 2009; Sartorius et al., 2010). We find activity in the LHb complex as an interaction effect of sex during anticipation period, which is not present during picture period. Characteristics of nucleus accumbens activation potentially related to habenular activation could have served to clarify this issue. However, slice coverage did not include nucleus accumbens.

The exact attribution of the observed interaction effect thus remains open for final clarification in future studies. At this stage, it has to be noted that a task-by-sex interaction leads to significant effects during anticipation in CM but not in MD.

Investigating preceding attention toward sexually and emotional stimuli, we could show that CM/PF is integrated into a cortical network which has previously been characterized as relevant for attention.

In contrast to this latter subcortical network mainly involved in attentional processes related to erotic processing, e.g., to direct attention and orchestrate occulomotor responses, we could confirm the role of mediodorsal thalamus for the processing of emotional tone during actual erotic stimulation (Walter et al., 2008a,b).

Specific thalamic nuclei: the mediodorsal thalamic nucleus mediates emotional salience of stimuli

The mediodorsal thalamic nucleus forms a main part of the limbic thalamus (Vogt et al., 1987a,b). Activation in this nucleus was described according to processing of emotional stimuli (Oyoshi et al., 1996; Price et al., 1996; Price, 1999; Vertes, 2006) and it seems to be impaired in mood disorders like depression (Drevets et al., 1992). It was, moreover, described as part of the salience network (Seeley et al., 2007). Despite insufficient spatial resolution, peak activations during erotic stimulation have been reported in MD (Redouté et al., 2000; Arnow et al., 2002; Karama et al., 2002; Heinzel et al., 2006). However, a specific component analysis revealed that these activations were rather caused by the emotional content of sexual stimuli, which often increases during erotic stimulation, than by the specific erotic intensity itself (Walter et al., 2008a).

Activation of the mediodorsal thalamic nucleus was found in our study during both, emotional and erotic picture perception. This activation supports the involvement of MD in emotional processing. In contrast to other studies, no differential effect between erotic

and non-erotic picture perception was found in MD in our study. We attribute this to the specific picture set used in our study, which, based on our previous investigations, was purposely matched for GEA. Consequently, since the two picture samples did not differ in their emotional intensity ratings, no significant differential effect was expected for MD during the picture conditions.

This finding supports the hypothesis that MD strongly detects the emotional content of a stimulus and thus provides a necessary component for sexual processing. Therefore the MD is not considered a specific but rather a supportive sexual core region. This would explain the observation of sexual dysfunction after MD lesions and underlines the importance of the affective component during a multidimensional processing of sexual arousal (Redouté et al., 2000; Temel et al., 2004).

The MD was described as a relay nucleus of the thalamus, linking basal ganglia and cortex (McFarland and Haber, 2002). Subcortical connections of MD were reported to periaqueductal gray, ventral tegmental area, and claustrum in monkeys (Erickson et al., 2004) as well as to amygdala, area innominata, LHb, lateral hypothalamus, the ventral tegmental area, and the dorsal tegmental gray in rats (Krettek and Price, 1977; Cornwall and Phillipson, 1988a). Main cortical projections were found to the PFC in humans, primates and cats (Alexander and Fuster, 1973; Vogt et al., 1987a,b; Price, 1999; Erickson and Lewis, 2004). Reciprocal connections to the anterior insula have been described in rats (Allen et al., 1991), hamsters (Reep and Winans, 1982a,b), and in monkeys (Ray and Price, 1992, 1993). Functional connectivity of MD with the anterior insula has also been shown in humans (Seeley et al., 2007) and anatomical studies *in vivo*, using DTI, showed MD connections to anterior insula and PFC (Eckert et al., 2009; Klein et al., 2010; Zhang et al., 2010).

In this study, we found a coactivation of the supracallosal and MCC and MD thalamus during picture perception. These activations were paralleled by mid-insular activations, though it has to be noted that most ventral aspects of the anterior insula were not covered by our functional acquisition. This coactivation is in line with the findings by Seeley and colleagues which suggest an involvement of MD in salience processing. Other than task- or goal-directed attention, salience describes a stimulus property to result in reallocation and binding of attentional resources to itself. In response to an external stimulus, this process was shown to be mediated by exactly these regions, namely the supracallosal dACC and midcingulate as well as anterior insula together with peak activations most likely to represent mediodorsal thalamus (Seeley et al., 2007). While this network was best described using an independent component approach on resting-state data, we were able to replicate this network using our task data. Interestingly, we did not find significant differences during the actual picture conditions, suggesting comparable salience of emotionally matched stimuli after they have both been announced by expectancy cues. MD activations may thus be equally related to the emotional tone of the pictures or to their (comparable) salience. This is also be indicated by similar BOLD responses in anterior insula and supracallosal ACC for both conditions, which have recently been shown to specifically code salience processing (Litt et al., 2010). At the current stage, the involvement of MD in salience processing or emotional processing during erotic visual stimulation cannot be ultimately clarified by our results. The absence of clear effects of salience during erotic picture conditions, however, should be interpreted with caution given our specific experimental design using cued conditions. It has to be noted that our paradigm uses expectancy conditions to induce preceding attention toward upcoming stimuli. This restricts the interpretation of attention effects to a rather cognitive construct of attention and may not be confused with other concepts of stimulus-driven attention. Nonetheless, the work in animals to which we related our findings in CM/PF, was mainly done on tasks which rather involve the latter type of attention. Despite these critical limitations to our findings, it can still be stated that MD activations, in contrast to CM/PF activations are more related to processing of aspects that lead to allocation of attention (such as emotion or salience), than to processing of attention itself (**Figure 7**).

Laterodorsal and parataenial thalamic nucleus are specific to the task independent effect of sexual arousal

An effect of stimulus value has to be considered for erotic stimuli. It was expected in thalamic regions that are connected to structures that have previously been reported to be associated with SSI or stimulus value such as the case for hypothalamus and ventral striatum (Walter et al., 2008a).

*Laterodorsal thalamic nucleus***.** We could show that thalamic activations that were located at anatomical locations of LD and PT were specific to sexual processing as ascertained by the analysis of the main effects of sex. The activation of the LD thalamus specific to visual erotic stimulation that we detected in our study is supported by several findings on connectivity of LD to the limbic cortex (Thompson and Robertson, 1987; van Groen and Wyss, 1992; Shibata, 2000) and to the lateral hypothalamus (Ryszka and Heger, 1979) found specifically active during erotic perception (Walter et al., 2008a) and erotic stimulation (Georgiadis et al., 2010).

*Parataenial thalamic nucleus***.** The PT nucleus belongs to the medial thalamic nuclei group (Rose and Woolsey, 1949).

Its projections to the nucleus accumbens (Powell and Cowan, 1954; Berendse and Groenewegen, 1991) are in accordance with the activation of the reward system found in most studies investigating sexual processing (Walter et al., 2008a). The activation of the PT nucleus specific to the perception of sexual stimuli as found in our study supports these previous findings.

It is worth noting that the analysis of the peristimulus time histograms in PT revealed that the peaks for erotic expectancy conditions lagged those for the erotic picture conditions by 3 s, which was the actual lag of expectancy and subsequent picture conditions (**Figure 4**). This indicates at least a confound on the expectancy effects by their subsequent picture conditions. According to these results, activation in PT is rather caused by sexual perception than by sexual attention although an influence of sexual attention on this area is likely, but cannot fully be explained by our results.

Ventral anterior thalamic nucleus and motor preparation

A greater role in motor preparation is hypothesized for VA, which showed effects of anticipation similar to the intralaminar CM/PF complex. Different from the latter nuclei, we found stronger activations during erotic as compared to emotional expectancies and no main effect of expectancy.

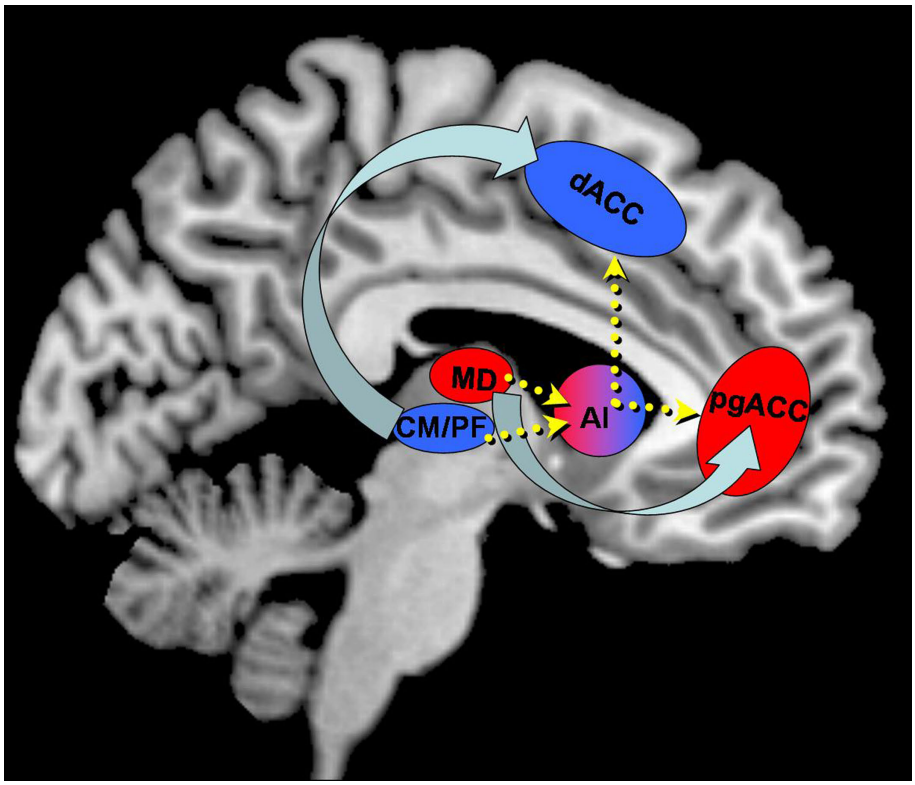

FIGURE 7 | Schematic representation of segregated and integrated processing of emotional and attentional dimensions during sexual stimulation. Distinct parcellations within thalamus reflect a rostro-caudal parcellation of ACC with direct connections of corresponding portions. The anterior insula (AI) may serve as an integrator with connections to both networks thus orchestrating functionally distinct processes. Arrow width does not indicate the strength of directionality.

The VA thalamic nucleus is one of the relay nuclei of the thalamus that have bidirectional connections both to basal ganglia and cortex (Haber and McFarland, 2001; McFarland and Haber, 2002; Haber and Calzavara, 2009). It is part of the oculomotor loop linking parts of the basal ganglia (caudate nucleus, globus pallidus, substantia nigra) and FEF (Alexander et al., 1986). Being associated with motor control and motor learning (Haber and McFarland, 2001), it holds cortical connections with the supplementary motor area, cingulate motor area, premotor cortex, PFC, and FEF.

In our experiment, we find main activation in VA and FEF during anticipation of sexual stimuli. This can be due to the motor preparatory component of the anticipation task, which is triggered by the sexual (motor relevant) content of the subsequent stimulus. This hypothesis is supported by our finding of coactivation of the precentral gyrus during the perception of erotic stimuli which has also been shown by previous studies investigating sexual perception (Mouras et al., 2003; Moulier et al., 2006).

EXTRATHALAMIC ACTIVATIONS

While hypothalamus and ventral striatum were not covered by our investigation, we could monitor sex effects in more dorsal parts of the ventral striatum:

Caudate and putamen

The caudate nucleus is part of several thalamocortical circuits as described by Alexander and colleagues (Alexander et al., 1986). It was shown to be involved in learning and memory (Oberg and Divac, 1975; Chozick, 1983; Packard and Knowlton, 2002). Moreover, an involvement in goal-directed behavior during erotic stimulation was suggested by Aron and colleagues (Aron et al., 2005). Studies investigating sexual processing found different activations in the caudate nucleus during erotic and emotional picture perception (Walter et al., 2008b). We found coactivation with both MD as well as CM/PF in the medial head of caudate nucleus in both anticipation and picture period, which supports its involvement in separate thalamocortical circuits processing, among others, attention and emotion. As revealed by our results, sex during both anticipation and picture period elicited greater results in the right medial head of caudate nucleus, which underlines its involvement not only in sexual perception but also in sexual attention.

Connections from caudate nucleus have been described to CM/PF in cats (Royce, 1978, 1983) and to MD in cats and monkeys (Showers, 1958; Macchi et al., 1984). We found a functional connectivity of the head of caudate nucleus with CM during anticipation period and with MD during picture perception. Our findings lend support to the previously described anatomical connections.

Claustrum

It has been suggested that the claustrum is involved in consciousness (Crick and Koch, 2005) and studies investigating sexual processing have been able to underline these findings (Mouras et al., 2003; Georgiadis et al., 2009, 2010).

As revealed by previous studies, activation in the claustrum was often hard to differentiate from insular activity due to poor special resolution (Arnow et al., 2002). Making use of a high spatial resolution approach, we were able to distinguish between claustrum and insular activity. We found activation in the claustrum for erotic, but not for non-erotic picture perception. This finding supports the hypothesis of a previous study by Walter and colleagues that the claustrum is mainly involved in the processing of erotic stimuli (Walter et al., 2008b). As our stimuli were matched for emotional intensity, we even suggest the specificity of claustral activation to erotic stimuli, independent of their emotional content.

LIMITATIONS

While this is, to our knowledge, the first group study at ultra high field, enabling group inference on small anatomical structures, our group size was limited to a population of 10 male subjects. This group setting was chosen in a way to maximize homogeneity. Since some authors have proposed differences in sex-related activations in males and females (Hamann et al., 2004), the extrapolation of our results to a female population is thus questionable. However it was shown that specific activations were similar between males and females when different degrees of subjective ratings were controlled for (Karama et al., 2002) or when stimuli with equal sexual intensity were chosen (Walter et al., 2008a). We wanted to use an optimized paradigm of one single run and thus maximized sexual arousal of pictures for a male group, which also limited possible confounds of gender on the main effects. It remains subject to future studies to explore the newly described activation patterns in a female population. However, future studies may address a greater population not only to account for gender effects, but also to improve inference accuracy about a global population.

At the current stage, the subject selection criteria on ultra high fields are comparably strict which may change with the increase in safety experience on such systems and which will further allow the definition of an optimal sample size for group studies. While there are currently efforts to include increasing numbers of subjects into the same study on lower fields to tackle the same issue of generalizability (Fox and Lancaster, 2002), other scales may probably be more sensible given that larger smoothing in large scale population studies counteract the high spatial specificities for which our setup was chosen.

Despite the above mentioned advantages of high resolution fMRI at 7 Tesla, it suffers from reduced accuracy due to signal dropouts in ventral subcortical structures, such as parts of nucleus accumbens and hypothalamus, which are caused by magnetic field (B0) inhomogeneities at air–tissue boundaries. As these structures have been shown to be involved especially during sexual arousal (Mouras et al., 2003; Moulier et al., 2006; Walter et al., 2007), functional imaging sequences that reduce the signal loss in these structures are highly desirable. Higher resolution is possible and has been demonstrated (Speck et al., 2008). On the other hand, high temporal resolution needed for event-related studies is obtained at the cost of reduced volume coverage.

Other structures like the ventral pallidum show extremely short native T2* at 7 Tesla due to iron deposits (Hallgren and Sourander, 1958; Aoki et al., 1989) and thus very low signal intensity. The covered scan volume was based on strong prior hypotheses from previous similar studies of our own group (Heinzel et al., 2006; Walter et al., 2007, 2008a,b) and showed convincing effects within the regions of interest including thalamus, dACC and anterior insula. Due to this limited volume coverage, this study cannot answer specific question regarding sexual processing in distinct parts of the ventral and dorsal attention network or functional connectivity that would help to further defining small structures such as LHb.

It should also be noted that despite additional smoothing, we strongly emphasized our analysis on the detection of small structures such as thalamic subregions, while larger clusters of activation in bigger anatomical (e.g., cortical) regions could have benefited from greater smoothing kernels. Our approach was aimed at high spatial specificity necessary for the detection of small subcortical structures. As group statistics dependent on spatial smoothing to increase intersubject overlap we accepted that this may lead to reduced sensitivity in cortical areas.

For the performance of group statistics, all anatomical and functional data were transferred into Talairach space as offered by BrainVoyager QX. The anatomical alignment requires additional anatomical information which leads to an optimized overlap of subcortical regions and allows for a clear differentiation between distinct subcortical structures. In cortical areas, however, other means of non-linear normalization as provided by other software distributions may have been better suited.

CONCLUSION

Our results show the involvement of subcortical structures, especially the thalamus and caudate nucleus, in the core components of sexual processing and embed them into predescribed thalamocortical loops.

The emotional component of sexual stimuli is reflected in the coactivation of the mediodorsal thalamic nucleus together with the pregenual ACC that has been shown to encode the emotional component of sexual arousal on cortical level (Walter et al., 2008a).

In contrast, sexual attention is represented by activation in the centromedian/parafascicular and the lateral habenula complex. The CM/PF that is connected to dACC, showed a functional connectivity with the anterior insula during erotic anticipation, which we point to trigger the salience component of sexual attention.

The paraventricular thalamus, including the paraventricular MD, LD, and PT showed sex-specific activation, in accordance with connections to lateral hypothalamus, nucleus accumbens and cingulate cortex. This brings small parts of the thalamus back to a specific involvement in sexual processing which was so far not detectable using standard fMRI at lower fields.

For the first time, different components of sexual processing described in the literature and observed on cortical level could be related to distinct thalamocortical circuits involving the basal ganglia as a relay station of sexual arousal and attention.

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