BRAIN CHOLINERGIC MECHANISMS

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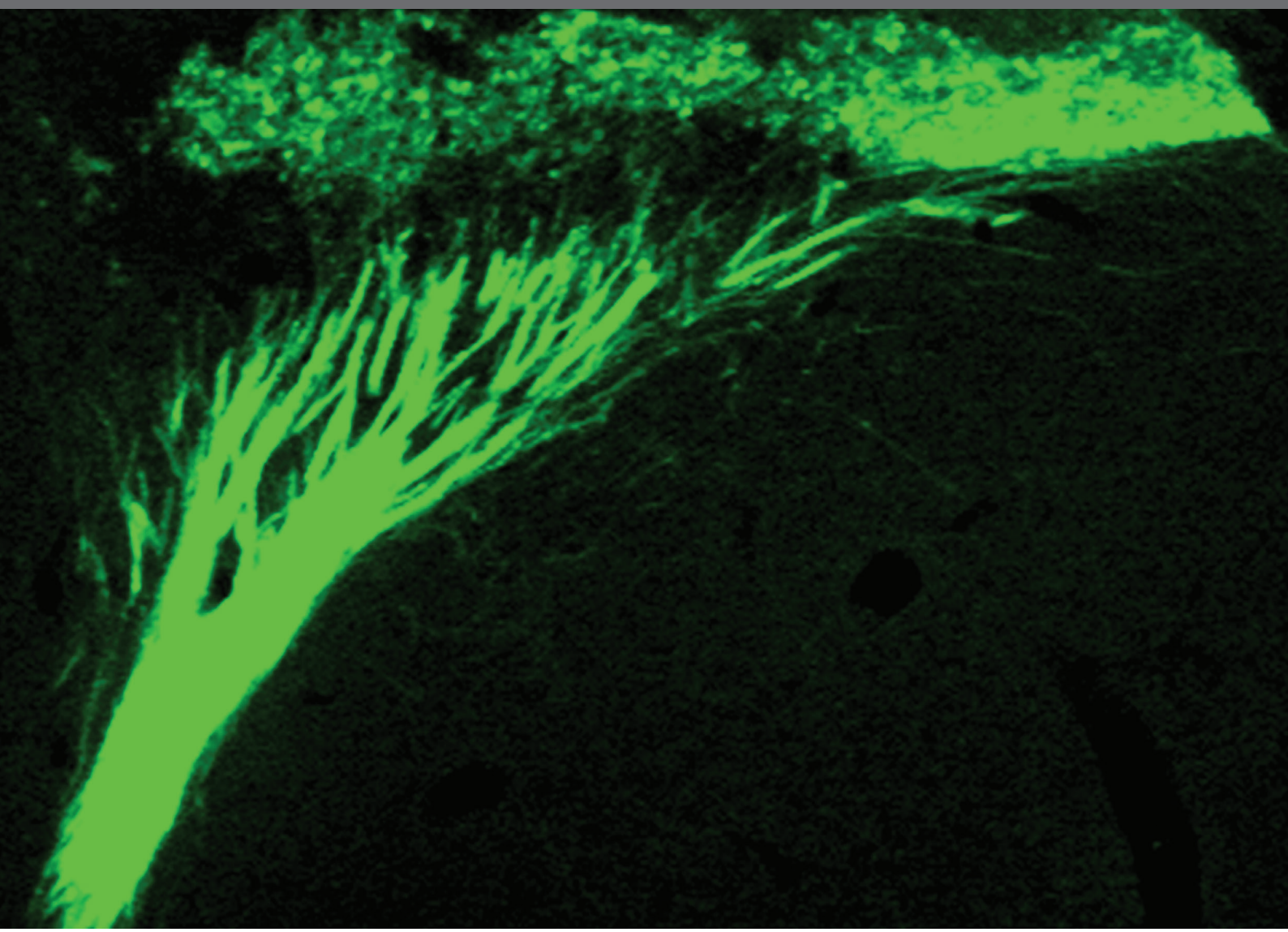

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BRAIN CHOLINERGIC MECHANISMS

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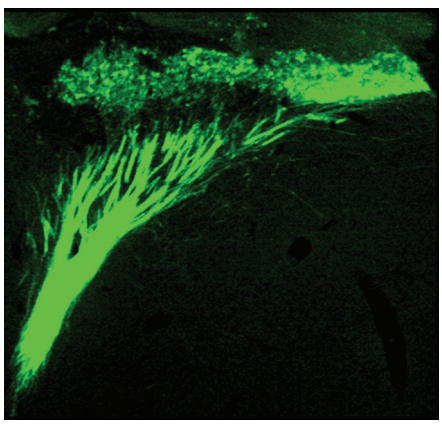

The Figure shows cholinergic neurons of the medial habenula and their projections through the fasciculus retroflexus. The cholinergic neurons express a tauGFP fusion protein driven by the choline acetyltransferase promoter.

Image by: Wesley Perrine and Sukumar Vijayaraghavan

Much of our understanding of brain physiology has focused on what one might call, first order processes. These essentially include the primary synaptic mechanisms underlying excitation (mainly glutamate) and inhibition (mainly GABA). Our attention has focused on how the balance of excitation and inhibition regulates the timing, patterns, and extent of information flow across various circuits. A lot less is understood regarding second order processes that sculpt and modify these primary interactions. One such modulatory transmitter in the brain is acetylcholine (ACh). The importance of ACh in modulating various behaviors related to learning, memory, and attention has been recognized over the last four decades as has its involvement in various neurodegenerative and psychiatric disorders. However, our understanding of the mechanistic bases for these actions is at its infancy, at best and much remains to be understood. The array of receptor subtypes for nicotinic and muscarinic receptors, their different locations, and complex signal transduction mechanisms remain a puzzle. Transmitter (ACh) release sites and their relationship to receptor loci are poorly understood. Overall, we lack a unifying framework for conceptualizing how disparate actions of the transmitter on receptors lead to circuit modulation and, eventually, influences on cognition.

By its very nature, reports on cholinergic signaling are quite scattered, presented in journals across sub-disciplines and in the context of the systems they modulate. Hence, there is need for consolidation of these studies under a single cover that would allow one to compare and contrast the effects of this transmitter across systems and contexts. This special issue represents one such compilation. The issue addresses cholinergic modulation of defined circuits that lead to specific behaviors and consists of a judicious mixture of review articles and primary papers. The articles focus on three aspects of the system: 1) Cellular targets of cholinergic signaling. 2) Receptor mechanisms. 3) Endogenous transmitter distribution and action.

While no common mechanism emerges that can explain cholinergic actions on brain functions, on can postulate that the transmitter system is dynamic, modulating the balance of excitation and inhibition in various circuits. This modulation sets up timed network oscillations and it is tempting to speculate that these oscillations form a template for better encoding of afferent inputs. One can broadly envision the role of the cholinergic system as facilitating processes that allow for more efficient acquisition of learning and engraving of memories. Thus, understanding the mechanisms underlying tonic and stimulus-dependent release of ACh and how it alters firing templates of neuronal networks would be the first step towards elucidating its role in learning and memory.

This special topics edition provides clues to some of the actions of ACh. It is hoped that the articles allow the reader to extract common themes and potential mechanisms of cholinergic regulation that will lead to elucidation of general principles governing the actions of this important neuromodulator.

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EDITORIAL
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Editorial: Brain cholinergic mechanisms

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Keywords: acetylcholine, nicotinic, muscarinic, interneurons, synaptic

While historically acetylcholine (ACh) holds a central place in the discovery of chemical transmission in the nervous system, progress in our knowledge of the mechanistic underpinnings of cholinergic transmission in the central nervous system (CNS) has lagged behind its sister transmitters. For example, unlike what we know about glutamatergic transmission, neither the prevalence of fast cholinergic transmission or postsynaptic specializations at cholinergic synapses, is well understood. Every level of inquiry into the cholinergic system reveals a bewildering complexity in signaling and transduction mechanisms. Receptor localizations within and among neurons, along with transmitter source and access to receptors, provide for a system that is capable of neuromodulation at multiple time scales that allow for short- and long-term regulation of circuit output in the brain. The compilation of reviews and primary papers in this research topic provides a sampling of findings at different levels of integration that highlight both the current status of our understanding of CNS cholinergic mechanisms as well as reveals the gaps in our knowledge that need to be filled.

How ACh mediates signaling in the brain is still an unresolved issue at the level of synaptic physiology. Cholinergic innervation in the brain arises from two main loci- the basal forebrain and the pendunculo-pontine area of the hindbrain. In both instances a relatively small cluster of neurons send diffuse projections into various target areas. The diffuse nature of the projections, as well as the non-planar relationship between the cholinergic neurons and their targets precludes traditional slice electrophysiology approaches to examining signaling. Recent advances in optogenetic techniques offer a potential solution to this problem and some of the advances made thus far are reviewed in this research topic (Luchicchi et al., 2014). Some of the relevant issues that need to be resolved are the prevalence of synaptic vs. non-synaptic modes of activation, the role of transmitter diffusion, relationship between ACh release sites and the distribution of acetylcholine esterases, and the potential co-release of other transmitters from cholinergic terminals.

In addition to the centrifugal cholinergic inputs, areas of the cortex also express their own, resident, cholinergic interneurons. Well studied among these are the cholinergic interneurons of the striatum, relevant to disorders like Parkinson's disease. These are large aspiny neurons that are small in number (about 1% of the total neuronal population) but send dense projections throughout the area. Recent findings that are summarized here (Lim et al., 2014) show that these neurons themselves are subject to complex regulation, both excitatory and inhibitory, from a vast area of other transmitter systems. Another area, where cholinergic interneurons have been described is the hippocampus (Frotscher et al., 2000). Thus, far, these neurons have remained an oddity, with no functional attribution. In this research topic, work done by the group of Josh Lawrence (Yi et al., 2015) has begun the examination of these neurons using transgenic mice where GFP or YFP is expressed driven by the choline acetyltransferase promoter. Interestingly, these neurons might themselves be subject to cholinergic modulation, presumably from basal forebrain innervation.

We have a long way to go before we understand the complex variables of cholinergic signaling. Differential expression of various subtypes of the ionotropic nicotinic acetylcholine receptors (nAChRs) and the metabotropic muscarinic acetylcholine receptors (mAChRs), their expression in multiple neuronal types within a region, and varying locations within a neuron (i.e., somatic,

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dendritic, synaptic etc.) all orchestrate a complex symphony of neuromodulation. A review of receptor localization and function within hippocampal CA 1 interneurons (McQuiston, 2014) illustrates how complex expression of the two classes of receptors can allow for differential control of principal cell activity under varying concentrations of the transmitter.

These local control mechanisms can regulate circuit outputs in various regions of the brain, potentially mediating the attention and learning-specific behaviors ascribed to ACh-driven modulation. In the olfactory system, nAChR activation can filter odor signals such that weak inputs are eliminated while strong ones are allowed through, thus providing a mechanism for altering the gain function of a circuit (D'Souza and Vijayaraghavan, 2014) and, perhaps, regulating the effects of these receptors on odor discrimination (Hellier et al., 2010). In the visual cortex, differential functional expression of mAChRs might explain neuronal synchrony and gamma oscillations allowing for the tuning of network output during tasks involving perceptual learning (Groleau et al., 2015). In the hippocampus, a potential role for mAChR-regulated release of endocannabinoid in circuit oscillation highlight the complex mechanisms in play that underlie the effects of the cholinergic system in regulation of behavior (Alger et al., 2014).

Cholinergic receptors are a potential gold mine as targets for therapies and pharmaceutical companies have not been diffident about exploring various receptor ligands as potential therapies. Emerging studies implicate cholinergic receptors in various addictive mechanisms: the direct interaction between cocaine and nAChRs reported in this research topic (Acevedo-Rodriguez et al., 2014) illustrates how these receptors could play an important role in the reinforcement properties of cocaine. The cholinergic system appears to be involved in specific behavioral endophenotypes of a number of diseases such as Alzheimer's disease (Oddo and LaFerla, 2006), schizophrenia (Wallace and Bertrand, 2013), and Parkinson's disease (Bohnen et al., 2010). More recent animal model studies also implicate the transmitter system in autism (Amodeo et al., 2014).

This is prime time for the development of clinical therapies that target the cholinergic system for a host of brain disorders. At the same time, as the collection of studies in this research topic illustrates, much remains to be understood regarding the physiology of cholinergic transmission and modulation. There is a risk of pharmacological advances outpacing our knowledge of cholinergic signaling mechanisms in the brain. History tells us that such a disconnect between therapeutic advances and our knowledge of physiology can often lead to unintended complications from novel therapies, a classic example being the aggressive marketing of heroin as a cough remedy at the turn of the twentieth century. There needs to be significant investment in examining the basic biology of cholinergic modulation of brain circuits in order to develop more rational and safe therapeutic targets that the cholinergic system offers.

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References

Acevedo-Rodriguez, A., Zhang, L., Zhou, F., Gong, S., Gu, H., De, B. M., et al. (2014). Cocaine inhibition of nicotinic acetylcholine receptors influences dopamine release. *Front. Synaptic Neurosci.* 6:19. doi: 10.3389/fnsyn.2014.00019

Alger, B. E., Nagode, D. A., and Tang, A. H. (2014). Muscarinic cholinergic receptors modulate inhibitory synaptic rhythms in hippocampus and neocortex. *Front. Synaptic Neurosci.* 6:18. doi: 10.3389/fnsyn.2014.00018

Amodeo, D. A., Yi, J., Sweeney, J. A., and Ragozzino, M. E. (2014). Oxotremorine treatment reduces repetitive behaviors in BTBR T+ tf/J mice. *Front. Synaptic Neurosci.* 6:17. doi: 10.3389/fnsyn.2014.00017

Bohnen, N. I., Müller, M. L., Kotagal, V., Koeppe, R. A., Kilbourn, M. A., Albin, R. L., et al. (2010). Olfactory dysfunction, central cholinergic integrity and cognitive impairment in Parkinson's disease. *Brain* 133, 1747–1754. doi: 10.1093/brain/awq079

D'Souza, R. D., and Vijayaraghavan, S. (2014). Paying attention to smell: cholinergic signaling in the olfactory bulb. *Front. Synaptic Neurosci.* 6:21. doi: 10.3389/fnsyn.2014.00021

Frotscher, M., Vida, I., and Bender, R. (2000). Evidence for the existence of non-GABAergic, cholinergic interneurons in the rodent hippocampus. *Neuroscience* 96, 27–31. doi: 10.1016/S0306-4522(99)00525-4

Groleau, M., Kang, J. I., Huppé-Gourgues, F., and Vaucher, E. (2015). Distribution and effects of the muscarinic receptor subtypes in the primary visual cortex. *Front. Synaptic Neurosci.* 7:10. doi: 10.3389/fnsyn.2015.00010

Hellier, J. L., Arevalo, N. L., Blatner, M. J., Dang, A. K., Clevenger, A. C., Adams, C. E., et al. (2010). Olfactory discrimination varies in mice with different levels of alpha7-nicotinic acetylcholine receptor expression. *Brain Res.* 1358, 140–150. doi: 10.1016/j.brainres.2010.08.027

Lim, S. A., Kang, U. J., and McGehee, D. S. (2014). Striatal cholinergic interneuron regulation and circuit effects. *Front. Synaptic Neurosci.* 6:22. doi: 10.3389/fnsyn.2014.00022

Luchicchi, A., Bloem, B., Viaña, J. N., Mansvelder, H. D., and Role, L. W. (2014). Illuminating the role of cholinergic signaling in circuits of attention and emotionally salient behaviors. *Front. Synaptic Neurosci.* 6:24. doi: 10.3389/fnsyn.2014.00024

McQuiston, A. R. (2014). Acetylcholine release and inhibitory interneuron activity in hippocampal CA1. *Front. Synaptic Neurosci.* 6:20. doi: 10.3389/fnsyn.2014.00020

Oddo, S., and LaFerla, F. M. (2006). The role of nicotinic acetylcholine receptors in Alzheimer's disease. *J. Physiol. Paris* 99, 172–179. doi: 10.1016/j.jphysparis.2005.12.080

Wallace, T. L., and Bertrand, D. (2013). Alpha7 neuronal nicotinic receptors as a drug target in schizophrenia. *Expert. Opin. Ther. Targets* 17, 139–155. doi: 10.1517/14728222.2013.736498

Yi, F., Catudio-Garrett, E., Gábriel, R., Wilhelm, M., Erdelyi, F., Szabo, G., et al. (2015). Hippocampal "cholinergic interneurons" visualized with the choline acetyltransferase promoter: anatomical distribution, intrinsic membrane properties, neurochemical characteristics, and capacity for cholinergic modulation. *Front. Synaptic Neurosci.* 7:4. doi: 10.3389/fnsyn.2015.00004

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REVIEW ARTICLE
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Illuminating the role of cholinergic signaling in circuits of attention and emotionally salient behaviors

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Acetylcholine (ACh) signaling underlies specific aspects of cognitive functions and behaviors, including attention, learning, memory and motivation. Alterations in ACh signaling are involved in the pathophysiology of multiple neuropsychiatric disorders. In the central nervous system, ACh transmission is mainly guaranteed by dense innervation of select cortical and subcortical regions from disperse groups of cholinergic neurons within the basal forebrain (BF; e.g., diagonal band, medial septal, nucleus basalis) and the pontine-mesencephalic nuclei, respectively. Despite the fundamental role of cholinergic signaling in the CNS and the long standing knowledge of the organization of cholinergic circuitry, remarkably little is known about precisely how ACh release modulates cortical and subcortical neural activity and the behaviors these circuits subserve. Growing interest in cholinergic signaling in the CNS focuses on the mechanism(s) of action by which endogenously released ACh regulates cognitive functions, acting as a neuromodulator and/or as a direct transmitter via nicotinic and muscarinic receptors. The development of optogenetic techniques has provided a valuable toolbox with which we can address these questions, as it allows the selective manipulation of the excitability of cholinergic inputs to the diverse array of cholinergic target fields within cortical and subcortical domains. Here, we review recent papers that use the light-sensitive opsins in the cholinergic system to elucidate the role of ACh in circuits related to attention and emotionally salient behaviors. In particular, we highlight recent optogenetic studies which have tried to disentangle the precise role of ACh in the modulation of cortical-, hippocampal- and striatal-dependent functions.

Keywords: acetylcholine, optogenetics, nicotinic receptors, limbic circuitries, attention

INTRODUCTION

Acetylcholine (ACh) is essential to normal CNS function, modulating cognitive, emotional and behavioral functions, including learning and memory (Kilgard and Merzenich, 1998; Hasselmo and Giocomo, 2006), reward (Leslie et al., 2013), wakefulness and attention (Klinkenberg et al., 2011; see Picciotto et al., 2012 for a recent review). Appropriate levels of ACh are required to process relevant sensory information and for encoding environmental cues that drive goal-directed behavior (Sarter et al., 2009). Disruptions of cholinergic transmission contribute to the pathophysiology of neuropsychiatric disorders, including Alzheimer's disease, schizophrenia and drug addiction (Court et al., 2001; Dani and Harris, 2005; Martin and Freedman, 2007). To support its prominent role in the brain, the cholinergic system sends dense projections from sparse cholinergic nuclei, that include the basal forebrain (BF), laterodorsal tegmental nucleus (LDTg), peduculopontine tegmentum (PPTg), and medial habenula (MHb; Woolf, 1991; Mesulam, 1995; Zaborszky et al., 1999; Ren et al., 2011). In addition, there is a small population of choline acetyltransferase (ChAT) positive interneurons in areas including the striatum and neocortex (Woolf, 1991; Mesulam, 1995; von Engelhardt et al., 2007). Cholinergic projections, from the BF, LDTg and PPTg nuclei extend throughout the main telencephalic and limbic structures delivering ACh to broad terminal fields. Released ACh activates via both ionotropic nicotinic and metabotropic muscarinic ACh receptors (nAChRs, and mAChRs, respectively) that vary in terms of cellular localization (pre- and/or postsynaptic), subunit composition, signaling mechanism(s) and affinity for ACh (for recent reviews see Wess, 2003; Gotti and Clementi, 2004; Changeux, 2010; Picciotto et al., 2012).

Although our understanding of the organization of the cholinergic system and its role in modulating certain behaviors is growing, many questions remain to be answered to understand the dynamics of ACh action and its involvement in (patho) physiology. The role of ACh in specific behaviors has been addressed using lesions of cholinergic projections or pharmacological interventions with ACh receptor activation. Such approaches, though informative, are confounded by issues of bioavailability, lack of complete reversibility and the fact that such interventions act on time scales of unknown relevance for cholinergic driven changes

in excitability *in vivo*. Our understanding of how cholinergic projections innervate and modulate target circuitry remains rudimentary. In fact, it is not even clear whether ACh acts as a classic synaptic neurotransmitter—on the millisecond to tens of millisecond time scale—or whether it acts as a neuromodulator (at the hundreds of milliseconds to seconds time scale) or both (see Picciotto et al., 2012; Sarter et al., 2014). The latter hypothesis is supported by several investigations that emphasize the predominant role of ACh in modifying cell excitability and activity of entire networks of neurons (Wonnacott, 1997; Kawai et al., 2007). Moreover, a relatively modest specificity of the cholinergic system exists in terms of connectivity in crucial target regions such as the cortex (see Sarter et al., 2009, for a review). On the other hand, the presence of point-to-point sites of ACh release juxtaposed to cholinergic receptors suggests that the cholinergic system may also utilize fast synaptic signaling, typical of classic neurotransmitters (Smiley et al., 1997; Turrini et al., 2001). Indeed, the complexity of results obtained to date has led to the conclusion that ACh signaling may occur over a range of different time courses due, in part, to varied release mechanisms and proximity of release and receptive sites as well as to the involvement of distinct signaling cascades downstream of both nicotinic and muscarinic AChRs (e.g see Arroyo et al., 2014; Jiang et al., 2014). A lack of high-temporal resolution and accurate detection methods for ACh release has hampered our understanding of whether endogenous cholinergic signaling is mediated by rapid, transient release (millisecond time-scale) and/or by a more diffuse transmission (from second to minute time-scale).

With the exponential rise in the number and type of optogenetic tools developed over the last decade it is now possible to selectively stimulate or inhibit specific populations of CNS cholinergic neurons and/or their axonal terminal fields through the activation of light-sensitive opsins (for reviews see: Deisseroth, 2011; Yizhar et al., 2011; Poorthuis et al., 2014). Here, we review the recent studies that have used the expression of photo-sensitive opsins in the cholinergic system to elucidate the role of endogenous ACh signaling in different brain regions related to attention and emotionally salient/ limbic behaviors.

BASAL FOREBRAIN ACH AND NEOCORTICAL FUNCTION

The mechanisms by which ACh release in the neocortex influences cognitive functions and behaviors are still poorly understood. While early microdialysis studies in the medial prefrontal cortex (mPFC) reported a long-lasting ACh increase during attention-related performance tasks (Passetti et al., 2000), more recent works with faster, dynamic, electrochemical detection of choline, have shown that ACh can also be released briefly in concert with cue detection in a cued appetitive response task (Parikh et al., 2007; Parikh and Sarter, 2008). Thus, while the microdialysis assays are consistent with the idea that ACh release could promote a general state of cortical arousal, due to sustained levels of ACh over long time-scales, recent and more sensitive electrochemical assays highlight a faster, and more transient release of ACh. The latter observation modifies the prior view that ACh only acts through "volume transmission" (Sarter et al., 2009), and underscores the possibility of faster components of ACh action in the modulation of specific cholinergic functions. For example, the phasic release of ACh would support more rapid transitions of cortical states, consistent with cholinergic regulation of an animal's ability to incorporate the detection of a cue into new goal-directed behaviors (Sarter et al., 2014).

CHOLINERGIC FAST SYNAPTIC TRANSMISSION IN CORTEX

The application of optogenetic tools to the analysis of central cholinergic signaling using ChAT-Cre lines in either mice (Kalmbach et al., 2012; Huang and Zeng, 2013) or rats (Witten et al., 2011; see **Figure 1**) allows selective activation and silencing of cholinergic neurons and axonal projections, both *in vitro* and *in vivo*. Using this approach, several studies have now shown that ACh signaling occurs through direct, fast synaptic transmission—as well as over longer time scales consistent with more diffuse transmission—in the cortex (Letzkus et al., 2011; Arroyo et al., 2012, 2014; Bennett et al., 2012; Kimura et al., 2014). Activating channelrhodopsin (ChR2) in fibers from the BF elicited a barrage of inhibitory synaptic inputs to layer (L) 2/3 pyramidal cells, which depended on nAChR activation (Arroyo et al., 2012, 2014; Bennett et al., 2012; Kimura et al., 2014). Pyramidal neurons in L2/3 apparently do not express nAChRs themselves, but L2/3 interneurons do (Poorthuis et al., 2013). Activation of BF fibers produced cell type-specific responses in cortical interneurons. L1 and L2/3 LS neurons exhibited both a fast and a slow response, while L2/3 ChAT bipolar neurons exhibited only a slow response. Activation of L2/3 interneurons by ACh via both nicotinic and muscarinic receptors depressed pyramidal neuron firing thereby curtailing visual responses (Kimura et al., 2014). ACh-induced excitatory postsynaptic currents were generated by a mixed population of nAChRs (Arroyo et al., 2012). In addition to a slow dihydro-β-erythroidine (DHβE) sensitive non-α7*-mediated current, a fast component of excitatory postsynaptic potentials (EPSCs) was abolished by methyllycaconitine (MLA) in both L1 and 2/3 interneurons but not in ChAT+ cells. Comparing the reported time course of the inhibitory barrage received by L2/3 pyramidal neurons upon light-induced ACh release with the time course of the two different EPSC components, suggested that L2/3 pyramidal neuron inhibition is more likely dependent on the slow component, rather than the fast component of cholinergic activation. This was confirmed by bath application of DhβE, which prevented the inhibitory drive onto pyramidal cells. In a follow-up study, the same authors found a large trial-to trial variability of the fast component of the ACh-induced current components, indicative of direct synaptic transmission which they propose is mediated by synaptic α7*-containing (α7*) receptors. This was confirmed by lack of effect of AChE inhibitors on the amplitude or kinetics of this fast current component (Bennett et al., 2012; Arroyo et al., 2014). The slow component showed much less trial-to-trial variability and was sensitive to manipulation of AChE activity. From this, the authors conclude that the slow, non-α7* component involves diffusion of ACh over some distance, and arises from the effects of ACh on extra synaptic α4β2* nAChRs, while the faster nAChR EPSCs are mediated by direct transmission via synaptic or peri-synaptic α7* AChRs (Arroyo et al., 2012, 2014). These experiments demonstrate that in superficial layers of the somatosensory, visual and auditory cortex, L1 and L2/3 interneurons receive both direct and

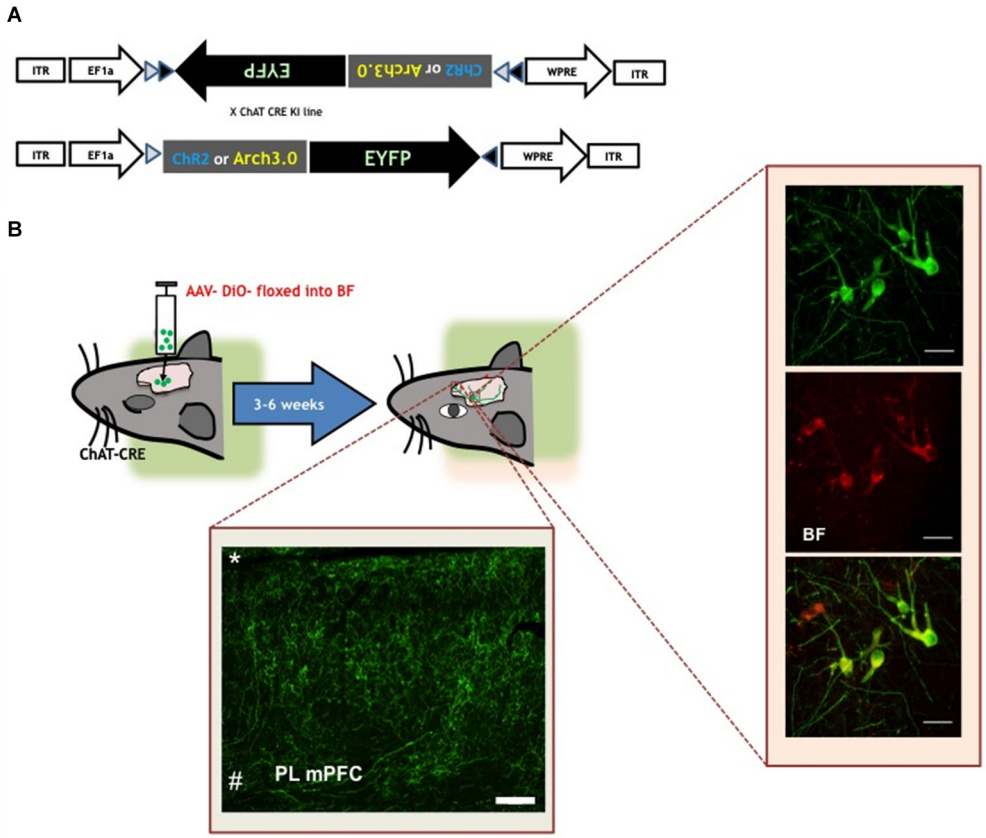

FIGURE 1 | Visualizing the cholinergic system in rodents. (A) Viral construct used to achieve selective expression of functional opsins (ChR2 or Arch3.0) in ChAT+ cells (Witten et al., 2011). Top panel shows the construct that is sterotactically injected in the basal forebrain region of ChAT-cre mice or rats. The portion of the construct encoding the opsin and fluorophore (Enhanced yellow fluorescent protein-EYFP) is inverted and flanked by double LoxP sites (black and white triangles). After virus delivery in the brain, and in presence of cre-recombinase, the coding fragment is oriented in the right direction, allowing the expression of functional light-sensitive opsins in the ChAT+ neurons. **(B)** Schematic representation of virus delivery and opsin expression in ChAT-cre mice/rats. Rodents undergo surgery to infuse the adeno-associated virus (AAV) construct with the coding information for opsins and/or fluorophores. After 3 to 6 weeks rodents have sustained expression of the flourophore and/or excitatory (ChR2, ChIEF etc.) or inhibitory (halorhodopsin or arch 3.0) in ChAT+ cell soma and fibers. Left inset is a confocal micrograph of the basal forebrain of a ChAT-cre rat. Green cells in the top panel express EYFP as result of the AAV-floxed EYFP injection. Middle panel is a confocal micrograph of ChAT+ neurons, confirmed by the presence of anti-ChAT antibody staining. Bottom panel is a confocal micrograph indicating that the EYFP probe is expressed only in ChAT+ cells. Scale bar is 40 μm (Luchicchi and Mansvelder, unpublished observations).Bottom inset confocal micrograph of EYFP+ labeled basal forebrain terminal fields within the mPFC (*pia; # white matter). Scale bar is 200 μm (Luchicchi and Mansvelder, unpublished observations). Figure is adapted from Jiang et al. (submitted).

diffuse cholinergic inputs, that enable the cholinergic system to manipulate neocortical processing on a millisecond time scale as well as on slower time scales (Arroyo et al., 2012, 2014; Kimura et al., 2014).

On a network level, BF stimulation in anesthetized animals results in a desynchronized state of field potentials (Goard and Dan, 2009; Kalmbach et al., 2012; reviewed in Bloem et al., 2014) and neuronal firing in the BF is correlated with a reduction in low frequency, and an increase of high frequency, oscillations in the cortex (Duque et al., 2000; Manns et al., 2000). Since the frequency band activity is related to the state of arousal and the extent of cortical activation (Uhlhaas et al., 2008; Wang, 2010; Deco and Thiele, 2011; Cachope et al., 2012), this supports the idea that ACh acts as a neuromodulator involved in setting the state of arousal. Mechanistically, it was shown that ACh activated mAChRs on cortical pyramidal neurons (Gulledge et al., 2009), thereby shifting firing modes from bursting to tonic and changing low frequency high amplitude oscillatory activity to high frequency low amplitude activity on a network level (Metherate et al., 1992).

Other studies have looked at the effect of ACh on the direction of the flow of information in the cortex. Again, these studies have been performed in sensory areas because in these regions, neuronal responses could be related to sensory stimulation. In this regard it is reasonably well established that ACh is directly involved in the enhancement of feed-forward thalamic input into the sensory cortical areas (see Bloem et al., 2014, for a review). In L4 of visual cortex, ACh increases the gain and reliability

of neuronal responses (Goard and Dan, 2009; Soma et al., 2012, 2013), an effect that is mediated by heteromeric nAChRs (Roberts et al., 2005; Disney et al., 2007). A similar effect of ACh is observed in the barrel cortex (Oldford and Castro-Alamancos, 2003).

In L2/3, the picture is more complex. In general, cholinergic modulation reduces firing rate in these layers by increasing GABAergic inhibition through mAChRs and nAChRs (Disney et al., 2012; Alitto and Dan, 2013; Soma et al., 2013; Kimura et al., 2014). The ACh modulation in firing rates was associated with enhancement of the reliability of encoding and modulation by stimuli presented (Goard and Dan, 2009; Soma et al., 2013).

The cortical depression associated with whisker trimming is accompanied by an increase of heteromeric nAChRs on interneurons in L2/3 and blockade of these nAChRs can prevent the cortical depression. These observations support the contention that heteromeric nAChRs in L2/3 are required for regulating the input- dependent responsiveness of the somatosensory cortex (Brown et al., 2012a).

Intra-cortical projections that connect superficial layers between different cortical columns are also inhibited by ACh through activation of mAChRs (Kimura and Baughman, 1997). Based on this finding and the reduced activity in the superficial layers, it has been suggested that ACh reduces horizontal processing through cortico-cortical interactions (Hasselmo and Giocomo, 2006). Indeed it has been observed in slices, and *in vivo* animal experiments as well as in humans, that the spatial spread of excitation in response to stimuli is reduced in the presence of elevated levels of ACh (Kimura et al., 1999; Silver et al., 2008). Such a modulation of excitation could have a sharpening effect on tuning curves of receptive fields and on discrimination of sensory stimuli (Roberts et al., 2005; Thiele et al., 2012). The combined effects of ACh—e.g., reduction of lateral interactions and increased sensitivity to thalamic inputs, would be expected to increase network sensitivity to incoming information and enhance signal to noise. A similar selective gain-control effect of ACh is observed with enhanced attention (Briggs et al., 2013) and could be one of the core mechanisms through which ACh modulates selective attention (Hasselmo and Giocomo, 2006; Deco and Thiele, 2011; Hasselmo and Sarter, 2011).

The functional impact of ACh on the deeper L5 and 6 is less well understood. It is clear that deep layer pyramidal and interneurons are modulated by both nAChRs and mAChRs (Gulledge et al., 2007; Kassam et al., 2008; Poorthuis et al., 2013). ACh is associated with both response suppression and response facilitation, although the net effect of endogenous cholinergic signaling is not clear (Soma et al., 2013). In L1, most (if not all) interneurons contain α7* and /or non-α7* nAChRs (Christophe et al., 2002; Alitto and Dan, 2013). Since these neurons inhibit both L1-3 interneurons and L2/3 pyramidal cells, the effect of cholinergic L1 activation appears to be complex with both net inhibition as well as disinhibition of pyramidal cells in deeper layers, and it is likely dependent on the source and extent of ACh release in L1 (Letzkus et al., 2011; Bennett et al., 2012; Cruikshank et al., 2012; Jiang et al., 2013; Arroyo et al., 2014).

Thalamic inputs to L5 neurons are strongly regulated by nicotinic receptor activation (Lambe et al., 2003; Couey et al., 2007; Poorthuis et al., 2013). Whether these are targeted by direct cholinergic inputs is not known. However, within the thalamic reticular nucleus, neurons receive biphasic fast cholinergic inputs mediated by non-α7* nAChRs and mAChRs (Sun et al., 2013).

MANIPULATING THE CORTICAL CHOLINERGIC SYSTEM DURING BEHAVIOR

Despite new insights as to how rapidly ACh levels may rise and fall in prefrontal cortex during cue detection (Sarter et al., 2014), there is still no direct demonstration of the cellular and synaptic mechanisms by which ACh controls attentional behaviors. Hints emerge from the optogenetic data on the disinhibitory circuit mechanisms in superficial layers of sensory areas (Letzkus et al., 2011; Arroyo et al., 2014), but the architecture of the somatosensory cortex differs substantially from that of prefrontal cortical regions. Indeed, L4 is absent from rodent medial PFC (Uylings et al., 2003), and projections from the mediodorsal thalamus target all layers of mPFC, in contrast to the more discrete segregation of thalamo—cortical input seen in somatosensory areas (Douglas and Martin, 2004; Constantinople and Bruno, 2013). Few studies have appeared that manipulate the cholinergic system using optogenetics during cognitive tasks. In the primary visual cortex (V1) optogenetic stimulation of BF projections improved visual discrimination, a hallmark of visual attention, in a go-no-go task (Pinto et al., 2013). Inhibiting the BF cholinergic projections to the visual cortex with either halo-rhodopsin (NpHR) or archaerhodopsin (Arch) impaired mouse performance on the same tasks (Pinto et al., 2013; Arroyo et al., 2014 for review).

In a recent report of unpublished observations, Sarter et al. (2014) optogenetically manipulated the excitability of BF projections to the PFC in mice performing a sustained attention task (SAT). This would be the first report of optogenetic manipulation of ACh release in the PFC and modulation of attention performance. Using ChAT-Cre mice expressing ChR2 in the BF, the authors report that brief blue light stimulation during cue presentation increases detection of the cue. Optogenetic stimulation of BF fibers in the absence of a cue, which predict the presentation of reward, results in a higher number of false-positive responses in cue detection of ChR2 mice. Inhibition of ACh fibers with NpHR stimulation reduced cue detection (Sarter et al., 2014). Previous studies from the same group have identified transient release of ACh in the mPFC as a modulator of cue-directed attention. In particular, fast ACh release occurred when the cue trial was preceded by an actual or perceived non-cue trial (Howe et al., 2013). Therefore, cholinergic transients may be involved in state-shifting: i.e., in regulating the shift from generalized monitoring to one of cue-directed attention (Sarter et al., 2014). In this sense, the optogenetic increase in false-positive responses, where the animal responds incorrectly to a non-cue trial, might reveal the mechanism by which transient release of ACh in the mPFC determines the transition from cue detection to a behavioral response. Full appreciation of the data underlying these conclusions awaits publication of the primary data referred to in the Sarter review (Sarter et al., 2014).

OPTOGENETIC CONTROL OF CHOLINERGIC PROJECTIONS TO HIPPOCAMPUS AND AMYGDALA: SYNAPTIC PLASTICITY AND OSCILLATIONS

Hippocampal control over specific behaviors, such as learning and memory, is potently modulated by cholinergic signaling. Antagonists to both nicotinic and muscarinic AChRs impair performance in hippocampal-dependent memory tasks in rodents (Levin et al., 2002), as well as the ability to encode spatial information (Blokland et al., 1992). The majority of cholinergic inputs to the hippocampus (up to 90%) come from the medial septum and diagonal band via the fimbria/fornix, and enter the hippocampus through the stratum oriens (SO; Frotscher and Léránth, 1985; Dutar et al., 1995). In addition, sparse cholinergic interneurons have been reported in some regions of the hippocampus, where they usually impinge on GABAergic interneurons (Griguoli and Cherubini, 2012). Both nicotinic and muscarinic AChRs are involved in regulating hippocampal network activity, such as synchronization of neuronal activity and altering of synaptic weights, thereby influencing hippocampal support of cognitive function (Yakel, 2012). Exogenous application of nicotinic agonists in hippocampal slices affects synaptic plasticity in nearly all hippocampal areas (Tu et al., 2009; Yakel, 2012), and muscarinic agonists induce fast network oscillations (Mann et al., 2005). However, it is still not completely clear how cholinergic receptors regulate rhythmic and phasic oscillations and synaptic plasticity *in vivo*, during hippocampal-dependent cognitive functions.

By stimulating septal cholinergic projecting neurons to the SO using both electrical and optogenetic methods, Gu and Yakel disentangled the temporal requirements for ACh release in the cholinergic modulation of synaptic strength of Schaffer's collateral (SC) to CA1 synapses (Gu and Yakel, 2011). With precisely timed activation of septal cholinergic neurons in ChAT-cre mice expressing ChR2, Yakel et al. showed that when the light-evoked increase of ACh release in the SO preceded the SC stimulation by 100 ms, long-term potentiation (LTP) in the CA1 was triggered. This effect was dependent on the activation of α7* nAChRs in postsynaptic neurons. On the other hand, ChR2 activation of cholinergic terminals only 10 ms before the SC stimulation resulted in hippocampal short-term depression. In the latter case the effect was due to an α7* subunit-dependent inhibition of presynaptic glutamate release. Even more intriguing, the α7* component also altered synaptic plasticity when light pulses were delivered 10 ms *after* the SC activation. In fact, this latter protocol caused LTD in SO neurons by a mechanism which was attributed to mAChR activation, although whether the muscarinic component was pre- or postsynaptic is not clear (Gu and Yakel, 2011).

Muscarinic AChRs also modulate hippocampal activity by acting on interneurons (Bell et al., 2013). This is in line with the role of these receptors in orchestrating network oscillation within the hippocampus (Mann et al., 2005). Interestingly, interneuron network responses to light-evoked ACh release from the septum varied according to the level of cholinergic activity. In particular, low-intensity stimulation of cholinergic inputs was more likely to inhibit certain classes of interneurons via a mechanism dependent on the M4 type of mAChRs, whereas higher levels of ACh release triggered depolarization in other interneurons via broader muscarinic signaling. Cholinergic inputs from BF can also activate GABAergic interneurons through activation of α4β2* nAChRs in specific layers of the hippocampus (Bell et al., 2011).

Combining optogenetic stimulation of medial septum/diagonal band of Broca (MS/DBB) projections to the hippocampus with whole-cell patch clamp recordings and voltage sensitive dye (VSD) imaging it has been shown that inhibitory interneurons in the hippocampus receive cholinergic EPSPs in response to light stimulation of septal cholinergic fibers that are sensitive to DhβE, but not MLA (Bell et al., 2011). These light-evoked EPSPs have slow kinetics similar to the non-α7* component seen in interneurons in the somatosensory cortex (Arroyo et al., 2012). The interneurons that express α4β2* have their somata or dendrites in the SO or stratum lacunosum-moleculare (SLM) of the hippocampus. Finally, another recent optogenetic study implicates ACh release from the MS/DBB in the modulation of synaptic plasticity triggered by GABAergic interneurons of the stratum oriens lacunosum-maculare (OLM) in the SC-CA1 (Leão et al., 2012). Taken together, these data show that ACh inputs from the septum can influence hippocampal oscillations and plasticity in a highly specialized manner, resulting in a fine-tuning of hippocampal network activity in a layer specific manner and with millisecond timing. We still lack knowledge on the exact timing of activation of hippocampal cholinergic inputs during behavior. This will require both optogenetic manipulation of cholinergic projections, and concurrent visualization of activity of the BF projections in the hippocampus of awake-behaving animals.

Recently, optogenetic studies have been carried out to study the influence of other neuromodulatory systems interacting with cholinergic signaling to modulate hippocampal network activity. A set of studies conducted by Alger's group have very elegantly demonstrated that both the endocannabinoid (eCb) and endogenous opioid systems may participate in the generation of ACh-dependent modulation of hippocampal oscillatory activity (Nagode et al., 2011, 2014). With brief stimulation of the MS/DBB fibers in the CA1, Nagode et al. (2011) reported rhythmic inhibitory post-synaptic currents (IPSCs) in pyramidal neurons, accompanied by low frequency oscillation in hippocampal slices. Interestingly, the IPSCs, which were likely evoked by interneurons impinging on the pyramidal cells, were abolished by either GABA or mAChR antagonists. Moreover, the same events were also eCb-sensitive, supporting the presence of active cannabinoid receptor (CB-R1) in the presynaptic interneuron terminal. It is widely known that CB-Rs are expressed in the hippocampus, where they drive different forms of plasticity and mediate aspects of neuroprotection (Wilson and Nicoll, 2001). Only cholecystokinin (CCK) + interneurons in hippocampus have functional CB1-Rs; CB-Rs are not present on PV+ interneurons (Katona et al., 1999). For this reason, it is likely that the cholinergic modulation of low frequency oscillations observed in this study depends solely on CCK+ cell activity. Optogenetic inhibition of either PV+ interneurons or glutamic-acid decarboxylase-2 (GAD2)+ cells in the CA1 confirmed that the PV− population of GABAergic interneurons were required for ACh induction of low frequency oscillations. Surprisingly the ability of these PV− cells to trigger

low frequency rhythms in the hippocampus was blocked by a mu-opioid receptor antagonist, and subsequent induction of IPSCs in pyramidal neurons by ACh release in ChAT-Cre mice was shown to be sensitive to both CB1 and mu-receptor blockade (Nagode et al., 2014). Overall, these studies provide new insights on the possible cross-communication between the eCb and cholinergic modulatory systems in the regulation of hippocampal network activity and perhaps, in memory functions.

The effects of cholinergic input in general, and of nAChRs in particular, in the basolateral amygdala is also under study with optogenetic labeling of the neurons and projections of the nucleus basalis (Role, 2014). These studies have revealed that cholinergic signaling potently modulates the plasticity of cortical synapses on basolateral amygdale (BLA) pyramidal neurons, decreasing the threshold for induction of LTP. Excitatory effects of nucleus basalis stimulation on BLA firing is confirmed in *in vivo* recording and, most striking, the rate of extinction of responses to a cue-associated fear conditioning paradigm is slowed by brief optogenetic activation of the cholinergic terminal fields in BLA during training (Role, 2014). These findings are consistent with the idea that cholinergic signaling reinforces amygdala-based memories, perhaps rendering them less susceptible to subsequent extinction (Role, 2014).

MODULATION OF STRIATAL CIRCUITS BY ACH

CHOLINERGIC INTERNEURONS MODULATE THE RELEASE OF MULTIPLE STRIATAL NEUROTRANSMITTERS

In addition to the robust modulatory activities of cholinergic signaling in cortex and hippocampus ACh is renowned for its strong regulatory role in subcortical brain regions within the midbrain and striatum. In particular, the core of the brain reward circuitry, comprising the ventral tegmental area (VTA) and the nucleus accumbens (NAc), is strongly modulated by ACh. The main source of ACh to the VTA neurons in the midbrain arises from the brainstem structures LDTg and PPTg, which play a role in acquisition of reward, and reward-related locomotor activity (Corrigall et al., 2002; Champtiaux et al., 2006). The main source of ACh for the NAc/ventral striatum, as well as for the dorsal striatum, is the cholinergic interneurons which comprise less than 2–5% of the total striatal neuron population (Descarries et al., 1997). Notwithstanding the paucity of striatal cholinergic interneurons, ACh signaling is directly involved in the modulation of (1) striatal dopamine (DA) release (Rice and Cragg, 2004; Exley and Cragg, 2008; Wang et al., 2014); (2) local network functionality (Galarraga et al., 1999; Koós and Tepper, 2002); and (3) striatal-dependent behaviors related to reward (Joshua et al., 2008).

The release of DA in striatum is crucial for functions such as motivation, reward and locomotor activity (see Cachope and Cheer, 2014, for a recent review) and cholinergic transmission can drive striatal DA release (Exley and Cragg, 2008). A recent study showed that selective optogenetic activation of accumbal cholinergic interneurons is sufficient to trigger DA release in the same region, and that this effect is independent of the suprathreshold activation of VTA DA neurons *per se* (Cachope et al., 2012; Threlfell et al., 2012; Wang et al., 2014). As such, the activity of cholinergic interneurons might boost the release of DA to encode aspects of reward-related events. This proposal is in line with studies in which photostimulation of cholinergic interneurons drove striatal DA release via activation of presynaptic nAChRs (Threlfell et al., 2012; Wang et al., 2014). On the other hand, Cachope et al. (2012) reported that the direct effect of cholinergic interneuron activation on DA release was only partially mediated by activation of AChRs. Combining optogenetic manipulation with *in vitro* pharmacology, revealed the collaboration of both nicotinic (β2*) and muscarinic receptors, together with the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors in the enhancement of striatal DA release. Thus, a synergy exists between ACh and glutamate in modulating the activity of the striatal network. The source of glutamate may be the striatal cholinergic interneurons themselves (Gras et al., 2008). Activating ChR2 in cholinergic striatal interneurons triggers postsynaptic responses onto medium spiny neurons (MSNs), the most abundant striatal cell type. Under the stimulation conditions used by Higley et al., the direct postsynaptic responses were blocked by glutamate receptor antagonists alone and were insensitive to AChR blockade (Higley et al., 2011). This suggests that at low levels of stimulation direct control of MSN firing by "cholinergic" interneurons may also rely on fast glutamatergic transmission.

A follow-up of this study was conducted looking at the connections between ACh interneurons and other local interneurons. including the PV+ interneurons, that also contact MSNs directly (Koós and Tepper, 1999; Gittis et al., 2010). Activating dorsal striatal ACh interneurons triggers the co-release of ACh and glutamate on PV+ interneurons, activating slow non-α7* nAChR currents, and both AMPA and N-methyl-D-aspartate (NMDA) receptors (Nelson et al., 2014). ACh and glutamate co-release was dependent on the presence of the vesicular glutamate transporter VGLUT3. This transporter is also involved in enhancing the vesicular loading of ACh and is important for di-synaptic inhibition of MSNs after PV+ excitation, a common feature in striatal information processing (Gras et al., 2008).

Activation of ChR2 in cholinergic interneurons in striatum also triggered GABA-A receptor-mediated postsynaptic currents in MSNs both *in vivo* and *in vitro* (Witten et al., 2010). Optogenetic stimulation of striatal cholinergic interneurons activated di-synaptic inhibitory responses in MSNs *in vitro* (Nelson et al., 2014). This effect was still present when PV+ neurons were ablated leading the authors to suggest that the di-synaptic inhibition of MSNs might be mediated by GABA release from DA terminals, that are studded with β2* nAChRs and targeted by cholinergic interneuronal projections. Thus, striatal network activity could be orchestrated by cholinergic interneurons through simultaneous regulation of DA and GABA release in the same striatal area.

OPTOGENETIC STUDIES OF ROLE OF CHOLINERGIC INTERNEURONS IN STRIATAL-DEPENDENT BEHAVIORS

Optogenetic studies have helped to define the role of striatal cholinergic interneurons in multiple aspects of motor control, associative learning and reward (see Jiang et al., 2014, for review; Exley and Cragg, 2008). During reward-related events, cholinergic interneurons initially increase their firing activity, and then pause, after which they start firing in a third phase of

elevated activity (e.g., Morris et al., 2004). Most likely, these phasic activity periods support DA release from VTA projections, with the nAChR mediated component being *independent* of VTA action potential firing (e.g., see Wang et al., 2014). By combining pharmacological, optogenetic and electrophysiological techniques, Straub et al. (2014) recently suggested reward coding resides in the pause in striatal ACh interneuron activity that results from the direct effect of nigrostriatal DA projections via D2 dopamine receptors on cholinergic interneurons. The authors did not identify which neurotransmitter is involved in the rebound phase. Other studies have suggested that the pause of ACh interneuron firing may be caused by a GABA component. Activating VTA GABA neurons that project to the striatum with ChR2 and recording activity on ACh interneurons resulted in a pause of ACh interneuron firing (Van Bockstaele and Pickel, 1995; Tan et al., 2012; Van Zessen et al., 2012). Interestingly this effect was only observed in striatal ACh interneurons, sparing the other cell population and it was insensitive to DA receptor blockade. Behavioral studies have confirmed that GABA mediated inhibition of cholinergic interneurons is a requisite component of stimulus-outcome association under relevant learning conditions, pinpointing the pivotal role of ACh interneurons in goal directed behaviors (Brown et al., 2012b). In addition, Witten et al. (2010) have reported that cholinergic interneuron silencing by NpHR stimulation reduced cocaine preference in behaving mice.

Taken together, these findings support the idea that cholinergic interneurons play a crucial role in the modulation of striatal activity, and striatal-dependent behavior. Recent anatomical studies have also underscored the potential importance of direct projections from the brainstem (PPTg and LDTg) to striatal cells (Dautan et al., 2014). Hence, it will be interesting to learn how cooperation between these different elements of the cholinergic system modulates striatal activity.

SUMMARY AND CONCLUSIONS

The application of optogenetic tools has accelerated the acquisition of precise information about the varied modulatory and direct synaptic signaling by ACh in an array of brain regions and behaviors. Selective expression of optogenetic probes in ChAT+ neurons allows studies of the connectivity, functionality and anatomy of cholinergic neurons and circuits throughout the rodent brain (Atasoy et al., 2008; Witten et al., 2011; for reviews see: Arroyo et al., 2014; Jiang et al., 2014; Poorthuis et al., 2014). The application of these techniques has unveiled novel contributions of previously un-identified ChAT-positive neurons to activity-dependent proliferation and neurogenesis (Paez-Gonzalez et al., 2014) as well as implicating the co-storage—and perhaps co-release—of ACh and glutamate (e.g., see Higley et al., 2011). Many important challenges and new areas of exploration are now accessible to the cholinergic enthusiast. It will be particularly important to establish the precise mechanisms by which ACh modulates attention and contributes to top down executive control of directed behaviors. With the increasing number of research groups that have adopted the optogenetic toolbox, we can expect to learn more about these exciting topics in the not-so-distant future.

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REFERENCES

Alitto, H. J., and Dan, Y. (2013). Cell-type-specific modulation of neocortical activity by basal forebrain input. *Front. Syst. Neurosci.* 6:79. doi: 10.3389/fnsys.2012.00079

Arroyo, S., Bennett, C., Aziz, D., Brown, S. P., and Hestrin, S. (2012). Prolonged disynaptic inhibition in the cortex mediated by slow, non-α7 nicotinic excitation of a specific subset of cortical interneurons. *J. Neurosci.* 32, 3859–3864. doi: 10.1523/JNEUROSCI.0115-12.2012

Arroyo, S., Bennett, C., and Hestrin, S. (2014). Nicotinic modulation of cortical circuits. *Front. Neural Circuits* 8:30. doi: 10.3389/fncir.2014.00030

Atasoy, D., Aponte, Y., Su, H. H., and Sternson, S. M. (2008). A FLEX switch targets channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. *J. Neurosci.* 28, 7025–7030. doi: 10.1523/jneurosci.1954-08.2008

Bell, L. A., Bell, K. A., and McQuiston, A. R. (2013). Synaptic muscarinic response types in hippocampal CA1 interneurons depend on different levels of presynaptic activity and different muscarinic receptor subtypes. *Neuropharmacology* 73, 160–173. doi: 10.1016/j.neuropharm.2013.05.026

Bell, K. A., Shim, H., Chen, C. K., and McQuiston, A. R. (2011). Nicotinic excitatory postsynaptic potentials in hippocampal CA1 interneurons are predominantly mediated by nicotinic receptors that contain α4 and β2 subunits. *Neuropharmacology* 61, 1379–1388. doi: 10.1016/j.neuropharm.2011.08.024

Bennett, C., Arroyo, S., Berns, D., and Hestrin, S. (2012). Mechanisms generating dual-component nicotinic EPSCs in cortical interneurons. *J. Neurosci.* 32, 17287–17296. doi: 10.1523/jneurosci.3565-12.2012

Bloem, B., Poorthuis, R. B., and Mansvelder, H. D. (2014). Cholinergic modulation of the medial prefrontal cortex: the role of nicotinic receptors in attention and regulation of neuronal activity. *Front. Neural Circuits* 8:17. doi: 10.3389/fncir.2014.00017

Blokland, A., Honig, W., and Raaijmakers, W. G. M. (1992). Effects of intra-hippocampal scopolamine injections in a repeated spatial acquisition task in the rat. *Psychopharmacology (Berl)* 109, 373–376. doi: 10.1007/bf02245886

Briggs, F., Mangun, G. R., and Usrey, W. M. (2013). Attention enhances synaptic efficacy and the signal-to-noise ratio in neural circuits. *Nature* 499, 476–480. doi: 10.1038/nature12276

Brown, C. E., Sweetnam, D., Beange, M., Nahirney, P. C., and Nashmi, R. (2012a). α4* nicotinic acetylcholine receptors modulate experience-based cortical depression in the adult mouse somatosensory cortex. *J. Neurosci.* 32, 1207–1219. doi: 10.1523/JNEUROSCI.4568-11.2012

Brown, M. T. C., Tan, K. R., O'Connor, E. C., Nikonenko, I., Muller, D., and Lüscher, C. (2012b). Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature* 492, 452–456. doi: 10.1038/nature11657

Cachope, R., and Cheer, J. F. (2014). Local control of striatal dopamine release. *Front. Behav. Neurosci.* 8:188. doi: 10.3389/fnbeh.2014.00188

Cachope, R., Mateo, Y., Mathur, B. N., Irving, J., Wang, H. L., Morales, M., et al. (2012). Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: setting the tone for reward processing. *Cell Rep.* 2, 33–41. doi: 10.1016/j.celrep.2012.05.011

Champtiaux, N., Kalivas, P. W., and Bardo, M. T. (2006). Contribution of dihydro-beta-erythroidine sensitive nicotinic acetylcholine receptors in the ventral tegmental area to cocaine-induced behavioral sensitization in rats. *Behav. Brain Res.* 168, 120–126. doi: 10.1016/j.bbr.2005.10.017

Changeux, J.-P. (2010). Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nat. Rev. Neurosci.* 11, 389–401. doi: 10.1038/nrn2849

Christophe, E., Roebuck, A., Staiger, J. F., Lavery, D. J., Charpak, S., and Audinat, E. (2002). Two types of nicotinic receptors mediate an excitation of neocortical layer I interneurons. *J. Neurophysiol.* 88, 1318–1327. doi: 10.1152/jn.00199.2002

Constantinople, C. M., and Bruno, R. M. (2013). Deep cortical layers are activated directly by thalamus. *Science* 340, 1591–1594. doi: 10.1126/science.1236425

Corrigall, W. A., Coen, K. M., Zhang, J., and Adamson, L. K. (2002). Pharmacological manipulations of the pedunculopontine tegmental nucleus in the rat reduce self-administration of both nicotine and cocaine. *Psychopharmacology (Berl)* 160, 198–205. doi: 10.1007/s00213-001-0965-2

Couey, J. J., Meredith, R. M., Spijker, S., Poorthuis, R. B., Smit, A. B., Brussaard, A. B., et al. (2007). Distributed network actions by nicotine increase the threshold for spike-timing-dependent plasticity in prefrontal cortex. *Neuron* 54, 73–87. doi: 10.1016/j.neuron.2007.03.006

Court, J., Martin-Ruiz, C., Piggott, M., Spurden, D., Griffiths, M., and Perry, E. (2001). Nicotinic receptor abnormalities in Alzheimer's disease. *Biol. Psychiatry* 49, 175–184. doi: 10.1016/s0006-3223(00)01116-1

Cruikshank, S. J., Ahmed, O. J., Stevens, T. R., Patrick, S. L., Gonzalez, A. N., Elmaleh, M., et al. (2012). Thalamic control of layer 1 circuits in prefrontal cortex. *J. Neurosci.* 32, 17813–17823. doi: 10.1523/JNEUROSCI.3231-12.2012

Dani, J. A., and Harris, R. A. (2005). Nicotine addiction and comorbidity with alcohol abuse and mental illness. *Nat. Neurosci.* 8, 1465–1470. doi: 10.1038/nn1580

Dautan, D., Huerta-Ocampo, I., Witten, I. B., Deisseroth, K., Bolam, J. P., Gerdjikov, T., et al. (2014). A major external source of cholinergic innervation of the striatum and nucleus accumbens originates in the brainstem. *J. Neurosci.* 34, 4509–4518. doi: 10.1523/jneurosci.5071-13.2014

Deco, G., and Thiele, A. (2011). Cholinergic control of cortical network interactions enables feedback-mediated attentional modulation. *Eur. J. Neurosci.* 34, 146–157. doi: 10.1111/j.1460-9568.2011.07749.x

Deisseroth, K. (2011). Optogenetics. *Nat. Methods* 8, 26–29. doi: 10.1038/nmeth.f.324

Descarries, L., Gisiger, V., and Steriade, M. (1997). Diffuse transmission by acetylcholine in the CNS. *Prog. Neurobiol.* 53, 603–625. doi: 10.1016/s0301-0082(97)00050-6

Disney, A. A., Aoki, C., and Hawken, M. J. (2007). Gain modulation by nicotine in macaque V1. *Neuron* 56, 701–713. doi: 10.1016/j.neuron.2007.09.034

Disney, A. A., Aoki, C., and Hawken, M. J. (2012). Cholinergic suppression of visual responses in primate V1 is mediated by GABAergic inhibition. *J. Neurophysiol.* 108, 1907–1923. doi: 10.1152/jn.00188.2012

Douglas, R. J., and Martin, K. A. C. (2004). Neuronal circuits of the neocortex. *Annu. Rev. Neurosci.* 27, 419–451. doi: 10.1146/annurev.neuro.27.070203.144152

Duque, A., Balatoni, B., Detari, L., and Zaborszky, L. (2000). EEG correlation of the discharge properties of identified neurons in the basal forebrain. *J. Neurophysiol.* 84, 1627–1635.

Dutar, P., Bassant, M. H., Senut, M. C., and Lamour, Y. (1995). The septohippocampal pathway: structure and function of a central cholinergic system. *Physiol. Rev.* 75, 393–427.

Exley, R., and Cragg, S. J. (2008). Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. *Br. J. Pharmacol.* 153(Suppl. 1), S283–S297. doi: 10.1038/sj.bjp.0707510

Frotscher, M., and Léránth, C. (1985). Cholinergic innervation of the rat hippocampus as revealed by choline acetyltransferase immunocytochemistry: a combined light and electron microscopic study. *J. Comp. Neurol.* 239, 237–246. doi: 10.1002/cne.902390210

Galarraga, E., Hernández-López, S., Reyes, A., Miranda, I., Bermudez-Rattoni, F., Vilchis, C., et al. (1999). Cholinergic modulation of neostriatal output: a functional antagonism between different types of muscarinic receptors. *J. Neurosci.* 19, 3629–3638.

Gittis, A. H., Nelson, A. B., Thwin, M. T., Palop, J. J., and Kreitzer, A. C. (2010). Distinct roles of GABAergic interneurons in the regulation of striatal output pathways. *J. Neurosci.* 30, 2223–2234. doi: 10.1523/jneurosci.4870-09.2010

Goard, M., and Dan, Y. (2009). Basal forebrain activation enhances cortical coding of natural scenes. *Nat. Neurosci.* 12, 1444–1449. doi: 10.1038/nn.2402

Gotti, C., and Clementi, F. (2004). Neuronal nicotinic receptors: from structure to pathology. *Prog. Neurobiol.* 74, 363–396. doi: 10.1016/j.pneurobio.2004.09.006

Gras, C., Amilhon, B., Lepicard, E. M., Poirel, O., Vinatier, J., Herbin, M., et al. (2008). The vesicular glutamate transporter VGLUT3 synergizes striatal acetylcholine tone. *Nat. Neurosci.* 11, 292–300. doi: 10.1038/nn2052

Griguoli, M., and Cherubini, E. (2012). Regulation of hippocampal inhibitory circuits by nicotinic acetylcholine receptors. *J. Physiol.* 590, 655–666. doi: 10.1113/jphysiol.2011.220095

Gu, Z., and Yakel, J. L. (2011). Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. *Neuron* 71, 155–165. doi: 10.1016/j.neuron.2011.04.026

Gulledge, A. T., Bucci, D. J., Zhang, S. S., Matsui, M., and Yeh, H. H. (2009). M1 receptors mediate cholinergic modulation of excitability in neocortical pyramidal neurons. *J. Neurosci.* 29, 9888–9902. doi: 10.1523/jneurosci.1366-09.2009

Gulledge, A. T., Park, S. B., Kawaguchi, Y., and Stuart, G. J. (2007). Heterogeneity of phasic cholinergic signaling in neocortical neurons. *J. Neurophysiol.* 97, 2215–2229. doi: 10.1152/jn.00493.2006

Hasselmo, M. E., and Giocomo, L. M. (2006). Cholinergic modulation of cortical function. *J. Mol. Neurosci.* 30, 133–136. doi: 10.1385/JMN:30:1:133

Hasselmo, M. E., and Sarter, M. (2011). Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology* 36, 52–73. doi: 10.1038/npp.2010.104

Higley, M. J., Gittis, A. H., Oldenburg, I. A., Balthasar, N., Seal, R. P., Edwards, R. H., et al. (2011). Cholinergic interneurons mediate fast VGluT3-dependent glutamatergic transmission in the striatum. *PLoS One* 6:e19155. doi: 10.1371/journal.pone.0019155

Howe, W. M., Berry, A. S., Francois, J., Gilmour, G., Carp, J. M., Tricklebank, M., et al. (2013). Prefrontal cholinergic mechanisms instigating shifts from monitoring for cues to cue-guided performance: converging electrochemical and fMRI evidence from rats and humans. *J. Neurosci.* 33, 8742–8752. doi: 10.1523/jneurosci.5809-12.2013

Huang, Z. J., and Zeng, H. (2013). Genetic approaches to neural circuits in the mouse. *Annu. Rev. Neurosci.* 36, 183–215. doi: 10.1146/annurev-neuro-062012-170307

Jiang, L., López-Hernández, G. Y., Lederman, J., Talmage, D. A., and Role, W. L. (2014). Optogenetic studies of nicotinic contributions to cholinergic signaling in the central nervous system. *Rev. Neurosci.* doi: 10.1515/revneuro-2014-0032. [Epub ahead of print].

Jiang, X., Wang, G., Lee, A. J., Stornetta, R. L., and Zhu, J. J. (2013). The organization of two new cortical interneuronal circuits. *Nat. Neurosci.* 16, 210–218. doi: 10.1038/nn.3305

Joshua, M., Adler, A., Mitelman, R., Vaadia, E., and Bergman, H. (2008). Midbrain dopaminergic neurons and striatal cholinergic interneurons encode the difference between reward and aversive events at different epochs of probabilistic classical conditioning trials. *J. Neurosci.* 28, 11673–11684. doi: 10.1523/jneurosci.3839-08.2008

Kalmbach, A., Hedrick, T., and Waters, J. (2012). Selective optogenetic stimulation of cholinergic axons in neocortex. *J. Neurophysiol.* 107, 2008–2019. doi: 10.1152/jn.00870.2011

Kassam, S. M., Herman, P. M., Goodfellow, N. M., Alves, N. C., and Lambe, E. K. (2008). Developmental excitation of corticothalamic neurons by nicotinic acetylcholine receptors. *J. Neurosci.* 28, 8756–8764. doi: 10.1523/JNEUROSCI.2645-08.2008

Katona, I., Sperlágh, B., Sík, A., Käfalvi, A., Vizi, E. S., Mackie, K., et al. (1999). Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* 19, 4544–4558.

Kawai, H., Lazar, R., and Metherate, R. (2007). Nicotinic control of axon excitability regulates thalamocortical transmission. *Nat. Neurosci.* 10, 1168–1175. doi: 10.1038/nn1956

Kilgard, M. P., and Merzenich, M. M. (1998). Cortical map reorganization enabled by nucleus basalis activity. *Science* 279, 1714–1718. doi: 10.1126/science.279.5357.1714

Kimura, F., and Baughman, R. W. (1997). Distinct muscarinic receptor subtypes suppress excitatory and inhibitory synaptic responses in cortical neurons. *J. Neurophysiol.* 77, 709–716.

Kimura, F., Fukuda, M., and Tsumoto, T. (1999). Acetylcholine suppresses the spread of excitation in the visual cortex revealed by optical recording: possible differential effect depending on the source of input. *Eur. J. Neurosci.* 11, 3597–3609. doi: 10.1046/j.1460-9568.1999.00779.x

Kimura, R., Safari, M.-S., Mirnajafi-Zadeh, J., Kimura, R., Ebina, T., Yanagawa, Y., et al. (2014). Curtailing effect of awakening on visual responses of cortical neurons by cholinergic activation of inhibitory circuits. *J. Neurosci.* 34, 10122–10133. doi: 10.1523/jneurosci.0863-14.2014

Klinkenberg, I., Sambeth, A., and Blokland, A. (2011). Acetylcholine and attention. *Behav. Brain Res.* 221, 430–442. doi: 10.1016/j.bbr.2010.11.033

Koós, T., and Tepper, J. M. (1999). Inhibitory control of neostriatal projection neurons by GABAergic interneurons. *Nat. Neurosci.* 2, 467–472. doi: 10.1038/8138

Koós, T., and Tepper, J. M. (2002). Dual cholinergic control of fast-spiking interneurons in the neostriatum. *J. Neurosci.* 22, 529–535.

Lambe, E. K., Picciotto, M. R., and Aghajanian, G. K. (2003). Nicotine induces glutamate release from thalamocortical terminals in prefrontal cortex. *Neuropsychopharmacology* 28, 216–225. doi: 10.1038/sj.npp.1300032

Leão, R. N., Mikulovic, S., Leão, K. E., Munguba, H., Gezelius, H., Enjin, A., et al. (2012). OLM interneurons differentially modulate CA3 and entorhinal inputs to hippocampal CA1 neurons. *Nat. Neurosci.* 15, 1524–1530. doi: 10.1038/nn.3235

Leslie, F. M., Mojica, C. Y., and Reynaga, D. D. (2013). Nicotinic receptors in addiction pathways. *Mol. Pharmacol.* 83, 753–758. doi: 10.1124/mol.112.083659

Letzkus, J. J., Wolff, S. B. E., Meyer, E. M. M., Tovote, P., Courtin, J., Herry, C., et al. (2011). A disinhibitory microcircuit for associative fear learning in the auditory cortex. *Nature* 480, 331–335. doi: 10.1038/nature10674

Levin, E. D., Bradley, A., Addy, N., and Sigurani, N. (2002). Hippocampal alpha 7 and alpha 4 beta 2 nicotinic receptors and working memory. *Neuroscience* 109, 757–765. doi: 10.1016/s0306-4522(01)00538-3

Mann, E. O., Suckling, J. M., Hajos, N., Greenfield, S. A., and Paulsen, O. (2005). Perisomatic feedback inhibition underlies cholinergically induced fast network oscillations in the rat hippocampus in vitro. *Neuron* 45, 105–117. doi: 10.1016/j.neuron.2004.12.016

Manns, I. D., Alonso, A., and Jones, B. E. (2000). Discharge profiles of juxtacellularly labeled and immunohistochemically identified GABAergic basal forebrain neurons recorded in association with the electroencephalogram in anesthetized rats. *J. Neurosci.* 20, 9252–9263.

Martin, L. F., and Freedman, R. (2007). Schizophrenia and the alpha7 nicotinic acetylcholine receptor. *Int. Rev. Neurobiol.* 78, 225–246. doi: 10.1016/S0074-7742(06)78008-4

Mesulam, M. M. (1995). Cholinergic pathways and the ascending reticular activating system of the human brain. *Ann. N Y Acad. Sci.* 757, 169–179. doi: 10.1111/j.1749-6632.1995.tb17472.x

Metherate, R., Cox, C. L., and Ashe, J. H. (1992). Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J. Neurosci.* 12, 4701–4711.

Morris, G., Arkadir, D., Nevet, A., Vaadia, E., and Bergman, H. (2004). Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron* 43, 133–143. doi: 10.1016/j.neuron.2004.06.012

Nagode, D. A., Tang, A. H., Karson, M. A., Klugmann, M., and Alger, B. E. (2011). Optogenetic release of ACh induces rhythmic bursts of perisomatic IPSCs in hippocampus. *PLoS One* 6:e27691. doi: 10.1371/journal.pone.0027691

Nagode, D. A., Tang, A.-H., Yang, K., and Alger, B. E. (2014). Optogenetic identification of an intrinsic cholinergically driven inhibitory oscillator sensitive to cannabinoids and opioids in hippocampal CA1. *J. Physiol.* 592, 103–123. doi: 10.1113/jphysiol.2013.257428

Nelson, A. B., Hammack, N., Yang, C. F., Shah, N. M., Seal, R. P., and Kreitzer, A. C. (2014). Striatal cholinergic interneurons drive GABA release from dopamine terminals. *Neuron* 82, 63–70. doi: 10.1016/j.neuron.2014.01.023

Oldford, E., and Castro-Alamancos, M. A. (2003). Input-specific effects of acetylcholine on sensory and intracortical evoked responses in the "barrel cortex" in vivo. *Neuroscience* 117, 769–778. doi: 10.1016/s0306-4522(02)00663-2

Paez-Gonzalez, P., Asrican, B., Rodriguez, E., and Kuo, C. T. (2014). Identification of distinct ChAT(+) neurons and activity-dependent control of postnatal SVZ neurogenesis. *Nat. Neurosci.* 17, 934–942. doi: 10.1038/nn.3734

Parikh, V., Kozak, R., Martinez, V., and Sarter, M. (2007). Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron* 56, 141–154. doi: 10.1016/j.neuron.2007.08.025

Parikh, V., and Sarter, M. (2008). Cholinergic mediation of attention. *Ann. N Y Acad. Sci.* 1129, 225–235. doi: 10.1196/annals.1417.021

Passetti, F., Dalley, J. W., O'Connell, M. T., Everitt, B. J., and Robbins, T. W. (2000). Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. *Eur. J. Neurosci.* 12, 3051–3058. doi: 10.1046/j.1460-9568.2000.00183.x

Picciotto, M. R., Higley, M. J., and Mineur, Y. S. (2012). Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron* 76, 116–129. doi: 10.1016/j.neuron.2012.08.036

Pinto, L., Goard, M. J., Estandian, D., Xu, M., Kwan, A. C., Lee, S.-H., et al. (2013). Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat. Neurosci.* 16, 1857–1863. doi: 10.1038/nn.3552

Poorthuis, R. B., Bloem, B., Schak, B., Wester, J., de Kock, C. P. J., and Mansvelder, H. D. (2013). Layer-specific modulation of the prefrontal cortex by nicotinic acetylcholine receptors. *Cereb. Cortex* 23, 148–161. doi: 10.1093/cercor/bhr390

Poorthuis, R. B., Enke, L., and Letzkus, J. J. (2014). Cholinergic circuit modulation through differential recruitment of neocortical interneuron types during behaviour. *J. Physiol.* 592, 4155–4164. doi: 10.1113/jphysiol.2014.273862

Ren, J., Qin, C., Hu, F., Tan, J., Qiu, L., Zhao, S., et al. (2011). Habenula "cholinergic" neurons corelease glutamate and acetylcholine and activate postsynaptic neurons via distinct transmission modes. *Neuron* 69, 445–452. doi: 10.1016/j.neuron.2010.12.038

Rice, M. E., and Cragg, S. J. (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nat. Neurosci.* 7, 583–584. doi: 10.1038/nn1244

Roberts, M. J., Zinke, W., Guo, K., Robertson, R., McDonald, J. S., and Thiele, A. (2005). Acetylcholine dynamically controls spatial integration in marmoset primary visual cortex. *J. Neurophysiol.* 93, 2062–2072. doi: 10.1152/jn.00911.2004

Role, L. W. (2014). "Cholinergic modulation of cortical-amygdala circuits and behavior. ABS S25," in *Nicotinic Acetylcholine Receptors, Wellcome Trust Scientific Conference*, eds I. Bermudez, N. Millar and S. Wonnacott.

Sarter, M., Lustig, C., Howe, W. M., Gritton, H., and Berry, A. S. (2014). Deterministic functions of cortical acetylcholine. *Eur. J. Neurosci.* 39, 1912–1920. doi: 10.1111/ejn.12515

Sarter, M., Parikh, V., and Howe, W. M. (2009). Phasic acetylcholine release and the volume transmission hypothesis: time to move on. *Nat. Rev. Neurosci.* 10, 383–390. doi: 10.1038/nrn2635

Silver, M. A., Shenhav, A., and D'Esposito, M. (2008). Cholinergic enhancement reduces spatial spread of visual responses in human early visual cortex. *Neuron* 60, 904–914. doi: 10.1016/j.neuron.2008.09.038

Smiley, J. F., Morrell, F., and Mesulam, M. M. (1997). Cholinergic synapses in human cerebral cortex: an ultrastructural study in serial sections. *Exp. Neurol.* 144, 361–368. doi: 10.1006/exnr.1997.6413

Soma, S., Shimegi, S., Osaki, H., and Sato, H. (2012). Cholinergic modulation of response gain in the primary visual cortex of the macaque. *J. Neurophysiol.* 107, 283–291. doi: 10.1152/jn.00330.2011

Soma, S., Shimegi, S., Suematsu, N., Tamura, H., and Sato, H. (2013). Modulation-specific and laminar-dependent effects of acetylcholine on visual responses in the rat primary visual cortex. *PLoS One* 8:e68430. doi: 10.1371/journal.pone.0068430

Straub, C., Tritsch, N. X., Hagan, N. A., Gu, C., and Sabatini, B. L. (2014). Multiphasic modulation of cholinergic interneurons by nigrostriatal afferents. *J. Neurosci.* 34, 8557–8569. doi: 10.1523/jneurosci.0589-14.2014

Sun, Y.-G., Pita-Almenar, J. D., Wu, C.-S., Renger, J. J., Uebele, V. N., Lu, H.-C., et al. (2013). Biphasic cholinergic synaptic transmission controls action potential activity in thalamic reticular nucleus neurons. *J. Neurosci.* 33, 2048–2059. doi: 10.1523/JNEUROSCI.3177-12.2013

Tan, K. R., Yvon, C., Turiault, M., Mirzabekov, J. J., Doehner, J., Labouèbe, G., et al. (2012). GABA neurons of the VTA drive conditioned place aversion. *Neuron* 73, 1173–1183. doi: 10.1016/j.neuron.2012.02.015

Thiele, A., Herrero, J. L., Distler, C., and Hoffmann, K.-P. (2012). Contribution of cholinergic and GABAergic mechanisms to direction tuning, discriminability, response reliability and neuronal rate correlations in macaque middle temporal area. *J. Neurosci.* 32, 16602–16615. doi: 10.1523/jneurosci.0554-12.2012

Threlfell, S., Lalic, T., Platt, N. J., Jennings, K. A., Deisseroth, K., and Cragg, S. J. (2012). Striatal dopamine release is triggered by synchronized activity in

cholinergic interneurons. *Neuron* 75, 58–64. doi: 10.1016/j.neuron.2012.04.038

Tu, B., Gu, Z., Shen, J.-X., Lamb, P. W., and Yakel, J. L. (2009). Characterization of a nicotine-sensitive neuronal population in rat entorhinal cortex. *J. Neurosci.* 29, 10436–10448. doi: 10.1523/jneurosci.2580-09.2009

Turrini, P., Casu, M. A., Wong, T. P., De Koninck, Y., Ribeiro-da-Silva, A., and Cuello, A. C. (2001). Cholinergic nerve terminals establish classical synapses in the rat cerebral cortex: synaptic pattern and age-related atrophy. *Neuroscience* 105, 277–285. doi: 10.1016/s0306-4522(01)00172-5

Uhlhaas, P. J., Haenschel, C., Nikolić, D., and Singer, W. (2008). The role of oscillations and synchrony in cortical networks and their putative relevance for the pathophysiology of schizophrenia. *Schizophr. Bull.* 34, 927–943. doi: 10.1093/schbul/sbn062

Uylings, H. B. M., Groenewegen, H. J., and Kolb, B. (2003). Do rats have a prefrontal cortex? *Behav. Brain Res.* 146, 3–17. doi: 10.1016/j.bbr.2003.09.028

Van Bockstaele, E. J., and Pickel, V. M. (1995). GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res.* 682, 215–221. doi: 10.1016/0006-8993(95)00334-m

Van Zessen, R., Phillips, J. L., Budygin, E. A., and Stuber, G. D. (2012). Activation of VTA GABA neurons disrupts reward consumption. *Neuron* 73, 1184–1194. doi: 10.1016/j.neuron.2012.02.016

von Engelhardt, J., Eliava, M., Meyer, A. H., Rozov, A., and Monyer, H. (2007). Functional characterization of intrinsic cholinergic interneurons in the cortex. *J. Neurosci.* 27, 5633–5642. doi: 10.1523/jneurosci.4647-06.2007

Wang, X.-J. (2010). Neurophysiological and computational principles of cortical rhythms in cognition. *Physiol. Rev.* 90, 1195–1268. doi: 10.1152/physrev.00035.2008

Wang, L., Shang, S., Kang, X., Teng, S., Zhu, F., Liu, B., et al. (2014). Modulation of dopamine release in the striatum by physiologically relevant levels of nicotine. *Nat. Commun.* 5:3925. doi: 10.1038/ncomms4925

Wess, J. (2003). Novel insights into muscarinic acetylcholine receptor function using gene targeting technology. *Trends Pharmacol. Sci.* 24, 414–420. doi: 10.1016/s0165-6147(03)00195-0

Wilson, R. I., and Nicoll, R. A. (2001). Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 410, 588–592. doi: 10.1038/35069076

Witten, I. B., Lin, S.-C., Brodsky, M., Prakash, R., Diester, I., Anikeeva, P., et al. (2010). Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science* 330, 1677–1681. doi: 10.1126/science.1193771

Witten, I. B., Steinberg, E. E., Lee, S. Y., Davidson, T. J., Zalocusky, K. A., Brodsky, M., et al. (2011). Recombinase-driver rat lines: tools, techniques and optogenetic application to dopamine-mediated reinforcement. *Neuron* 72, 721–733. doi: 10.1016/j.neuron.2011.10.028

Wonnacott, S. (1997). Presynaptic nicotinic ACh receptors. *Trends Neurosci.* 20, 92–98. doi: 10.1016/s0166-2236(96)10073-4

Woolf, N. J. (1991). Cholinergic systems in mammalian brain and spinal cord. *Prog. Neurobiol.* 37, 475–524. doi: 10.1016/0301-0082(91)90006-m

Yakel, J. L. (2012). Nicotinic ACh receptors in the hippocampus: role in excitability and plasticity. *Nicotine Tob. Res.* 14, 1249–1257. doi: 10.1093/ntr/nts091

Yizhar, O., Fenno, L. E., Davidson, T. J., Mogri, M., and Deisseroth, K. (2011). Optogenetics in neural systems. *Neuron* 71, 9–34. doi: 10.1016/j.neuron.2011.06.004

Zaborszky, L., Pang, K., Somogyi, J., Nadasdy, Z., and Kallo, I. (1999). The basal forebrain corticopetal system revisited. *Ann. N Y Acad. Sci.* 877, 339–367. doi: 10.1111/j.1749-6632.1999.tb09276.x

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Paying attention to smell: cholinergic signaling in the olfactory bulb

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The tractable, layered architecture of the olfactory bulb (OB), and its function as a relay between odor input and higher cortical processing, makes it an attractive model to study how sensory information is processed at a synaptic and circuit level. The OB is also the recipient of strong neuromodulatory inputs, chief among them being the central cholinergic system. Cholinergic axons from the basal forebrain modulate the activity of various cells and synapses within the OB, particularly the numerous dendrodendritic synapses, resulting in highly variable responses of OB neurons to odor input that is dependent upon the behavioral state of the animal. Behavioral, electrophysiological, anatomical, and computational studies examining the function of muscarinic and nicotinic cholinergic receptors expressed in the OB have provided valuable insights into the role of acetylcholine (ACh) in regulating its function. We here review various studies examining the modulation of OB function by cholinergic fibers and their target receptors, and provide putative models describing the role that cholinergic receptor activation might play in the encoding of odor information.

Keywords: muscarinic, nicotinic, glomerular, GABAergic, filter

INTRODUCTION

The network of cholinergic fibers acts as a major neuromodulatory system in the brain. It is not only implicated in the pathophysiology of neurodegenerative disorders like Alzheimer's disease, but it also plays a central role in the functioning of the healthy brain. The release of ACh by these fibers is involved in the enhancement of sensory perception during wakefulness, particularly during periods of sustained attention (Himmelheber et al., 2000; Jones, 2005). Studying how ACh controls various brain systems at the level of circuits and synaptic transmission is critical for the understanding of how ACh affects brain function, both in health and in disease. The mammalian main OB provides for a convenient model system to study the modulatory control of sensory circuits. It is located centrally in the olfactory pathway (only one synapse away from odor input into the nose and one synapse away from higher cortical processing), and its excitatory and inhibitory neurons are relatively well-segregated. Importantly, its circuits and function are strongly modulated by ACh. Cholinergic input to the OB is provided primarily by axons of neurons whose cell bodies reside in the HDB in the basal forebrain (Wenk et al., 1980; Senut et al., 1989). While a more recent study has demonstrated the presence of choline acetyltransferase (ChAT)-expressing neurons within the OB itself (Krosnowski et al., 2012), a functional role has yet to be ascribed to these cholinergic interneurons. ACh released by HDB cholinergic neurons acts on both, nicotinic and muscarinic receptors (nAChR and mAChR, respectively) resulting in the control of olfactory function that is dependent upon the brain state of the animal – whether it is sleeping, performing a task, or simply awake and immobile. In this review, we focus on studies that have helped us gain better insights into how the release of ACh in the OB affects olfaction at the cellular, circuit, and behavioral level, and discuss how it might modulate odor coding during attentional control of OB circuits.

MULTIPLE, COMPLEX MECHANISMS INVOLVED IN OLFACTORY CODING

The OB represents a convergence point for incoming odor signals and contains the synapse transferring odor information between the ORNs and higher cortical regions. ORNs send their axons (which form the ON) into defined structures called glomeruli (**Figure 1**). Projections from ORNs that recognize the same odor epitope converge onto about two (of about two thousand) glomeruli in the ipsilateral bulb (Vassar et al., 1994). Within the glomerular neuropil, these neurons provide direct (Najac et al., 2011) and indirect (Najac et al., 2011; Gire et al., 2012) synaptic inputs onto the MCs, the principal output neurons of the OB. Modulation of odor information provided by these inputs occurs in the glomerulus as well as in the other layers of the bulb by a number of bulbar interneurons. Two key neuronal cell types that modulate glomerular output are the GABAergic PG cells and the glutamatergic external tufted (ET) cells, both of which are

Abbreviations: ACh, acetylcholine; ET cell, external tufted cell; HDB, horizontal limb of the diagonal band of Broca; M/T cells, mitral and/or tufted cells; mAChR, muscarinic acetylcholine receptor; MC, mitral cell; nAChR, nicotinic acetylcholine receptor; OB, olfactory bulb; ON, olfactory nerve; ORN, olfactory receptor neuron; PG cell, periglomerular cell; GC, Granule cells.

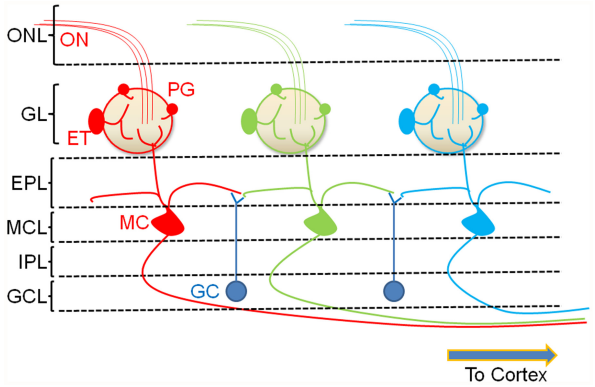

FIGURE 1 | A simplified cartoon of the bulbar circuit. ORNs in the nasal epithelium send their axons ON to the glomerular layer of the OB. Each glomerulus receives convergent ON input from ORNs that recognize the same odor epitope (color coded). Resident interneurons in the juxtaglomerular region receive ON input and modulate glomerular signaling. The two major glomerular interneuron classes are the glutamatergic external tufted (ET) cells and the GABAergic PG cells. Together, these cells regulate the glomerular output resulting in the excitation of MCs, the principal neurons of the OB. A second interneuron type, the granule cell (GC), forms dendrodendritic synapses with the lateral dendrites of the MCs. These neurons exert GABAergic control over multiple MCs resulting in lateral inhibition. The modulated output of the MC is then transmitted to the pyriform cortex. ONL, olfactory nerve layer; GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; IPL, internal plexiform layer; GCL, granule cell layer.

also directly excited by ON input (Gire and Schoppa, 2009). The lateral dendrites of the MCs receive a second set of GABAergic inputs from granule cells (GCs) within another distinct layer called the external plexiform layer (EPL; **Figure 1**). Other interneuron types and subtypes have been described (Batista-Brito et al., 2008) but are not considered here in the context of cholinergic modulation.

Much of the information on odor representations (Xu et al., 2000; Bozza et al., 2004) and MCs responses to odor (Kashiwadani et al., 1999) comes from studies on anesthetized animals. However, recent studies have shown a much more complex scenario in awake animals, requiring re-evaluation of our notions of olfactory processing (Kato et al., 2012; Wachowiak et al., 2013). MCs in awake, behaving animals are spontaneously active (Rinberg and Gelperin, 2006; Rinberg et al., 2006; Davison and Katz, 2007) with firing that is often locked to the respiration cycle (Cury and Uchida, 2010; Wachowiak, 2011). Odor-evoked responses are not encoded in simple changes in firing frequencies; instead, the OB adopts various sophisticated mechanisms, involving the activity of MCs, to detect and encode odors. For example, upon odor onset, the latency of the first MC spike in response to the odor (Margrie and Schaefer, 2003; Junek et al., 2010), reduction in MC firing frequency (Rinberg and Gelperin, 2006; Rinberg et al., 2006; Davison and Katz, 2007), alterations in the relative temporal phase of individual spikes (Dhawale et al., 2010), relative timing of MC spikes (Haddad et al., 2013), and fine-scale changes in temporal spike patterns (Friedrich and Laurent, 2001; Cury and Uchida, 2010) are all thought to play important roles in odor coding. Each of these mechanisms is a potential target for modulation, thus leading to a multifold increase in the computational power of the OB.

It has now been demonstrated that the OB is not merely an encoder of odor information that is subsequently decoded downstream in the cortex, but that it is itself involved in "higher order" processing. The response of MCs to odors, for example, depends not only on the chemical structure of odorant molecules, but also on more behaviorally relevant properties. *In vivo* recordings have shown that synchrony between MC spiking, in response to an odor, can be altered depending on whether the odor is rewarded in a behavioral task or not (Doucette and Restrepo, 2008; Doucette et al., 2011). Such an associative cortex-like feature (Doucette et al., 2011) suggests an advanced role for the OB in sensory information processing. This is consistent with studies which show that the activity of OB neurons can be profoundly affected by feedback inputs from the cortex (Gao and Strowbridge, 2009; Markopoulos et al., 2012). Task-dependent control of circuits in the OB thus plays a vital role in processing odor information.

THE OLFACTORY BULB AND ITS CHOLINERGIC INPUT

A cluster of cholinergic neurons from the basal forebrain sends diffuse projections to the entire cortical mantle. All cortical areas receive cholinergic innervation, though there appears to be differences in the density of innervation across specific layers (Lysakowski et al., 1989; Mesulam et al., 1992). The lack of consistent topographic precision leads to the idea that cholinergic activation might lead to uniform effects across structures. However, there are different clusters of basal forebrain cholinergic neurons that have been identified and described that might suggest modality-specific control by the transmitter (Zaborszky, 2002).

The cholinergic input from the HDB is a major centrifugal projection into the OB. Cholinergic neurons of the basal forebrain regulate cortical activity in a state-dependent manner. These neurons fire bursts of action potentials during awake and paradoxical sleep states while remaining more or less silent during slow wave sleep (Jones, 2004, 2005). During active periods, the burst discharge of these neurons appears to be synchronized with gamma and theta oscillations (Lee et al., 2005).

Incoming fibers from the HDB show diffuse innervation across different layers of the bulb (Macrides et al., 1981; Zaborszky et al., 1986; Durand et al., 1998). This innervation is complete by postnatal day 12 (Salcedo et al., 2011). However, during further maturation, there is a distinct patterning of the innervation, with the predominant projections being directed to the glomerular layer (**Figures 2** and **3**) and sparser projections to other OB layers (Macrides et al., 1981; Salcedo et al., 2011). Within the glomerular layer, there are variations in projections (**Figure 2**) with some atypical glomeruli showing much denser innervation (Macrides et al., 1981; Gomez et al., 2005; Salcedo et al., 2011). The identity of odor inputs into these glomeruli, or the functional significance of their dense cholinergic innervation is, as yet, unclear. This suggests considerable pruning of cholinergic afferents during maturation (Salcedo et al., 2011).

Occluding sensory input to the bulb from one naris revealed that cholinergic input is modulated by olfactory activity (**Figure 3**). When unilateral naris occlusion was performed on

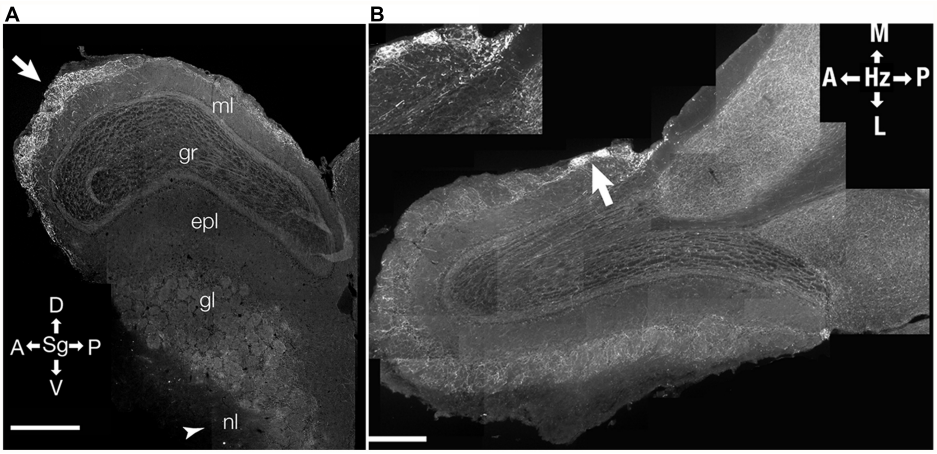

FIGURE 2 | Distribution of cholinergic innervation in the OB. Distribution of incoming cholinergic fibers from the HDB was examined in sections from a 3 month-old mouse expressing a tauGFP fusion protein under a choline acetyltransferase promoter (ChAT-tauGFP mouse). **(A)** Parasagittal section (Sg). *Arrow* points to region of relatively heavy GFP labeling in the anterior glomerular region of the bulb. *Arrow head* indicates the olfactory nerve layer (nl) where relatively little labeling is found. ml- mitral cell layer; gr, granule cell layer; epl, external plexiform layer; gl, glomerular layer; nl, olfactory nerve layer. **(B)** Micrograph of a horizontal (Hz) cross-section of the OB. Arrow points to heavily stained atypical glomeruli shown in inset. **(B** inset**)** High-resolution micrograph of two atypical glomeruli with a relatively high amount of GFP staining. D, dorsal; V, ventral; A, anterior; P, posterior; L, lateral; M, medial. Data, with permission, from Salcedo et al. (2011).

postnatal day 2, the pattern and intensity remained unchanged up to postnatal day 12. However, significant reductions in intensities were observed in the ipsilateral bulb of the adult (**Figure 3**). In addition, the patterning was lost during this period. These results suggest that odor-induced activity is required for the maintenance and patterning of the cholinergic innervation.

CHOLINERGIC RECEPTOR DISTRIBUTION IN THE OB

The anatomy and function of cholinergic receptor expression in the OB appear to be layer-specific. Quantitative autoradiography in rat OB slices point to the presence of presynaptic cholinergic terminals in the glomerular layer and in the EPL (Le Jeune et al., 1995), suggesting that the glomerulus and the secondary dendrites of MCs are important targets for cholinergic modulation. There is no evidence to suggest that cholinergic terminals form direct synaptic contacts on MCs. On the other hand, asymmetric cholinergic synapses have been described on dendrites of PG cells and GCs (Kasa et al., 1995). The prevalence of synaptic versus non-synaptic cholinergic signaling in the OB, like with other brain areas, remains unresolved to date.

The markers for the cholinergic receptors indicate an age-dependency of cholinergic receptor expression in the OB, with lowest levels of these markers observed at birth, and adult values observed by the end of 4–5 postnatal weeks (Le Jeune et al., 1996). This is consistent with the patterning of cholinergic innervation in the glomerular layer (Salcedo et al., 2011). The postnatal development of cholinergic innervation also extends to the EPL where GC dendrites make GABAergic contacts with the MCs.

Binding of [^{125}I] α-bungarotoxin, a marker for the α7-containing nAChRs, was observed in the glomerular neuropil, suggesting a role for the α7 nAChR subtype in glomerular signaling. On the other hand, [^{3}H] cytisine, which targets heteromeric nAChR subtypes, labeling α4β2*- and α3β4*-nAChRs (Xiao et al., 1998; Mao et al., 2008), binds to juxtaglomerular neurons and MCs. In addition to the ubiquitous α7 receptor, the rat OB also exhibits the presence of messenger RNA (mRNA) transcripts of nAChR genes that encode the α2, α3, α4, α5, α6, α9, β2, β3, and β4 subunits (Keiger and Walker, 2000), pointing to the possible expression of multiple receptor subtypes and, perhaps, indicating a diverse functional role of for nAChRs in the OB.

M1 and M2 mAChRs were shown to be highly expressed in the EPL indicating that mAChRs might be involved in regulating the dendrodendritic interactions between MCs and GCs. This has been experimentally verified by electrophysiological studies in acute slices (Castillo et al., 1999; Ghatpande et al., 2006; Pressler et al., 2007; Ghatpande and Gelperin, 2009), as well as *in vivo* (Tsuno et al., 2008).

CHOLINERGIC SIGNALING IN THE OB

A major site for nAChR regulation is the glomerulus of the OB. Consistent with autoradiographic studies (Le Jeune and Jourdan, 1993; Le Jeune et al., 1995) functional nAChRs have been described in MCs and ET cells (D'Souza and Vijayaraghavan, 2012; D'Souza et al., 2013). These functional receptors belong to the heteromeric α3β4*-nAChR and the α4β2*-nAChR subtypes. On MCs, nAChRs appear to be selectively clustered at the primary dendritic tuft within the glomerular neuropil. Removing the primary dendrite drastically attenuates ACh-induced nAChR currents (D'Souza and Vijayaraghavan, 2012). Overall, these results suggest that nAChRs are expressed primarily on excitatory neurons in the glomerular microcircuit. Further, activation of glomerular nAChRs leads to increased glutamate release within the neuropil, resulting in an excitation-dependent feedback inhibition onto the MCs and ET cells. This occurs via increased GABA release from activated juxtaglomerular interneurons, presumably the PG cells (D'Souza

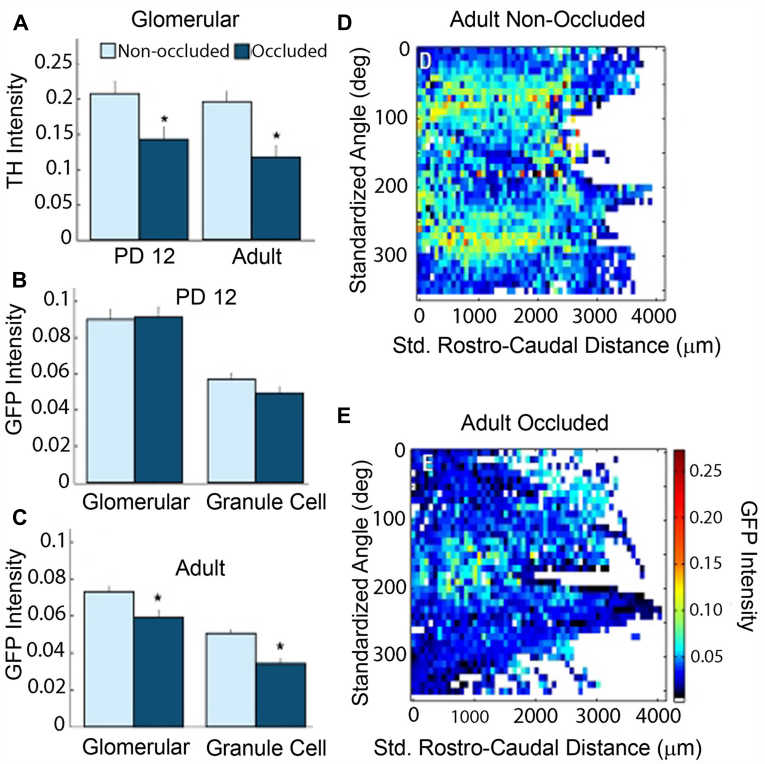

FIGURE 3 | Naris occlusion abolishes differential GFP staining pattern in adult animals. Background subtracted intensities from 12-bit images were converted to a 0–1 scale and plotted. Details of image processing are given in Salcedo et al. (2011). **(A)** Significantly lower tyrosine hydroxylase (TH) intensity in occluded bulbs as compared to non-occluded bulbs confirmed that the occluded bulbs had reduced olfactory activity in both PD12 animals and adult animals (*t*-test, *p*-value = 1×10^{-2} and 3.6×10^{-4}, respectively). **(B)** PD12 animals: mean GFP intensity did not vary significantly between occluded and non-occluded bulbs in either the glomerular or granule cell layers of PD12 bulbs. **(C)** Adult animals: mean GFP intensity fell significantly in both the glomerular and granule cell layers in the occluded bulbs of adult animals (*t*-test, *p*-values: 2.2×10^{-3} and 2.4×10^{-8}, respectively). **(D–E)** Average intensity map in the occluded bulb **(E)** showed a markedly reduced differential patterning of GFP intensity throughout the GL as compared to the non-occluded bulb **(D)**. Data, with permission, from Salcedo et al. (2011).

and Vijayaraghavan, 2012; D'Souza et al., 2013). The predominant effect of nAChR activation appears to be to inhibit incoming signals from the ORNs, leading to a "filtering" mechanism wherein only ORNs excited above a certain intensity threshold transmit their information to cortex (**Figure 4**). A possible mechanism for this inhibition is the shunting of ORN inputs due to the increase in the membrane conductance of MCs upon the opening of a large number of channels, particularly the nAChRs and GABA receptors. Thus, the receptors act as high pass filters that attenuate weak signals while allowing stronger ones to pass, thus setting odor detection thresholds.

Potentially important players in the increased GABA release within the glomerulus upon nAChR activation are the ET cells (D'Souza et al., 2013), a population of OB neurons whose physiological properties have been characterized over the last decade. ET cells are thought to be a major source of excitation for juxtaglomerular neurons (Hayar et al., 2004), as well as drivers of feed-forward MC excitation via glutamate release within the glomerulus (De Saint et al., 2009; Najac et al., 2011; Gire et al., 2012). As targets for neuromodulation by cholinergic (D'Souza et al., 2013), serotonergic (Liu et al., 2012) and endocannabinoid receptor-mediated (Wang et al., 2012) mechanisms, ET cells are well placed to play a vital role in the state-dependent control of OB function. Similar to MCs, glomerular nAChR activation leads to an enhancement of ET cell excitability. This excitation, along with MC excitation, is likely responsible for the increase in the frequency of GABA release within the glomerulus upon nAChR stimulation. There is one report suggesting that a subpopulation of PG cells might, themselves, express nAChRs (Castillo et al., 1999) but their contribution to the glomerular microcircuit is yet to be determined.

mAChRs, on the other hand, appear to mainly control a second inhibitory circuit in the OB, involving GCs and the lateral dendrites of MCs (see **Figure 5A**) within the EPL. Activation of M1-mAChRs, via mobilization of endoplasmic reticulum store calcium, release GABA onto the MCs at the dendrodendritic synapses between GCs and MCs (Castillo et al., 1999; Ghatpande et al., 2006; Ghatpande and Gelperin, 2009). At the same time, M1-mAChRs increase GC excitation thus providing an additional inhibitory drive on to MCs (Pressler et al., 2007). Similar mechanisms of cholinergic modulation were also observed in the accessory OB where M1-like mAChRs control GC-to-MC inhibition, while nAChR activation increases MC excitability (Smith and Araneda, 2010).

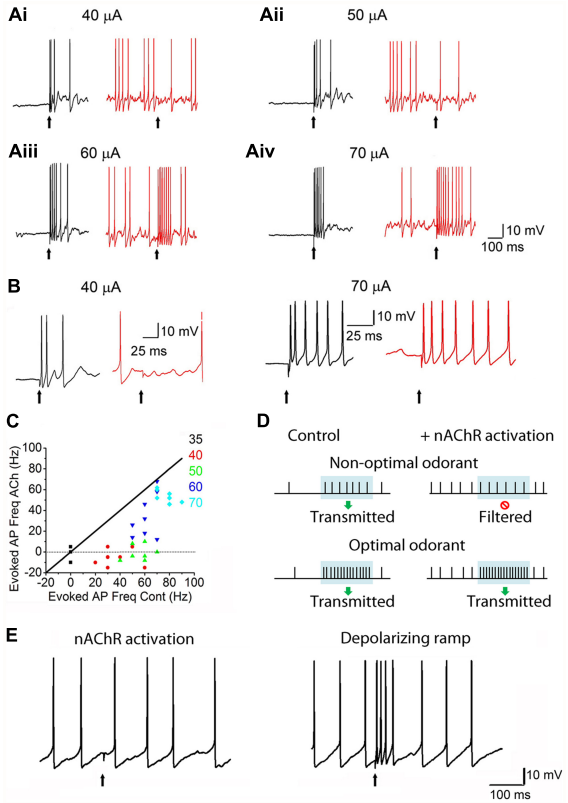

FIGURE 4 | nAChRs act as high-pass filters of glomerular output. **(Ai)**: Responses of MCs to ON stimulation, recorded under current-clamp. Left: **(A)** 40 μA ON stimulus causes an MC to exhibit a burst of spikes. Right: during a ACh/At-mediated depolarization (and enhanced background firing), the 40 μA stimulus fails to evoke a response in the same MC. ACh/At refers to a 1 s focal application of 1 mM ACh in the presence of 2 μM atropine, the latter also added in the bath in order to block mAChRs. **(Aii–iv)**: Similar data for 50, 60, and 70 μA stimuli, respectively. While the 50 μA stimulus also fails to evoke a response during the ACh/At-mediated spiking in the same MC as in Ai, the MC responds to higher stimuli with increased spiking. In all cases, control traces are in black and traces in the presence of ACh/At are in red. **(B)**: Expanded traces from Ai and Aiv. **(C)**: Scatter plot of net increase in spiking upon ON stimulation, during the ACh/At-mediated depolarization, plotted against the same during control conditions. Data is from the same cell as in **(A,B)**. Net increase in spiking was calculated by subtracting the mean firing frequency before ON stimulation from the mean firing frequency during the 100 ms window after ON stimulation. While responses to all stimulus intensities were suppressed during the ACh-mediated depolarization, lower intensity stimuli (up to 50 μA) show a filtering of MC responses (not different from 0). Diagonal line (slope = 1) is where the points would lie if there were no ACh-mediated filtering. **(D)**: Cartoon summarizing the effects of nAChRs on MC responses. Period of odor exposure shown by shaded box. Under non-optimal conditions (weak odor), a MC fires a burst of APs during odor exposure leading to signal transmission. On the other hand, nAChR activation, causes an increase in basal MC firing but shows no net change in firing patterns during the period of odor exposure thus resulting in filtering of the response. Under optimal conditions (i.e., strong odor), there is a net increase in MC firing during odor exposure both under control conditions and when nAChRs are activated. **(E)**: Filtering shown in the presence of ACh/At (left trace) is not seen when the same cell is depolarized via current injection to elicit APs in the absence of ACh/At (right trace, from the same cell). This suggests that optimal excitation-driven feedback inhibition requires the activation of more than one MC, and that the filtering is not merely a result of MC membrane depolarization. Figures **(A–C,E)** adapted, with permission, from D'Souza and Vijayaraghavan (2012).

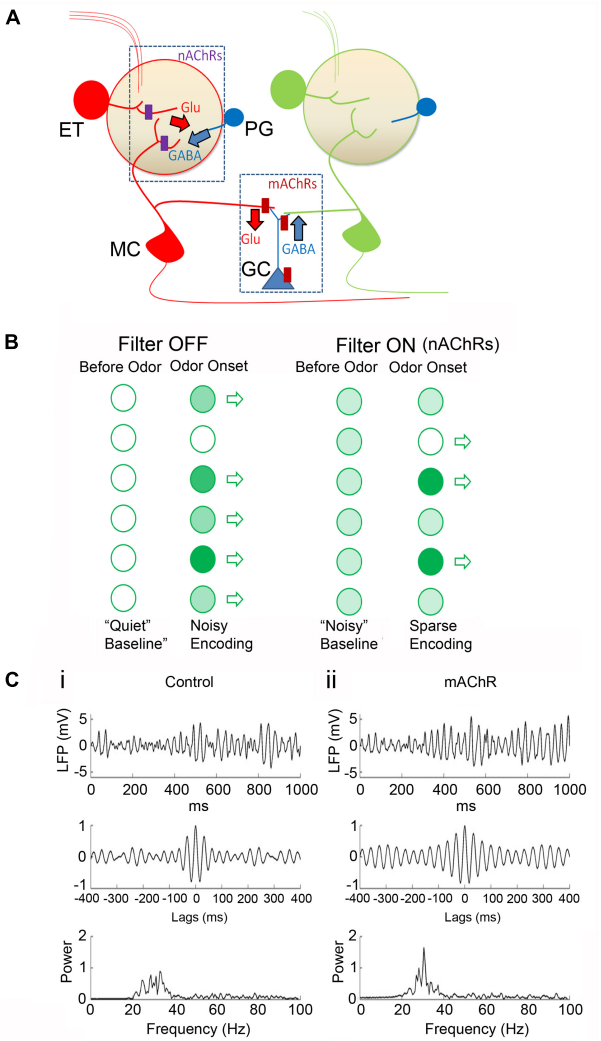

FIGURE 5 | A working model for cholinergic modulation of OB circuitry. **(A)** Cartoon of the OB circuit showing major sites of cholinergic inhibition. In the glomerular microcircuit, nAChRs (purple rectangles) are expressed in the primary dendritic tufts of MCs and on the ET cell (in red). Activation of these receptors depolarizes the neurons resulting in the release of glutamate (Glu). Released glutamate excites nearby PG cells (blue) eliciting a feedback GABA release on to the excitatory neurons. **(A)** Similar feedback circuit exists at the dendrodendritic synapses between GCs and secondary dendrites of MCs allowing for lateral inhibition of adjacent MC dendrites. **(B)** Cartoon showing glomerular output (open arrows). In the absence of receptor activation (Filter OFF) there is less baseline activity ("Quiet baseline.") Odor stimulations at different strengths (light and dark green circles for weak and strong stimulation, respectively) are transmitted through. Upon nAChR activation, (Filter ON), excitation of ET cells and MCs lead to a noisier baseline (i.e., all light green). However, upon odor input, excitation-driven inhibition results in filtering out of weaker inputs, such that only strong ones pass through. Further, increased basal activity also allows for potential "inhibitory readouts" (open circle) where net MC firing rates can be reduced to levels below that prior to odor onset (see **Figure 4C**). The time window of the nAChR-evoked inhibition will determine the efficacy of this filter. Thus determining the temporal patterns of ACh release, in relation to behavioral stages, is necessary in order to predict the direct consequences of this filter mechanism. **(C)** Modeling the activation of mAChRs in the OB (with permission from Li and Cleland, 2013). In this model, mAChR activation does not alter MC firing rates but the receptor

(Continued)

FIGURE 5 | Continued
activation enhances sLFP oscillatory power and imposes more stringent phase locking between MC spikes and sLFP oscillations. **(i)** Control responses: simulated sLFP during odor presentation **(top)** with autocorrelation **(middle)** and power spectrum **(bottom)**. **(ii)** Same as **(i)** but during active mAChR modulation. In response to odor, MC spikes were locked to the gamma frequencies under both control conditions and upon mAChR activation, but the responses were more tightly phase constrained when mAChRs were active. This is consistent with the idea that modulation of lateral inhibition by mAChR signaling at GC-MC synapses imposes a stronger synchronization of MC firing in the OB.

mAChRs AND nAChRs CONTROL OB FUNCTION VIA DISTINCT MECHANISMS

Results from a variety of behavioral and *in vivo* electrophysiological studies point to the importance of cholinergic receptor activation in modulating the detection and discrimination of odors, as well as in olfactory perceptual learning, i.e., learning to distinguish between two or more perceptually similar odors (Fletcher and Wilson, 2002; Wilson and Stevenson, 2003; Wilson et al., 2004; Fletcher and Chen, 2010). For instance, increasing the level of ACh in the OB results in the sharpening of the molecular "receptive field" of individual MCs in response to odors, while the addition of nAChR and mAChR blockers into the OB impairs the ability to distinguish between similar odors, both at the level of MC spike frequency, as well as in behavioral tests (Chaudhury et al., 2009).

In addition to modulating odor detection and discrimination (Chaudhury et al., 2009), nAChRs are also involved in olfactory working memory. Mice that lacked the α7 nAChR showed impairments in working memory when compared to wild type mice (Young et al., 2007a), while acute nicotine administration could fully restore deficits in olfactory working memory in a transgenic mouse model that overexpressed the caspase-3 protein (Young et al., 2007b). Olfactory working memory could also be enhanced in rats via subcutaneous administration of specific agonists for the α4β2 and the α7 receptor subtypes (Rushforth et al., 2010).

mAChRs have been shown to be involved in the behavioral state-dependent control of dendrodendritic synapses between MCs and GCs (Tsuno et al., 2008). Results from this work indicated that inhibition of MCs by GCs was most enhanced during the slow-wave sleep state and successively weaker during light sleep, awake immobility, and awake moving states. Activation of mAChRs were responsible for this inhibition. This supports the observation that ACh levels in the brain are higher in the awake state than when the animal is asleep. Real time monitoring of ACh levels demonstrate that the awake state is characterized by low levels of ACh (Parikh and Sarter, 2008), though relatively higher than that during slow wave sleep, which might signal via mAChRs to maintain a tonic GABAergic control on the basal firing rates of MCs. It appears, therefore, that an important function of cholinergic input to the OB is to inhibit the activity of GCs, thereby disinhibiting the MCs during wakefulness and behavior. *In vivo*, mAChRs have been suggested to play a role in potentiating the firing rates of MCs upon stimulation of the basal forebrain (Zhan et al., 2013), as well in olfactory perception and short-term olfactory memory (Chaudhury et al., 2009; Devore et al., 2012).

Interestingly, optogenetic excitation of cholinergic neurons in the HDB of anesthetized animals inhibits the basal firing rate of (M/T) cells, while also inhibiting the basal firing of the GABAergic granule and PG cells (Ma and Luo, 2012). This observation is quite surprising because, as described above, MCs have been shown to be excited by nAChR activation. Further, work from a number of labs using acute OB slices have demonstrated that mAChRs, in contrast to the optogenetic study, excite GCs, leading to increased GABAergic postsynaptic currents in MCs (Pressler et al., 2007). It is therefore unclear as to how cholinergic input inhibits GCs *in vivo*. It must be pointed out, however, that general anesthetics have effects on nAChR function and might, therefore, confound interpretations when testing cholinergic effects in anesthetized animals (Hara and Harris, 2002; Weber et al., 2005; Liu et al., 2009). Further, a more recent study demonstrated that exciting the cholinergic axons in the OB, instead of exciting the cell bodies in the HDB, leads to an enhancement of M/T cell firing (Rothermel et al., 2014). This observation suggests that activating cholinergic somata in the HDB may lead to indirect inhibition of M/T cells and other bulbar neurons, via pathways that remain to be elucidated (Rothermel et al., 2014).

Activation of basal forebrain cholinergic neurons also results in the sharpening of M/T cell responses so that when these inputs are activated, M/T cell responses to the optimal odorant (i.e., an odorant that elicits the maximal response in the M/T cell under control conditions) are enhanced, while responses to non-optimal odorants are suppressed (Ma and Luo, 2012). It should be noted, however, that direct activation of the cholinergic axons in the OB did not lead to such a suppression for non-optimal odors; instead, excitation of these fibers led to an enhancement of odor-evoked M/T cell responses independent of control response strengths (Rothermel et al., 2014). Thus, there appears to be qualitative differences between activating cholinergic cell bodies within the HDB and activating their fibers in the OB to study the effects of cholinergic input on bulbar function. Regardless of these differences, these results strongly imply that cholinergic input to the OB is responsible for enhanced olfactory function, potentially playing a central role in the detection of weak odors and in the discrimination of chemically similar odorants. Computational models based on experimental observations suggest that mAChRs are likely responsible for the generation of gamma oscillations in the OB while the activation of nAChRs sharpen the tuning curves of MCs in response to odor input (Li and Cleland, 2013), thus pointing to a role of mAChRs (via the modulation GC-MC interactions in the EPL) in controlling MC spike timing, and the role of nAChRs in enhancing contrast between activated glomeruli.

OTHER PLAYERS IN THE CHOLINERGIC MODULATION OF THE OB

Our knowledge of cholinergic modulation of the OB output is far from complete as we are still discovering the extent of receptor distribution and cell types that they can act upon. The functional role that the α7 receptor plays in modulating OB function is still unresolved. Anatomical studies have revealed that α7 nAChRs are highly expressed in the glomerulus (Le Jeune et al., 1995; Hellier et al., 2010), while behavioral studies point to an important role for this receptor subtype in olfactory function (Hellier

et al., 2010, 2012). However, electrophysiological studies suggest that α7 nAChRs do not play a significant role in modulating the spontaneous activity of MCs (D'Souza and Vijayaraghavan, 2012). It is possible that glomerular α7 nAChRs are expressed not on the glomerular tufts of MCs, but on the axon terminals of ORNs, which provide the input to the OB. Other possibilities include the expression of these receptors on glomerular astrocytes, or on centrifugal fibers that innervate the glomerulus. The observation that the release of other neuromodulators such as serotonin and noradrenaline can be altered by cholinergic activation (Decker and McGaugh, 1991; Levin and Simon, 1998), and that circuits in the OB are also modulated by these two neuromodulators (Fletcher and Chen, 2010; Devore and Linster, 2012; Liu et al., 2012), point to the possibility of a sophisticated interplay between these three neuromodulatory systems in regulating the output of the OB. While α7 nAChRs do not appear to play a significant role in inducing nicotinic currents in MCs, or altering the frequency of spontaneous postsynaptic currents on them, they might play a role in mediating plasticity in the OB. This is supported by observations that the receptor is important for olfactory learning (Hellier et al., 2010, 2012; Rushforth et al., 2010).

Similarly, we have no information on the role M2-mAChRs play in the cholinergic modulation of the bulbar output. Anatomical evidence suggests that these receptors are localized on GC synapses in the EPL, on second-order GABAergic neurons in the infra-mitral cell layer, and on some juxtaglomerular GABAergic interneurons, suggesting complex inhibition/disinhibition roles for these receptors on MC output (Crespo et al., 2000).

Our knowledge of the function and regulation of various juxtaglomerular interneurons is incomplete as well. For example, the short axon cells, a type of juxtaglomerular cells that mediates interglomerular inhibition (Aungst et al., 2003), have been studied with increased detail only in recent years (Kiyokage et al., 2010; Liu et al., 2013; Whitesell et al., 2013). Their role in cholinergic modulation remains unresolved, although it's possible that this cell type was previously identified as "bipolar PG cells" that exhibited prominent, slow, inward currents upon nicotinic activation (Castillo et al., 1999).

POSSIBLE MECHANISMS FOR CHOLINERGIC CONTROL OF OLFACTORY CODING

The vast repertoire of cholinergic receptor subtypes expressed throughout the brain exhibit a variety of physiological properties. These include the sensitivity of these receptors to ACh, as well as their desensitization rates. A major puzzle in the field of cholinergic function is to understand the roles played by these different receptor subtypes. Cholinergic transmission in the brain can be broadly classified as occurring via two modes, synaptic, and diffusion-based, resulting in the release of ACh with concentrations that vary over orders of magnitude. Varying concentrations of ACh acting on receptor subtypes exhibiting a myriad of sensitivity and desensitizing properties indicate a dynamic control of sensory processing over multiple timescales that is dependent on the behavioral state of the animal.

As we described earlier, behavioral and *in vivo* work have shown that during light sleep or awake immobility, a low, tonic level of ACh primarily activate mAChRs expressed on GCs (Tsuno et al., 2008). This sets a basal cholinergic tone for GABAergic control of OB output. In contrast, during the anticipational/attentional phase of behavior, there is a rapid and transient increase in MC firing, and it has been suggested that this spontaneous activity in the alert animals might be driven by basal forebrain cholinergic activity (Rinberg and Gelperin, 2006). Consistent with this finding, real-time measurements of ACh levels in the brains of rats performing attention-dependent tasks indicate that cholinergic activity acts on three distinct timescales depending on effort: (1) cue-evoked transient increases in ACh levels that act on the scale of seconds, (2) pre-cue cholinergic signals on the scale of tens of seconds when the rat is anticipating or predicting a cue, and (3) a tonic level of activity that lasts for minutes throughout the session (Parikh et al., 2007, 2008). The transient increase in ACh levels during sustained attention would be sufficient to activate the lower affinity nAChRs, especially the slowly desensitizing heteromeric receptor subtypes. This excitation, in conjunction with feedback GABAergic inhibition, could potentially result in the gating of odor input so that only MCs belonging to strongly activated glomeruli are excited. Such a mechanism would potentially filter out "noise" from weakly activated glomeruli, and lead to enhanced contrast between odor maps encoding chemically similar odors. Noise, in this context, refers to the non-optimal activation of glomeruli via weakly excited ORNs (see **Figure 5B**). This model, therefore, predicts a role for both tonic and phasic modulation for cholinergic inputs (Parikh and Sarter, 2008; Sarter et al., 2009a,b).

Direct excitation of MCs by cholinergic activation has important implications for odor processing. First, the depolarization of MCs can drive them to spike with a high basal firing rate (D'Souza and Vijayaraghavan, 2012). If attention-dependent cholinergic input leads to an increase in the basal firing rate of MCs, it would allow an odor input to alter the frequency, as well as the timing, of spikes. For instance, a decrease in spike frequency or changes in the fine temporal structure at the level of individual action potentials, upon odor input, would not be possible if the cells were not already firing in the first place. Having a baseline firing rate before odor input therefore provides a template for the incoming odor input to manipulate and provide more information to process. Second, depolarization of MCs *before* the onset of an odor signal would trigger the PG-cell driven feedback inhibition, such that, only MCs belonging to glomeruli that receive a strong odor input would transmit the information to the cortex. Third, increasing the basal firing frequency of MCs would increase the probability of coincident synaptic excitation of GCs. Since GCs form reciprocal dendrodendritic contacts with the lateral dendrites of MCs, an increase in the excitation of GCs would, in turn, increase lateral inhibition between MCs (Arevian et al., 2008). Slice and computational studies have implicated the role of GC-mediated lateral inhibition in the synchronization of MC action potentials (Galan et al., 2006; Schoppa, 2006). The cause of this synchrony is the near-simultaneous recovery of MCs from

synchronized GABAergic inhibition, and is thought to underlie synchronous neuronal oscillations in the gamma frequency (Schoppa, 2006). Computational modeling supports the idea that cholinergic excitation of the OB circuit increases the synchronization, as well as the sparseness, of MC action potentials in response to odor input (de Almeida et al., 2013). The observations that cholinergic influence mediate gamma frequency oscillations within neuronal populations (Dickson et al., 2000; Simon et al., 2011) suggest a possibility for a synchronized activity baseline in the OB prior to odor input that could be altered by a subsequent inhalation of odors, providing for a mechanism for allowing the detection and higher-order processing of olfactory information.

OB CHOLINERGIC MODULATION AND NEURODEGENERATIVE DISEASES

The notion that olfactory dysfunction is one of the early symptoms in neurodegenerative diseases is gaining recognition (Attems et al., 2014). As olfactory deficits have been shown to manifest themselves years prior to onset of characteristic symptoms, they might act as early biomarkers of these diseases (Barresi et al., 2012). Major deficits in odor detection, identification, and discrimination have been described in Parkinson's disease (PD) patients, prior to the onset of motor disturbances (Mesholam et al., 1998; Tissingh et al., 2001; Doty, 2009), even leading to a theory that PD might be a primary olfactory disorder (Hawkes et al., 1999).

Similar evidence exists for patients with Alzheimer's disease (AD) where early loss of olfactory discrimination and anosmia has been reported (Christen-Zaech et al., 2003; Djordjevic et al., 2008). Changes in the number of dopaminergic PG cells and loss of OB volume have been described in AD (Mundinano et al., 2011). In mouse models of AD, early onset of olfactory deficits also corresponds to early depositions of amyloid β protein prior to central pathology (Wesson et al., 2010; Kim et al., 2011).

The cholinergic hypothesis for diseases like AD has had dominance for many decades (Bartus et al., 1982; Coyle et al., 1983; Bartus, 2000) and has led to the development of the only approved drugs for the treatment for early and mild dementia. While cholinergic dysfunction is likely to be one of many causes for neurodegeneration (Craig et al., 2011), these studies nonetheless suggest a dominant role for this neurotransmitter system. Studies with patients suffering from Parkinson's disease have indicated that olfactory deficits, seen early in the disease process, correlates with cholinergic degeneration rather than the nigro-striatal dopaminergic neuron deficits (Bohnen et al., 2010), once again confirming the correlation between olfactory function and the cholinergic system.

Our studies indicate that distribution of cholinergic fibers in the OB is intricately connected to olfactory sensory input (**Figure 3**). Unilateral naris occlusion results in a loss of pruning of incoming cholinergic fibers in the adult and results in diffuse innervation of the OB similar to that seen in young (day 12) animals (Salcedo et al., 2011). Does disruption of axonal pruning in the bulb alter the survival of HDB cholinergic neurons? We do not know this, but if loss of axons results in "die-back" and delayed death of neuronal soma in the basal forebrain, it is possible to conceptualize a mechanism that connects sensory environment to neurodegeneration and memory loss observed in AD or other diseases.

CONCLUSION

In most mammals, the ability to discriminate, effectively, benign odors from those that could signal danger is an essential prerequisite for survival. It is, therefore, logical that systems signaling arousal and attention are brought to bear during tasks of odor discrimination. The key transmitter system invoked in these olfactory tasks involves the cholinergic projections from the basal forebrain, long thought to be involved in attention, arousal, learning, and memory. In the olfactory system, it is well accepted that significant processing of odor information occurs at the OB.

A simple model, based on current state of our knowledge, would postulate that nAChR activity dominates at the glomerular microcircuit, while mAChRs control the GC-driven modulation of MC firing (**Figure 5**). The key process in cholinergic modulation of OB functions, appears to be GABAergic signaling. Activation of nAChRs drives glomerular inhibition via the indirect excitation of PG cells. This allows for normalization of glomerular excitation, setting thresholds for transfer of information. The excitation of MCs and ET cells by nAChRs also increase baseline firing, potentially providing a template for net negative readouts in firing frequencies, as well (see **Figures 4C** and **5B**).

At the same time mAChR-driven excitation of GCs and their modulation of GABAergic signaling at the GC-MC dendrodendritic synapses allows for lateral inhibition. Recovery from inhibition across MCs aids in synchronizing firing which is thought to facilitate the integration of incoming information at a population level (**Figure 5C**). Ongoing efforts at further localizing the relevant receptors and at manipulating cholinergic inputs in awake animals performing olfactory tasks will shed more light on this important modulation of a sensory modality by cholinergic processes.

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REFERENCES

Arevian, A. C., Kapoor, V., and Urban, N. N. (2008). Activity-dependent gating of lateral inhibition in the mouse olfactory bulb. *Nat. Neurosci.* 11, 80–87. doi: 10.1038/nn2030

Attems, J., Walker, L., and Jellinger, K. A. (2014). Olfactory bulb involvement in neurodegenerative diseases. *Acta Neuropathol.* 127, 459–475. doi: 10.1007/s00401-014-1261-7

Aungst, J. L., Heyward, P. M., Puche, A. C., Karnup, S. V., Hayar, A., Szabo, G., et al. (2003). Centre-surround inhibition among olfactory bulb glomeruli. *Nature* 426, 623–629. doi: 10.1038/nature02185

Barresi, M., Ciurleo, R., Giacoppo, S., Foti, C. V., Celi, D., Bramanti, P., et al. (2012). Evaluation of olfactory dysfunction in neurodegenerative diseases. *J. Neurol. Sci.* 323, 16–24. doi: 10.1016/j.jns.2012.08.028

Bartus, R. T. (2000). On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Exp. Neurol.* 163, 495–529. doi: 10.1006/exnr.2000.7397

Bartus, R. T., Dean, R. L. III, Beer, B., and Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science* 217, 408–414. doi: 10.1126/science.7046051

Batista-Brito, R., Close, J., Machold, R., and Fishell, G. (2008). The distinct temporal origins of olfactory bulb interneuron subtypes. *J. Neurosci.* 28, 3966–3975. doi: 10.1523/JNEUROSCI.5625-07.2008

Bohnen, N. I., Muller, M. L., Kotagal, V., Koeppe, R. A., Kilbourn, M. A., Albin, R. L., et al. (2010). Olfactory dysfunction, central cholinergic integrity and cognitive impairment in Parkinson's disease. *Brain* 133, 1747–1754. doi: 10.1093/brain/awq079

Bozza, T., McGann, J. P., Mombaerts, P., and Wachowiak, M. (2004). In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron* 42, 9–21. doi: 10.1016/S0896-6273(04)00144-8

Castillo, P. E., Carleton, A., Vincent, J. D., and Lledo, P. M. (1999). Multiple and opposing roles of cholinergic transmission in the main olfactory bulb. *J. Neurosci.* 19, 9180–9191.

Chaudhury, D., Escanilla, O., and Linster, C. (2009). Bulbar acetylcholine enhances neural and perceptual odor discrimination. *J. Neurosci.* 29, 52–60. doi: 10.1523/JNEUROSCI.4036-08.2009

Christen-Zaech, S., Kraftsik, R., Pillevuit, O., Kiraly, M., Martins, R., Khalili, K., et al. (2003). Early olfactory involvement in Alzheimer's disease. *Can. J. Neurol. Sci.* 30, 20–25.

Coyle, J. T., Price, D. L., and DeLong, M. R. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* 219, 1184–1190. doi: 10.1126/science.6338589

Craig, L. A., Hong, N. S., and McDonald, R. J. (2011). Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. *Neurosci. Biobehav. Rev.* 35, 1397–1409. doi: 10.1016/j.neubiorev.2011.03.001

Crespo, C., Blasco-Ibanez, J. M., Brinon, J. G., Alonso, J. R., Dominguez, M. I., and Martinez-Guijarro, F. J. (2000). Subcellular localization of m2 muscarinic receptors in GABAergic interneurons of the olfactory bulb. *Eur. J. Neurosci.* 12, 3963–3974. doi: 10.1046/j.1460-9568.2000.00289.x

Cury, K. M., and Uchida, N. (2010). Robust odor coding via inhalation-coupled transient activity in the mammalian olfactory bulb. *Neuron* 68, 570–585. doi: 10.1016/j.neuron.2010.09.040

D'Souza, R. D., Parsa, P. V., and Vijayaraghavan, S. (2013). Nicotinic receptors modulate olfactory bulb external tufted cells via an excitation-dependent inhibitory mechanism. *J. Neurophysiol.* 110, 1544–1553. doi: 10.1152/jn.00865.2012

D'Souza, R. D., and Vijayaraghavan, S. (2012). Nicotinic receptor-mediated filtering of mitral cell responses to olfactory nerve inputs involves the alpha3beta4 subtype. *J. Neurosci.* 32, 3261–3266. doi: 10.1523/JNEUROSCI.5024-11.2012

Davison, I. G., and Katz, L. C. (2007). Sparse and selective odor coding by mitral/tufted neurons in the main olfactory bulb. *J. Neurosci.* 27, 2091–2101. doi: 10.1523/JNEUROSCI.3779-06.2007

de Almeida, L. B., Idiart, M. A., and Linster, C. (2013). A model of cholinergic modulation in olfactory bulb and piriform cortex. *J. Neurophysiol.* 109, 1360–1377. doi: 10.1152/jn.00577.2012

De Saint, J. D., Hirnet, D., Westbrook, G. L., and Charpak, S. (2009). External tufted cells drive the output of olfactory bulb glomeruli. *J. Neurosci.* 29, 2043–2052. doi: 10.1523/JNEUROSCI.5317-08.2009

Decker, M. W., and McGaugh, J. L. (1991). The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* 7, 151–168. doi: 10.1002/syn.890070209

Devore, S., and Linster, C. (2012). Noradrenergic and cholinergic modulation of olfactory bulb sensory processing. *Front. Behav. Neurosci.* 6:52. doi: 10.3389/fnbeh.2012.00052

Devore, S., Manella, L. C., and Linster, C. (2012). Blocking muscarinic receptors in the olfactory bulb impairs performance on an olfactory short-term memory task. *Front. Behav. Neurosci.* 6:59. doi: 10.3389/fnbeh.2012.00059

Dhawale, A. K., Hagiwara, A., Bhalla, U. S., Murthy, V. N., and Albeanu, D. F. (2010). Non-redundant odor coding by sister mitral cells revealed by light addressable glomeruli in the mouse. *Nat. Neurosci.* 13, 1404–1412. doi: 10.1038/nn.2673

Dickson, C. T., Biella, G., and de, C. M. (2000). Evidence for spatial modules mediated by temporal synchronization of carbachol-induced gamma rhythm in medial entorhinal cortex. *J. Neurosci.* 20, 7846–7854.

Djordjevic, J., Jones-Gotman, M., De Sousa, K., and Chertkow, H. (2008). Olfaction in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiol. Aging* 29, 693–706. doi: 10.1016/j.neurobiolaging.2006.11.014

Doty, R. L. (2009). The olfactory system and its disorders. *Semin. Neurol.* 29, 74–81. doi: 10.1055/s-0028-1124025

Doucette, W., Gire, D. H., Whitesell, J., Carmean, V., Lucero, M. T., and Restrepo, D. (2011). Associative cortex features in the first olfactory brain relay station. *Neuron* 69, 1176–1187. doi: 10.1016/j.neuron.2011.02.024

Doucette, W., and Restrepo, D. (2008). Profound context-dependent plasticity of mitral cell responses in olfactory bulb. *PLoS. Biol.* 6:e258. doi: 10.1371/journal.pbio.0060258

Durand, M., Coronas, V., Jourdan, F., and Quirion, R. (1998). Developmental and aging aspects of the cholinergic innervation of the olfactory bulb. *Int. J. Dev. Neurosci.* 16, 777–785. doi: 10.1016/S0736-5748(98)00087-2

Fletcher, M. L., and Chen, W. R. (2010). Neural correlates of olfactory learning: critical role of centrifugal neuromodulation. *Learn. Mem.* 17, 561–570. doi: 10.1101/lm.941510

Fletcher, M. L., and Wilson, D. A. (2002). Experience modifies olfactory acuity: acetylcholine-dependent learning decreases behavioral generalization between similar odorants. *J. Neurosci.* 22, RC201.

Friedrich, R. W., and Laurent, G. (2001). Dynamic optimization of odor representations by slow temporal patterning of mitral cell activity. *Science* 291, 889–894. doi: 10.1126/science.291.5505.889

Galan, R. F., Fourcaud-Trocme, N., Ermentrout, G. B., and Urban, N. N. (2006). Correlation-induced synchronization of oscillations in olfactory bulb neurons. *J. Neurosci.* 26, 3646–3655. doi: 10.1523/JNEUROSCI.4605-05.2006

Gao, Y., and Strowbridge, B. W. (2009). Long-term plasticity of excitatory inputs to granule cells in the rat olfactory bulb. *Nat. Neurosci.* 12, 731–733. doi: 10.1038/nn.2319

Ghatpande, A. S., and Gelperin, A. (2009). Presynaptic muscarinic receptors enhance glutamate release at the mitral/tufted to granule cell dendrodendritic synapse in the rat main olfactory bulb. *J. Neurophysiol.* 101, 2052–2061. doi: 10.1152/jn.90734.2008

Ghatpande, A. S., Sivaraaman, K., and Vijayaraghavan, S. (2006). Store calcium mediates cholinergic effects on mIPSCs in the rat main olfactory bulb. *J. Neurophysiol.* 95, 1345–1355. doi: 10.1152/jn.00757.2005

Gire, D. H., Franks, K. M., Zak, J. D., Tanaka, K. F., Whitesell, J. D., Mulligan, A. A., et al. (2012). Mitral cells in the olfactory bulb are mainly excited through a multistep signaling path. *J. Neurosci.* 32, 2964–2975. doi: 10.1523/JNEUROSCI.5580-11.2012

Gire, D. H., and Schoppa, N. E. (2009). Control of on/off glomerular signaling by a local GABAergic microcircuit in the olfactory bulb. *J. Neurosci.* 29, 13454–13464. doi: 10.1523/JNEUROSCI.2368-09.2009

Gomez, C., Brinon, J. G., Barbado, M. V., Weruaga, E., Valero, J., and Alonso, J. R. (2005). Heterogeneous targeting of centrifugal inputs to the glomerular layer of the main olfactory bulb. *J. Chem. Neuroanat.* 29, 238–254. doi: 10.1016/j.jchemneu.2005.01.005

Haddad, R., Lanjuin, A., Madisen, L., Zeng, H., Murthy, V. N., and Uchida, N. (2013). Olfactory cortical neurons read out a relative time code in the olfactory bulb. *Nat. Neurosci.* 16, 949–957. doi: 10.1038/nn.3407

Hara, K., and Harris, R. A. (2002). The anesthetic mechanism of urethane: the effects on neurotransmitter-gated ion channels. *Anesth. Analg.* 94, 313–318. doi: 10.1097/00000539-200202000-00015

Hawkes, C. H., Shephard, B. C., and Daniel, S. E. (1999). Is Parkinson's disease a primary olfactory disorder? *QJM.* 92, 473–480. doi: 10.1093/qjmed/92.8.473

Hayar, A., Karnup, S., Ennis, M., and Shipley, M. T. (2004). External tufted cells: a major excitatory element that coordinates glomerular activity. *J. Neurosci.* 24, 6676–6685. doi: 10.1523/JNEUROSCI.1367-04.2004

Hellier, J. L., Arevalo, N. L., Blatner, M. J., Dang, A. K., Clevenger, A. C., Adams, C. E., et al. (2010). Olfactory discrimination varies in mice with different levels of alpha7-nicotinic acetylcholine receptor expression. *Brain Res.* 1358, 140–150. doi: 10.1016/j.brainres.2010.08.027

Hellier, J. L., Arevalo, N. L., Smith, L., Xiong, K. N., and Restrepo, D. (2012). Alpha7-Nicotinic acetylcholine receptor: role in early odor learning preference in mice. *PLoS ONE* 7:e35251. doi: 10.1371/journal.pone.0035251

Himmelheber, A. M., Sarter, M., and Bruno, J. P. (2000). Increases in cortical acetylcholine release during sustained attention performance in rats. *Brain Res. Cogn. Brain Res.* 9, 313–325. doi: 10.1016/S0926-6410(00)00012-4

Jones, B. E. (2004). Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex. *Prog. Brain Res.* 145, 157–169. doi: 10.1016/S0079-6123(03)45011-5

Jones, B. E. (2005). From waking to sleeping: neuronal and chemical substrates. *Trends Pharmacol. Sci.* 26, 578–586. doi: 10.1016/j.tips.2005.09.009

Junek, S., Kludt, E., Wolf, F., and Schild, D. (2010). Olfactory coding with patterns of response latencies. *Neuron* 67, 872–884. doi: 10.1016/j.neuron.2010.08.005

Kasa, P., Hlavati, I., Dobo, E., Wolff, A., Joo, F., and Wolff, J. R. (1995). Synaptic and non-synaptic cholinergic innervation of the various types of neurons in the main olfactory bulb of adult rat: Immunocytochemistry of choline acetyltransferase. *Neuroscience* 67, 667–677. doi: 10.1016/0306-4522(95)00031-D

Kashiwadani, H., Sasaki, Y. F., Uchida, N., and Mori, K. (1999). Synchronized oscillatory discharges of mitral/tufted cells with different molecular receptive ranges in the rabbit olfactory bulb. *J. Neurophysiol.* 82, 1786–1792.

Kato, H. K., Chu, M. W., Isaacson, J. S., and Komiyama, T. (2012). Dynamic sensory representations in the olfactory bulb: modulation by wakefulness and experience. *Neuron* 76, 962–975. doi: 10.1016/j.neuron.2012.09.037

Keiger, C. J., and Walker, J. C. (2000). Individual variation in the expression profiles of nicotinic receptors in the olfactory bulb and trigeminal ganglion and identification of alpha2, alpha6, alpha9, and beta3 transcripts. *Biochem. Pharmacol.* 59, 233–240. doi: 10.1016/S0006-2952(99)00326-3

Kim, J., Choi, I. Y., Michaelis, M. L., and Lee, P. (2011). Quantitative in vivo measurement of early axonal transport deficits in a triple transgenic mouse model of Alzheimer's disease using manganese-enhanced MRI. *Neuroimage* 56, 1286–1292. doi: 10.1016/j.neuroimage.2011.02.039

Kiyokage, E., Pan, Y. Z., Shao, Z., Kobayashi, K., Szabo, G., Yanagawa, Y., et al. (2010). Molecular identity of periglomerular and short axon cells. *J. Neurosci.* 30, 1185–1196. doi: 10.1523/JNEUROSCI.3497-09.2010

Krosnowski, K., Ashby, S., Sathyanesan, A., Luo, W., Ogura, T., and Lin, W. (2012). Diverse populations of intrinsic cholinergic interneurons in the mouse olfactory bulb. *Neuroscience* 213, 161–178. doi: 10.1016/j.neuroscience.2012.04.024

Le Jeune, H., Aubert, I., Jourdan, F., and Quirion, R. (1995). Comparative laminar distribution of various autoradiographic cholinergic markers in adult rat main olfactory bulb. *J. Chem. Neuroanat.* 9, 99–112. doi: 10.1016/0891-0618(95)00070-N

Le Jeune, H., Aubert, I., Jourdan, F., and Quirion, R. (1996). Developmental profiles of various cholinergic markers in the rat main olfactory bulb using quantitative autoradiography. *J. Comp. Neurol.* 373, 433–450. doi: 10.1002/(SICI)1096-9861(19960923)373:3<433::AID-CNE8>3.0.CO;2-3

Le Jeune, H., and Jourdan, F. (1993). Cholinergic innervation of olfactory glomeruli in the rat: an ultrastructural immunocytochemical study. *J. Comp. Neurol.* 336, 279–292. doi: 10.1002/cne.903360209

Lee, M. G., Hassani, O. K., Alonso, A., and Jones, B. E. (2005). Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep. *J. Neurosci.* 25, 4365–4369. doi: 10.1523/JNEUROSCI.0178-05.2005

Levin, E. D., and Simon, B. B. (1998). Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology (Berl.)* 138, 217–230. doi: 10.1007/s002130050667

Li, G., and Cleland, T. A. (2013). A two-layer biophysical model of cholinergic neuromodulation in olfactory bulb. *J. Neurosci.* 33, 3037–3058. doi: 10.1523/JNEUROSCI.2831-12.2013

Liu, L. T., Willenbring, D., Xu, Y., and Tang, P. (2009). General anesthetic binding to neuronal alpha4beta2 nicotinic acetylcholine receptor and its effects on global dynamics. *J. Phys. Chem. B* 113, 12581–12589. doi: 10.1021/jp9039513

Liu, S., Aungst, J. L., Puche, A. C., and Shipley, M. T. (2012). Serotonin modulates the population activity profile of olfactory bulb external tufted cells. *J. Neurophysiol.* 107, 473–483. doi: 10.1152/jn.00741.2011

Liu, S., Plachez, C., Shao, Z., Puche, A., and Shipley, M. T. (2013). Olfactory bulb short axon cell release of GABA and dopamine produces a temporally biphasic inhibition-excitation response in external tufted cells. *J. Neurosci.* 33, 2916–2926. doi: 10.1523/JNEUROSCI.3607-12.2013

Lysakowski, A., Wainer, B. H., Bruce, G., and Hersh, L. B. (1989). An atlas of the regional and laminar distribution of choline acetyltransferase immunoreactivity in rat cerebral cortex. *Neuroscience* 28, 291–336. doi: 10.1016/0306-4522(89)90180-2

Ma, M., and Luo, M. (2012). Optogenetic activation of Basal forebrain cholinergic neurons modulates neuronal excitability and sensory responses in the main olfactory bulb. *J. Neurosci.* 32, 10105–10116. doi: 10.1523/JNEUROSCI.0058-12.2012

Macrides, F., Davis, B. J., Youngs, W. M., Nadi, N. S., and Margolis, F. L. (1981). Cholinergic and catecholaminergic afferents to the olfactory bulb in the hamster: a neuroanatomical, biochemical, and histochemical investigation. *J. Comp. Neurol.* 203, 495–514. doi: 10.1002/cne.902030311

Mao, D., Perry, D. C., Yasuda, R. P., Wolfe, B. B., and Kellar, K. J. (2008). The alpha4beta2alpha5 nicotinic cholinergic receptor in rat brain is resistant to up-regulation by nicotine in vivo. *J. Neurochem.* 104, 446–456. doi: 10.1111/j.1471-4159.2007.05011.x

Margrie, T. W., and Schaefer, A. T. (2003). Theta oscillation coupled spike latencies yield computational vigour in a mammalian sensory system. *J. Physiol.* 546, 363–374. doi: 10.1113/jphysiol.2002.031245

Markopoulos, F., Rokni, D., Gire, D. H., and Murthy, V. N. (2012). Functional properties of cortical feedback projections to the olfactory bulb. *Neuron* 76, 1175–1188. doi: 10.1016/j.neuron.2012.10.028

Mesholam, R. I., Moberg, P. J., Mahr, R. N., and Doty, R. L. (1998). Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Arch. Neurol.* 55, 84–90. doi: 10.1001/archneur.55.1.84

Mesulam, M. M., Hersh, L. B., Mash, D. C., and Geula, C. (1992). Differential cholinergic innervation within functional subdivisions of the human cerebral cortex: a choline acetyltransferase study. *J. Comp. Neurol.* 318, 316–328. doi: 10.1002/cne.903180308

Mundinano, I. C., Caballero, M. C., Ordonez, C., Hernandez, M., DiCaudo, C., Marcilla, I., et al. (2011). Increased dopaminergic cells and protein aggregates in the olfactory bulb of patients with neurodegenerative disorders. *Acta Neuropathol.* 122, 61–74. doi: 10.1007/s00401-011-0830-2

Najac, M., De Saint, J. D., Reguero, L., Grandes, P., and Charpak, S. (2011). Monosynaptic and polysynaptic feed-forward inputs to mitral cells from olfactory sensory neurons. *J. Neurosci.* 31, 8722–8729. doi: 10.1523/JNEUROSCI.0527-11.2011

Parikh, V., Kozak, R., Martinez, V., and Sarter, M. (2007). Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron* 56, 141–154. doi: 10.1016/j.neuron.2007.08.025

Parikh, V., Man, K., Decker, M. W., and Sarter, M. (2008). Glutamatergic contributions to nicotinic acetylcholine receptor agonist-evoked cholinergic transients in the prefrontal cortex. *J. Neurosci.* 28, 3769–3780. doi: 10.1523/JNEUROSCI.5251-07.2008

Parikh, V., and Sarter, M. (2008). Cholinergic mediation of attention: contributions of phasic and tonic increases in prefrontal cholinergic activity. *Ann. N. Y. Acad. Sci.* 1129, 225–235. doi: 10.1196/annals.1417.021

Pressler, R. T., Inoue, T., and Strowbridge, B. W. (2007). Muscarinic receptor activation modulates granule cell excitability and potentiates inhibition onto mitral cells in the rat olfactory bulb. *J. Neurosci.* 27, 10969–10981. doi: 10.1523/JNEUROSCI.2961-07.2007

Rinberg, D., and Gelperin, A. (2006). Olfactory neuronal dynamics in behaving animals. *Semin. Cell Dev. Biol.* 17, 454–461. doi: 10.1016/j.semcdb.2006.04.009

Rinberg, D., Koulakov, A., and Gelperin, A. (2006). Speed-accuracy tradeoff in olfaction. *Neuron* 51, 351–358. doi: 10.1016/j.neuron.2006.07.013

Rothermel, M., Carey, R. M., Puche, A., Shipley, M. T., and Wachowiak, M. (2014). Cholinergic inputs from Basal forebrain add an excitatory bias to odor coding in the olfactory bulb. *J. Neurosci.* 34, 4654–4664. doi: 10.1523/JNEUROSCI.5026-13.2014

Rushforth, S. L., Allison, C., Wonnacott, S., and Shoaib, M. (2010). Subtype-selective nicotinic agonists enhance olfactory working memory in normal rats: a novel use of the odour span task. *Neurosci. Lett.* 471, 114–118. doi: 10.1016/j.neulet.2010.01.022

Salcedo, E., Tran, T., Ly, X., Lopez, R., Barbica, C., Restrepo, D., et al. (2011). Activity-dependent changes in cholinergic innervation of the mouse olfactory bulb. *PLoS. ONE.* 6:e25441. doi: 10.1371/journal.pone.0025441

Sarter, M., Parikh, V., and Howe, W. M. (2009a). nAChR agonist-induced cognition enhancement: integration of cognitive and neuronal mechanisms. *Biochem. Pharmacol.* 78, 658–667. doi: 10.1016/j.bcp.2009.04.019

Sarter, M., Parikh, V., and Howe, W. M. (2009b). Phasic acetylcholine release and the volume transmission hypothesis: time to move on. *Nat. Rev. Neurosci.* 10, 383–390. doi: 10.1038/nrn2635

Schoppa, N. E. (2006). Synchronization of olfactory bulb mitral cells by precisely timed inhibitory inputs. *Neuron* 49, 271–283. doi: 10.1016/j.neuron.2005.11.038

Senut, M. C., Menetrey, D., and Lamour, Y. (1989). Cholinergic and peptidergic projections from the medial septum and the nucleus of the diagonal band of Broca to dorsal hippocampus, cingulate cortex and olfactory bulb: a combined wheatgerm agglutinin-apohorseradish peroxidase-gold immunohistochemical study. *Neuroscience* 30, 385–403. doi: 10.1016/0306-4522(89)90260-1

Simon, C., Kezunovic, N., Williams, D. K., Urbano, F. J., and Garcia-Rill, E. (2011). Cholinergic and glutamatergic agonists induce gamma frequency activity in dorsal subcoeruleus nucleus neurons. *Am. J. Physiol. Cell Physiol.* 301, C327–C335. doi: 10.1152/ajpcell.00093.2011

Smith, R. S., and Araneda, R. C. (2010). Cholinergic modulation of neuronal excitability in the accessory olfactory bulb. *J. Neurophysiol.* 104, 2963–2974. doi: 10.1152/jn.00446.2010

Tissingh, G., Berendse, H. W., Bergmans, P., DeWaard, R., Drukarch, B., Stoof, J. C., et al. (2001). Loss of olfaction in de novo and treated Parkinson's disease: possible implications for early diagnosis. *Mov. Disord.* 16, 41–46. doi: 10.1002/1531-8257(200101)16:1<41::AID-MDS1017>3.0.CO;2-M

Tsuno, Y., Kashiwadani, H., and Mori, K. (2008). Behavioral state regulation of dendrodendritic synaptic inhibition in the olfactory bulb. *J. Neurosci.* 28, 9227–9238. doi: 10.1523/JNEUROSCI.1576-08.2008

Vassar, R., Chao, S. K., Sitcheran, R., Nunez, J. M., Vosshall, L. B., and Axel, R. (1994). Topographic organization of sensory projections to the olfactory bulb. *Cell* 79, 981–991. doi: 10.1016/0092-8674(94)90029-9

Wachowiak, M. (2011). All in a sniff: olfaction as a model for active sensing. *Neuron* 71, 962–973. doi: 10.1016/j.neuron.2011.08.030

Wachowiak, M., Economo, M. N., Diaz-Quesada, M., Brunert, D., Wesson, D. W., White, J. A., et al. (2013). Optical dissection of odor information processing in vivo using GCaMPs expressed in specified cell types of the olfactory bulb. *J. Neurosci.* 33, 5285–5300. doi: 10.1523/JNEUROSCI.4824-12.2013

Wang, Z. J., Sun, L., and Heinbockel, T. (2012). Cannabinoid receptor-mediated regulation of neuronal activity and signaling in glomeruli of the main olfactory bulb. *J. Neurosci.* 32, 8475–8479. doi: 10.1523/JNEUROSCI.5333-11.2012

Weber, M., Motin, L., Gaul, S., Beker, F., Fink, R. H., and Adams, D. J. (2005). Intravenous anaesthetics inhibit nicotinic acetylcholine receptor-mediated currents and Ca^{2+} transients in rat intracardiac ganglion neurons. *Br. J. Pharmacol.* 144, 98–107. doi: 10.1038/sj.bjp.0705942

Wenk, H., Bigl, V., and Meyer, U. (1980). Cholinergic projections from magnocellular nuclei of the basal forebrain to cortical areas in rats. *Brain Res.* 2, 295–316. doi: 10.1016/0165-0173(80)90011-9

Wesson, D. W., Levy, E., Nixon, R. A., and Wilson, D. A. (2010). Olfactory dysfunction correlates with amyloid-beta burden in an Alzheimer's disease mouse model. *J. Neurosci.* 30, 505–514. doi: 10.1523/JNEUROSCI.4622-09.2010

Whitesell, J. D., Sorensen, K. A., Jarvie, B. C., Hentges, S. T., and Schoppa, N. E. (2013). Interglomerular lateral inhibition targeted on external tufted cells in the olfactory bulb. *J. Neurosci.* 33, 1552–1563. doi: 10.1523/JNEUROSCI.3410-12.2013

Wilson, D. A., Fletcher, M. L., and Sullivan, R. M. (2004). Acetylcholine and olfactory perceptual learning. *Learn. Mem.* 11, 28–34. doi: 10.1101/lm.66404

Wilson, D. A., and Stevenson, R. J. (2003). Olfactory perceptual learning: the critical role of memory in odor discrimination. *Neurosci. Biobehav. Rev.* 27, 307–328. doi: 10.1016/S0149-7634(03)00050-2

Xiao, Y., Meyer, E. L., Thompson, J. M., Surin, A., Wroblewski, J., and Kellar, K. J. (1998). Rat alpha3/beta4 subtype of neuronal nicotinic acetylcholine receptor stably expressed in a transfected cell line: pharmacology of ligand binding and function. *Mol. Pharmacol.* 54, 322–333.

Xu, F., Greer, C. A., and Shepherd, G. M. (2000). Odor maps in the olfactory bulb. *J. Comp. Neurol.* 422, 489–495. doi: 10.1002/1096-9861(20000710)422:4<489::AID-CNE1>3.0.CO;2-#

Young, J. W., Crawford, N., Kelly, J. S., Kerr, L. E., Marston, H. M., Spratt, C., et al. (2007a). Impaired attention is central to the cognitive deficits observed in alpha 7 deficient mice. *Eur. Neuropsychopharmacol.* 17, 145–155. doi: 10.1016/j.euroneuro.2006.03.008

Young, J. W., Kerr, L. E., Kelly, J. S., Marston, H. M., Spratt, C., Finlayson, K., et al. (2007b). The odour span task: a novel paradigm for assessing working memory in mice. *Neuropharmacology* 52, 634–645. doi: 10.1016/j.neuropharm.2006.09.006

Zaborszky, L. (2002). The modular organization of brain systems. Basal forebrain: the last frontier. *Prog. Brain Res.* 136, 359–372. doi: 10.1016/S0079-6123(02)36030-8

Zaborszky, L., Carlsen, J., Brashear, H. R., and Heimer, L. (1986). Cholinergic and GABAergic afferents to the olfactory bulb in the rat with special emphasis on the projection neurons in the nucleus of the horizontal limb of the diagonal band. *J. Comp. Neurol.* 243, 488–509. doi: 10.1002/cne.902430405

Zhan, X., Yin, P., and Heinbockel, T. (2013). The basal forebrain modulates spontaneous activity of principal cells in the main olfactory bulb of anesthetized mice. *Front. Neural. Circuits.* 7:148. doi: 10.3389/fncir.2013.00148

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Hippocampal "cholinergic interneurons" visualized with the choline acetyltransferase promoter: anatomical distribution, intrinsic membrane properties, neurochemical characteristics, and capacity for cholinergic modulation

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Release of acetylcholine (ACh) in the hippocampus (HC) occurs during exploration, arousal, and learning. Although the medial septum-diagonal band of Broca (MS-DBB) is the major extrinsic source of cholinergic input to the HC, cholinergic neurons intrinsic to the HC also exist but remain poorly understood. Here, ChAT-tauGFP and ChAT-CRE/Rosa26YFP (ChAT-Rosa) mice were examined in HC. The HC of ChAT-tauGFP mice was densely innervated with GFP-positive axons, often accompanied by large GFP-positive structures, some of which were Neurotrace/DAPI-negative and likely represent large axon terminals. In the HC of ChAT-Rosa mice, ChAT-YFP cells were Neurotrace-positive and more abundant in CA3 and dentate gyrus than CA1 with partial overlap with calretinin/VIP. Moreover, an anti-ChAT antibody consistently showed ChAT immunoreactivity in ChAT-YFP cells from MS-DBB but rarely from HC. Furthermore, ChAT-YFP cells from CA1 stratum radiatum/stratum lacunosum moleculare (SR/SLM) exhibited a stuttering firing phenotype but a delayed firing phenotype in stratum pyramidale (SP) of CA3. Input resistance and capacitance were also different between CA1 SR/LM and CA3 SP ChAT-YFP cells. Bath application of ACh increased firing frequency in all ChAT-YFP cells; however, cholinergic modulation was larger in CA1 SR/SLM than CA3 SP ChAT-YFP cells. Finally, CA3 SP ChAT-YFP cells exhibited a wider AP half-width and weaker cholinergic modulation than YFP-negative CA3 pyramidal cells. Consistent with CRE expression in a subpopulation of principal cells, optogenetic stimulation evoked glutamatergic postsynaptic currents in CA1 SR/SLM interneurons. In conclusion, the presence of fluorescently labeled hippocampal cells common to both ChAT-tauGFP and ChAT-Rosa mice are in good agreement with previous reports on the existence of cholinergic interneurons, but both transgenic mouse lines exhibited unexpected anatomical features that departed considerably from earlier observations.

Keywords: hippocampus, cholinergic modulation, glutamate transmission, optogenetics, transgenic mice

INTRODUCTION

Release of the neurotransmitter acetylcholine (ACh) in the HC is important for learning and memory (Micheau and Marighetto, 2011; Teles-Grilo Ruivo and Mellor, 2013). The major source of acetylcholine in the HC is extrinsic and supplied by the medial septum-diagonal band of Broca (MS-DBB) (Dutar et al., 1995). Lack of HC ACh is associated with cognitive deficits that are observed in Alzheimer's disease (Coyle et al., 1983).

In addition to the MS-DBB cholinergic projection, cholinergic interneurons intrinsic to the hippocampus have been found that may comprise an intrinsic source of ACh (Frotscher et al., 1986, 2000; Freund and Buzsáki, 1996; Romo-Parra et al., 2003). Although originally discovered almost 30 years ago using antibodies to the ACh synthesizing enzyme choline acetyltransferase (ChAT) (Frotscher et al., 1986), no information exists regarding their intrinsic membrane properties. Frotscher and colleagues demonstrated that this population does not contain mRNA for

the GABA synthesizing enzymes GAD67 and GAD65 (Frotscher et al., 2000), consistent with the idea that cholinergic interneurons are not GABAergic in nature. With the visualization of these cells using transgenic mouse technology, interest in ChAT-positive cells has resurfaced (von Engelhardt et al., 2007). A recent study in which EGFP was expressed under the control of the ChAT promoter observed that cortical interneurons are highly colocalized with calretinin and/or VIP, implying that cholinergic interneurons are a specialized subpopulation of HC interneurons (Bayraktar et al., 1997; Tricoire and Cea-Del Rio, 2007; von Engelhardt et al., 2007; Chamberland et al., 2010; Chamberland and Topolnik, 2012). GFP-positive neurons have been observed in the HC of transgenic mice in which GFP is driven by the ChAT-promoter (von Engelhardt et al., 2007; Grybko et al., 2011), yet no study has systematically examined this population using transgenic mouse technology in the HC. Here, we investigated fluorescently labeled ChAT-tauGFP and ChAT-YFP neurons in the HC of ChAT-tauGFP and ChAT-Rosa mice, respectively. Consistent with earlier reports, both ChAT-tauGFP and ChAT-YFP neurons labeled a subpopulation of HC neurons in CA1 and CA3 stratum radiatum/stratum lacunosum moleculare. However, we also made several unexpected observations, including cell-sized en passant boutons in ChAT-tauGFP mice and an electrophysiologically distinct subpopulation of CA3 pyramidal cells in ChAT-Rosa mice.

MATERIAL AND METHODS

ETHICS STATEMENT

All procedures were performed in accordance with the University of Montana Institutional Animal Care and Use Committee (AUP 017-14).

GENERATION OF ChAT-Rosa AND GAD65-GFP/ChAT-CRE TRANSGENIC MICE

Rosa26EYFP$^{+/-}$ mice (Soriano, 1999; Srinivas et al., 2001; Madisen et al., 2009) were purchased from Jackson Laboratories (stock no. #007920, Bar Harbor, ME) and bred to homozygosity (Yi et al., 2014). ChAT-CRE mice (GM24 founder line, MMRRC 017269-UCD; Gong et al., 2007; Ivanova et al., 2010) were bred to homozygosity and maintained as a homozygous line. WT, heterozygosity, and homozygosity of ChAT-CRE mice were determined through qPCR similarly to previously established protocols in PV-CRE mice (Tesson et al., 2002; Yi et al., 2014). Heterozygous ChAT-Rosa26EYFP mice were then generated by crossing homozygous ChAT-CRE and homozygous Rosa26EYFP mice. In the present study, ChAT-Rosa was used to refer to ChAT-Rosa26EYFP mouse line, while ChAT-YFP was used to refer to EYFP-positive cells in ChAT-Rosa mice. ChAT-tauGFP mice, in which a tauGFP fusion protein was driven by the ChAT promoter (Grybko et al., 2011), were obtained from Sukumar Vijayaraghavan at University of Colorado-Denver. Homozygous ChAT-CRE mice were crossed with GAD65-GFP mice (López-Bendito et al., 2004; Cea-del Rio et al., 2010). Heterozygous neonates (P1-P3) from this cross were pre-screened for GFP expression using miner's lamp goggles (FHS/F01, Biological Laboratory Equipment Maintenance and Service, Ltd., Budapest, Hungary) equipped with 460-495 nm light source (FHS/LS-1B) and GFP/YFP emission filters (FHS/EF-3GY2) (Cea-del Rio et al., 2010, 2011). GAD65-GFP/ChAT-CRE mice were then bred to homozygosity through this pre-screening method, combined with the determination of CRE zygosity through qPCR (Tesson et al., 2002; Yi et al., 2014). After wean, mice were socially housed in shoebox-style ventilated cages in gender-specific groups (4–5 littermates per cage). Previous studies with ChAT-CRE or ChAT-Rosa mice have shown a high degree of specificity with endogenous ChAT expression in many brain regions, though not complete co-localization (Gong et al., 2007; Ivanova et al., 2010; Witten et al., 2010; Lopes et al., 2012).

IMMUNOCYTOCHEMISTRY (ICC) IN ChAT-Rosa AND ChAT-tauGFP MICE

Anti-GFP ICC was conducted to intensify the YFP or tauGFP signal, which reduced the laser power required to obtain high quality images, similarly to previously described in PV-GFP (Cea-del Rio et al., 2010) and PV-Rosa mice (Yi et al., 2014). After mice were deeply anesthetized and non-responsive to toe pinch, mice were first transcardially perfused with 50 ml ice cold 0.1 M phosphate buffered saline (PBS), followed by 40–50 ml of ice cold 4% paraformaldehyde (PFA) in PBS, at an approximate rate of 10 ml/min. After clearing of the liver, the mouse was then decapitated with scissors. The brain was carefully removed from the skull and immersed in 4% PFA overnight. In lateralization experiments, an incision was made on the right side of the cortex to preserve orientation in subsequent experiments. On the following day, the tissue block was mounted on a vibratome stage against a block of agarose (4% in dH_2O) in a PBS bath and sectioned (50 μm thickness) using a vibrating blade microtome (VT1000 S, Leica Microsystems Inc., Buffalo Grove, IL USA). Coronal sections, collected between −1.34 and −2.30 mm from bregma, were collected sequentially in a 24 well plate containing 1 ml PBS per well. For anti-GFP staining, every other slice was chosen, yielding approximately 9–12 slices (each containing a left and right hippocampus) per mouse. On Day 1, HC slices were washed 3 times for 10 min in PBS. Slices were placed in 1 ml of antibody diluent (1% BSA, 0.1% sodium azide, and 0.3% Triton-X in PBS; Gábriel et al., 1992). Primary chicken anti-GFP antibody (directed against YFP; 1:4000, cat# GFP-1020, Aves Labs, Tigard, OR) was then added and left overnight on a shaker at 16°C. In a subset of experiments, goat anti-calretinin antibody (1:200; cat# CG1, Swant, Switzerland) and rabbit anti-VIP antibody (1:200; cat# 9535-0204, AbD Serotec, NC, US) were added to the antibody diluent. On Day 2, slices were washed 3 times in PBS for 10 min each. Slices were then placed in PBS containing secondary antibodies for 2–4 h, followed by 3 washes in PBS for 10 min each. Secondary antibodies included donkey anti-chicken Alexa 488 (1:500; cat# 703-545-155, Jackson ImmunoResearch), donkey anti-goat 647 (1:250, cat# 705-605-143, Jackson ImmunoResearch, West Grove, PA, US), and donkey anti-rabbit Alexa 555 (1:250, cat# A-31572, Life Technologies).

For quantification experiments in ChAT-tauGFP and ChAT-Rosa mice, to define HC layers and label neurons, slices were counterstained with Neurotrace 435/455 Blue Fluorescent Nissl Stain (1:100, cat# N-21479, Life Technologies, Grand Island, NY) or Neurotrace 640/660 (1:100 cat# N-21483, Life Technologies,) for 30–45 min. Slices were then mounted on ColorFrost Plus

microscope slides (cat# 9991011, Thermo Scientific) with VectaShield Hardset Mounting Medium (cat # H-1400, Vector Laboratories, Inc.) or Vectashiled Hardset Mounting Medium with DAPI (H-1200, Vector Laboratories, Inc.). Neurotrace 640/660 and DAPI staining exhibited consistent co-localization in all HC cells observed.

TYRAMIDE SIGNAL AMPLITIFCATION (TSA)

For anti-ChAT ICC, acute coronal MS-DBB and transverse HC slices at 300 μm were obtained (see Brain Slice Preparation section), incubated with colchicine (100 μg/mL, C9754-1G, Sigma-Aldrich, St. Louis, MO) in sucrose based cutting/storage (SBC) solution for 8 h, containing (in mM): 80 NaCl, 2.5 KCl, 24 $NaHCO_3$, 0.5 $CaCl_2$, 4 $MgCl_2$, 1.25 NaH_2PO_4, 25 glucose, 75 sucrose, 1 ascorbic acid, 3 sodium pyruvate, saturated with 95% O_2/5% CO_2 (carbogen), pH 7.4. Slices were then fixed with 2% paraformaldehyde (PFA, cat# 15714-S, Electron Microscopy Sciences, Hatfield, PA) for 1 h (von Engelhardt et al., 2007). Slices were then crytoprotected in 30% sucrose solution in PBS overnight. Brain slices were re-sectioned at 60 μm using a freezing sliding microtome (HM430, Thermo Scientific, Waltham, MA, USA). After 2 hours incubation at room temperature in a gelatin-containing PBS solution (0.2% gelatin; PBS-GT) containing 0.25% Triton X-100 and 10% normal donkey serum, re-sectioned slices were incubated with a goat anti-ChAT primary antibody (1:500; cat# AB144P, EMD Millipore, 3 days) followed by overnight incubation with anti-GFP primary antibody (see previous section). Slices were then incubated with donkey anti-chicken Alexa 488 (1:500) and donkey anti-goat HRP (1:500; cat# AB180P, EMD Millipore) for 60 min. After 10 min incubation with a Tyramide Signal Amplification (TSA) Plus Cyanine Kit (cat# NEL745001KT, PerkinElmer, Waltham, MA) applied directly to the slices and 3xPBS washes, slices were stained with Neurotrace 435/455 Blue Fluorescent Nissl Stain for 30–45 min and mounted on slides. For negative controls, slices containing anti-ChAT primary antibody only or anti-goat HRP secondary antibody and TSA only were processed in parallel (**Figure S1**).

IMAGE ACQUISITION, CELL COUNTING, AND STATISTICAL ANALYSIS

A total of four ChAT-Rosa mice (11–12 weeks of age, 2 males and 2 females) and five ChAT-tauGFP mice (11–12 weeks of age, 2 males and 3 females) were used for quantification. Images were acquired with a Fluoview FV-1000 confocal imaging system equipped with 10× and 60× objectives (Olympus Center Valley, PA). Blue (405 nm), green (488 nm), and/or red (647 nm) channels were acquired sequentially. Tiles were acquired with ∼10% overlap per field. Tiles were flat projected, saved as TIFF files, and either stitched automatically (as part of acquisition in Fluoview) or stitched manually (with Image J). HC ChAT-tauGFP or ChAT-YFP structures were scanned and marked with a color-coded symbol to ensure that they were not counted twice. Neurotrace-positive and Neurotrace-negative structures were distinguished in ChAT-tauGFP mice by toggling between channels in Fluoview or ImageJ. ChAT-tauGFP slices labeled with anti-GFP, DAPI, and Neurotrace 640/660 were selected for diameter measurements. In ImageJ, potential ChAT-tauGFP-positive cells were outlined with the polygon tool and added as objects to the ROI manager box. Feret's diameter (maximum caliper) was measured for each object and the results table was transferred to Excel.

Counting was performed in 11 areas: CA3 (SO, SP, SR, and SLM), CA1 (SO, SP, SR, and SLM), dentate gyrus (SM and SG), and hilus, comprising 11 groups. Each group (from 88 total hippocampi in ChAT-Rosa mice or 112 total hippocampi in ChAT-tauGFP mice) represented the total number of cells counted across all hippocampi. Groups were statistically compared on a per slice and per-layer basis using Prism 6 (Graphpad Software, Inc., La Jolla, CA). Most groups failed tests for normality (D'Agostino & Pearson omnibus normality test and Shapiro–Wilk normality test). Therefore, a Friedman test followed by Dunn's multiple comparisons test were used. The dentate granule cell layer and the hilus passed tests for normality; therefore, lateralization data were statistically compared with a One-Way ANOVA and Tukey's multiple comparison's test.

STEREOTAXIC INJECTION OF ChR2-mCHERRY AAV INTO DORSAL CA1 HC

AAV9 EF1.DIO.hChR2(H134R)-mCherry.WPRE.hGH (∼10^{12} vc/ml) was strereotaxically injected into the ventral CA1 HC of adult GAD65-GFP/ChAT-CRE mice. Equipment and procedures for stereotaxic injection of AAV into dorsal CA1 HC of GAD65-GFP/ChAT-CRE mice were as previously described (Yi et al., 2014). Stereotaxic coordinates for AAV injection into ventral CA1 HC (1.5 μL at 0.25 μL/min per hemisphere) were: AP 2.9 mm, ML 3.3 mm, and DV 2.3 mm. Injected mice were used for imaging or electrophysiological recordings at least 2 weeks after survival surgery.

BRAIN SLICE PREPARATION

Both male and female ChAT-Rosa (24–45 day old) or adult GAD65-GFP/ChAT-CRE mice were used. Mice were anesthetized with 4% isoflurane and transcardially perfused with oxygenated, ice-cold, SBC solution. After decapitation, the brain was immediately placed in SBC solution saturated with carbogen. Transverse HC slices or coronal MS-DBB slices were cut at 300 μm on a Leica 1200S Vibratome, using the Leica Vibrocheck device to minimize vibration of the blade in the z-direction prior to use (Geiger et al., 2002). After sectioning, slices were placed in a storage chamber and incubated with carbogen-saturated SBC solution at 36–37°C until use.

WHOLE-CELL PATCH CLAMP RECORDINGS

After incubation for at least 30 min with SBC solution at 36–37°C, a single slice was gently placed on poly-D-lysine-coated glass coverslips (12 mm diameter, 0.09–0.12 thickness, cat# 633009, Carolina Biological Supply Company, Burlington, NC) and perfused at 34–35°C (TC-324B, Warner Instruments, Hamden, CT, USA) with extracellular solution (in mM): 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4 and 20 glucose, saturated with carbogen, pH 7.4. Acute slices were then viewed using Infrapatch (Luigs and Neumann, Ratingen Germany) on an upright microscope (Axio Examiner D1, Carl Zeiss Microscopy, LLC, USA). Fluorescent YFP+ cells in slices from ChAT-Rosa mice or GAD65-GFP cells were identified using a Zeiss LED (505 nm for YFP; 470 nm for GFP; Colibri, Carl

Zeiss Microscopy, LLC, USA) and subsequently viewed under IR-Dodt contrast with a 63× water immersion objective (W Plan-Apo 63x/1.0 VIS-IR WD=2.1 M27, Carl Zeiss Microscopy, LLC, USA), similarly to previously described (Yi et al., 2014). Thin-wall glass capillaries (TW150F-3, World Precision Instruments; Sarasota, FL) were fabricated with a 2.5–4.5 MΩ tip resistance on a 2-step PC-10 Narishige vertical puller (East Meadow, NY, USA). Whole-cell recordings were obtained using a Multiclamp 700 B amplifier (Molecular Devices, Union City, CA), filtered at 4 kHz, and digitized at 20 kHz (Digidata 1440 A, Molecular Devices). Glass capillaries contained intracellular solution (IC; in mM): 110 potassium gluconate, 40 KCl, 10 HEPES, 0.1 EGTA, 4 MgATP, 0.3 Na_2GTP, 10 phosphocreatine and 0.2% biocytin, pH 7.2, osmolarity 290–300 mOsm. Seal resistances ranged from 1–2 GΩ and access resistance ranged from 4–20 MΩ. Bridge balance was used throughout current-clamp experiments and was monitored with a 100 ms long hyperpolarizing current step from −60 mV every 20 s. If access resistance changed by >20%, data were excluded from further analysis. For optogenetic stimulation, TTL-driven flashes from Zeiss LED were delivered to the whole slice to excite ChR2-mCherry-positive neurons. The AAV9-ChR2-mCherry infected ChAT-CRE cells in HC were reliably excited (9/9 cells) by delivery of 2 ms 470 nm light pulse at 5 Hz (**Figure S2**).

ANATOMICAL IDENTIFICATION OF RECORDED CELLS

Biocytin (0.2%) was included in the IC for *post-hoc* morphological identification of each recorded cell. Whole-cell mode was maintained for at least 15 min, the electrode was withdrawn slowly to allow cell membrane resealing to an outside-out patch, and the slice was perfused for an additional 10–15 min to allow biocytin to diffuse to distal intracellular compartments. Slices were fixed overnight at 4°C in PBS containing 4% PFA, transferred to PBS, and stored for up to 1 week at 4°C. After permeabilization with 0.3% Triton X-100 in PBS for 2 h at room temperature, slices were incubated in PBS overnight at 16°C with Alexa Fluor 633-conjugated streptavidin (cat # S-21375, final concentration 1 μg/ml; Life Technologies, Grand Island, NY) in PBS. Slices were cryopreserved in 30% sucrose containing PBS and resectioned at 100–150 μm thickness using a freezing sliding microtome. Resectioned slices underwent PBS washes, were incubated with Neurotrace 435/455 Blue Fluorescent Nissl Stain (1:100 in PBS) for 20 min, and were mounted on Colorfrost Plus slides (cat #99-910-11, Thermo Scientific) using Vectashield HardSet mounting medium (cat #H-1400, Vector Laboratories, Inc., Burlingame, CA). Sections were imaged with a Fluoview FV-1000 confocal imaging system (Olympus, Center Valley, PA) with a 25× objective (XLPL25XWMP, Olympus, Tokyo, Japan). Confocal stacks (800 × 800 pixels) of recorded cells were flat projected, rotated, and cropped in Photoshop 13.0 for display.

CHEMICAL REAGENTS

Acetylcholine chloride (A6625), DNQX (D0540), and gabazine (SR-95531; S106) were obtained from Sigma-Aldrich Inc. (St. Louis, MO). DL-APV was obtained from R&D Systems (Minneapolis, MN).

ANALYSIS OF ELECTROPHYSIOLOGICAL DATA

Electrophysiological data analysis was performed with Axograph X (Axograph Scientific, Sydney, Australia). All parameters, which included afterdeflection (ADF), input resistance (R_{in}) time constant (τ_m), cellular capacitance (C_m), action potential (AP) half width (measured from the first AP), and sag ratio (steady state (SS)/peak; in response to a 1 s long, −100 pA current step from −60 mV), were measured as described previously (Yi et al., 2014). A One-Way ANOVA and Tukey's multiple comparison test or a One-Way ANOVA and Dunn's multiple comparison test was used when appropriate.

RESULTS

REGION- AND LAMINA-SPECIFIC EXPRESSION IN THE HC OF ChAT-tauGFP AND ChAT-ROSA MICE

In ChAT-tauGFP mice, consistent with a previous study (Grybko et al., 2011), GFP labeling was highly visible as a dense plexus of axonal processes innervating every layer of the HC (**Figure 1A**). Occasionally, large, globular structures were observed among ChAT-tauGFP-positive fibers, suggestive of cholinergic interneurons (Grybko et al., 2011). To define HC layers and determine the cellular distribution of ChAT-tauGFP interneurons, sections were counterstained with DAPI and Neurotrace 640/660. As a positive control, ChAT-tauGFP cells were readily observed in the habenula (**Figures 1A–E**). In the HC, upon closer inspection, a subset of these cells were co-labeled with DAPI and Neurotrace (**Figures 1F–I**), consistent with a population of HC cholinergic interneurons (Frotscher et al., 1986, 2000; Grybko et al., 2011). Surprisingly, a subset of cell-sized ChAT-tauGFP-positive globular structures were negative for both DAPI and Neurotrace (**Figures 1J–M**). Axonal fibers were often observed leading into and out of these globular structures, suggesting that the were large, en passant boutons (see also **Figures S3E–J**).

We also examined ChAT-Rosa mice to visualize HC ChAT-YFP neurons. In contrast to the prominent axonal labeling in ChAT-tauGFP mice, YFP appeared to be localized predominantly to the somatodendritic domains, possibly due to the limited diffusion of cytosolic YFP into axon terminals. In ChAT-Rosa mice, ChAT-YFP structures in the HC were unambiguously neurons due to dendritic labeling and Neurotrace positivity (**Figure 2**). In accordance with previous studies (Frotscher et al., 1986, 2000), ChAT-YFP cells were distributed throughout the HC (**Figure 2A**). Consistent with expected expression of YFP in brain regions known to contain cholinergic neurons, ChAT-YFP cells were observed in the cortex (**Figure 2A**) (von Engelhardt et al., 2007) and medial habenula (**Figures 2A,B**) (Grybko et al., 2011; Ren et al., 2011). Within the HC, ChAT-YFP cells were found in the DG (**Figures 2A,C**), hilus (**Figures 2A,D**), CA3 (**Figures 2A,E,F**), and CA1 (**Figures 2A,G**). The diffuse YFP staining in the DG inner molecular layer, combined with the presence of YFP-positive cells in hilus (**Figures 2A,D**), suggests that mossy cells are labeled in ChAT-Rosa mice.

Theoretically, the distribution of ChAT-tauGFP and ChAT-YFP cells in the HC should be similar. However, due to the initial observation that some ChAT-tauGFP structures were negative for DAPI and Neurotrace (**Figures 1J–M**), we investigated

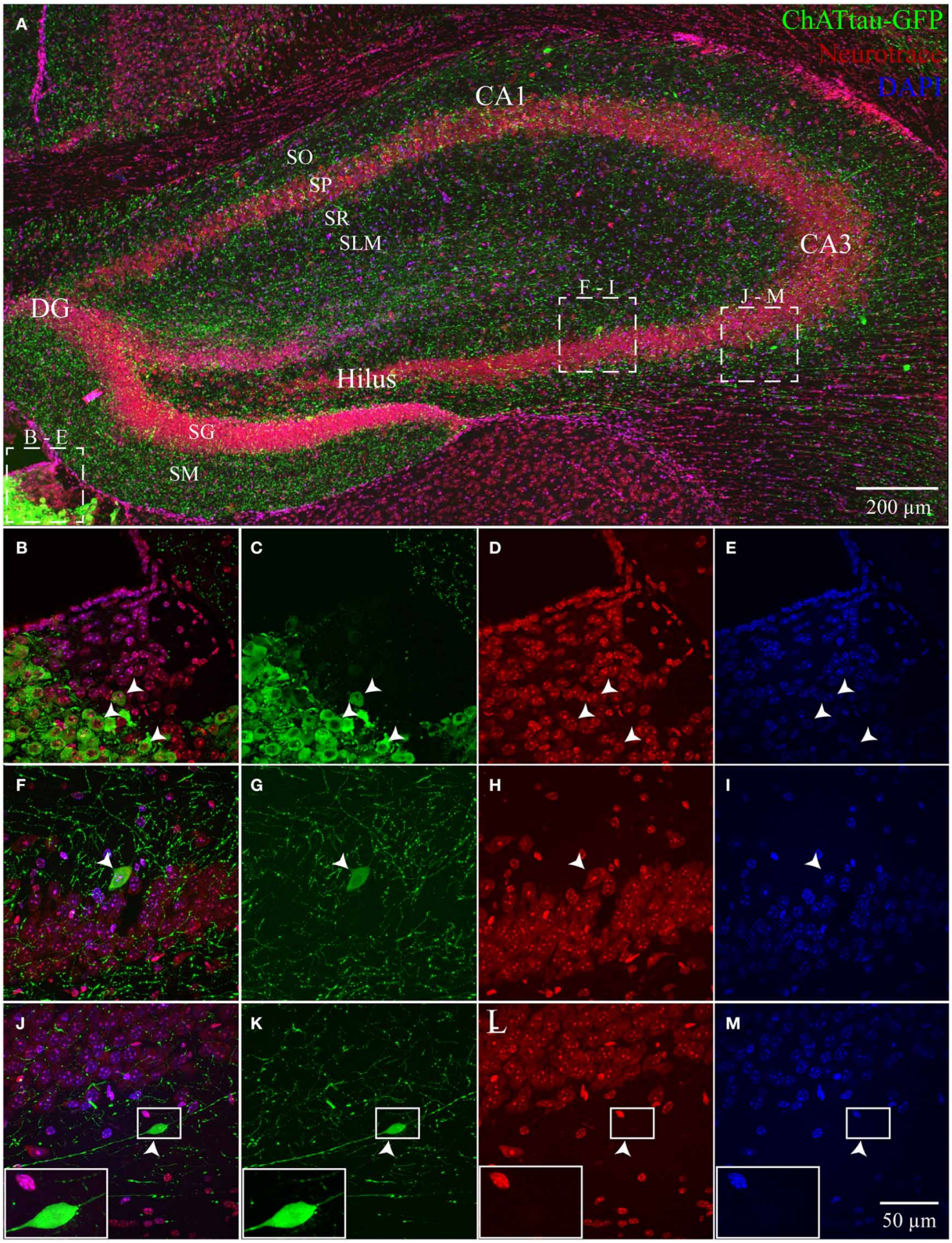

FIGURE 1 | Hippocampus and habenula in ChAT-tauGFP mice. (A) Flat-projected confocal image displaying (green) ChAT-tauGFP cells in the hippocampus. Cells and layers are counterstained with (red) Neurotrace 640/660 and DAPI (blue). Representative examples of Neurotrace/DAPI-positive ChAT-tauGFP cells in **(B–E)** habenula and **(F–I)** CA3 SP. **(J–M)** Representative example of a Neurotrace/DAPI-negative ChAT-tauGFP structure in the CA3 SO layer. (J-M, inset) Magnified view suggesting an en passant bouton. Dotted boxes in **(A)** show the relationship to **(B–M)**.

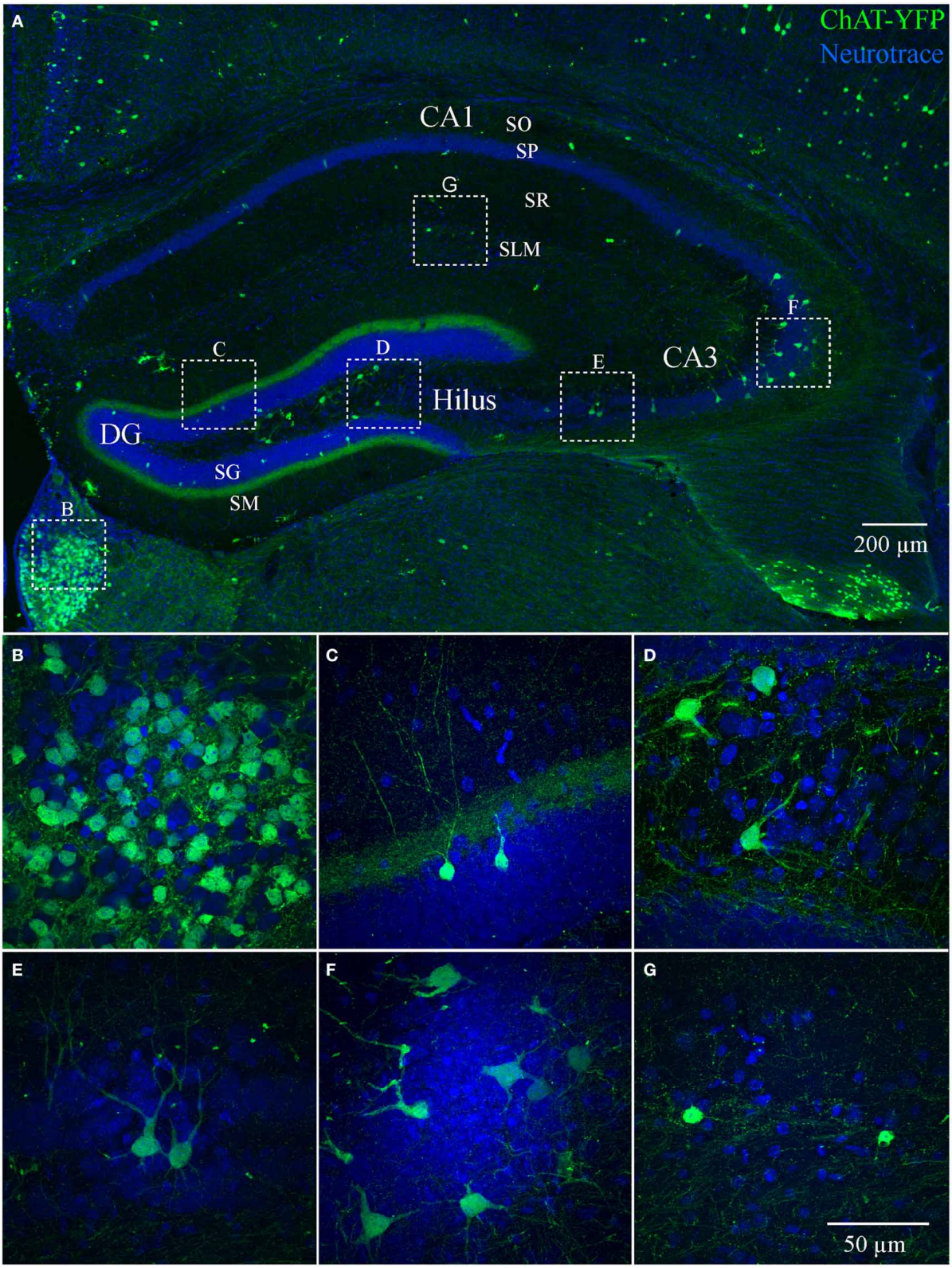

FIGURE 2 | Hippocampus and habenula in ChAT-Rosa mice. (A) Flat-projected confocal image displaying ChAT-YFP cells (green) in the hippocampus. Cells and layers are counterstained with Neurotrace 435/455 (blue). Magnified views of ChAT-YFP cells in **(B)** medial habenula and hippocampal subregions **(C)** DG, **(D)** hilus, **(E,F)** CA3, and **(G)** CA1. Dotted boxes in **(A)** show the relationship to **(B–G)**.

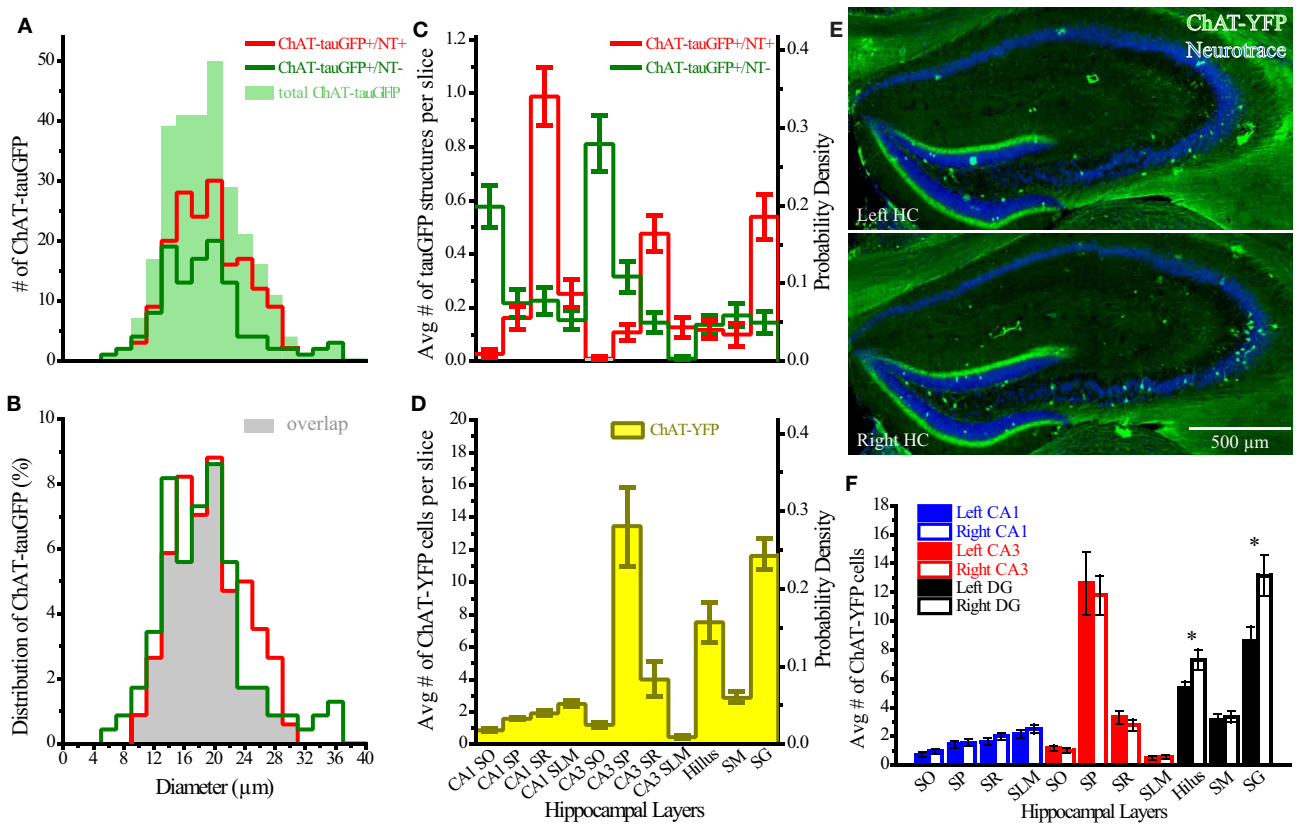

FIGURE 3 | Quantification of ChAT-tauGFP and ChAT-YFP cells. (A) Distribution of ChAT-tauGFP diameters (filled green; $n = 279$), categorized by presence (red, indicating true cells, $n = 167$) or absence (green, non-cells, $n = 116$) of Neurotrace 640/660 (2 μm bin). **(B)** Probability density; filled gray indicates the area of overlap. **(C)** Region and layer distribution for ChAT-GFP cells and non-cells. **(D)** Region and layer distribution of ChAT-YFP cells (all were Neurotrace-positive). Panels **(C,D)** are displayed both as average number per slice (left Y axis) and probability density function (right Y axis). **(E)** Flat-projected, confocal images of left (upper, horizontally flipped) and right (lower) HC from a ChAT-Rosa mouse, counterstained with (blue) Neurotrace 435/455. **(F)** Average number of cells in left (solid bars) or right (open bars) hippocampus (31 bilateral hippocampal sections across 3 ChAT-Rosa mice). *Denotes $p < 0.05$.

whether Neurotrace-negative and Neurotrace-positive ChAT-tauGFP structures could be differentiable based on size. Using the Ferret diameter as a measure (**Figures 3A,B**), size was not significantly different ($p = 0.16$, Mann–Whitney test; from 42 hippocampi of 2 mice) between of DAPI/Neurotrace-positive (18.4 ± 0.4 μm, $n = 163$) and DAPI/Neurotrace-negative structures (18.0 ± 0.6 μm; $n = 116$). These two groups had a similar distribution of diameters (**Figure 3A**). Moreover, the probability density function revealed a large region of overlap (80.6%; **Figure 3B**, gray), indicating that ChAT-tauGFP neuronal and non-neuronal structures could not readily be differentiated from each other based on size.

Because DAPI staining confirmed that Neurotrace was a reliable neuronal marker, we pooled quantification from 2 mice counterstained with DAPI/Neurotrace (**Figure 1**) and 3 mice counterstained with Neurotrace only (**Figure S3**). Consistent with the cellular distribution observed in ChAT-immunopositive cells in rat (Frotscher et al., 1986, 2000) and ChAT-EGFP cells in mouse (von Engelhardt and colleagues, unpublished observations), we found that the majority of Neurotrace-positive ChAT-tauGFP cells were observed in the stratum radiatum (SR) layer of CA1 ($34.1 \pm 3.7\%$, $p < 0.05$) and CA3 ($16.4 \pm 2.3\%$, $p < 0.05$), with fewer cells observed in other HC layers (**Figure 3C**, red; **Table 1**). Interestingly, the distribution of Neurotrace-negative structures was different, with a high percentage of these structures present in the stratum oriens of CA1 ($19.9 \pm 2.7\%$, $p < 0.05$) and CA3 ($28.0 \pm 4\%$, $p < 0.05$) (**Figure 3C**; **Table 1**). In summary, Neurotrace-positive ChAT-tauGFP cell counts are consistent with earlier work, but our results also reveal cell-sized Neurotrace-negative structures that resembled en passant boutons in ChAT-tauGFP mice.

We also quantified the distribution of ChAT-YFP cells in ChAT-Rosa mice. There was a larger number of ChAT-YFP cells in DG (22.3 ± 1.0, $p < 0.0001$) and CA3 (19.4 ± 1.2, $p < 0.0001$) than CA1 (6.9 ± 0.4, $n = 88$ hippocampi from 4 mice). Layer-specific differences were also observed within the HC of ChAT-Rosa mice ($p < 0.0001$, Friedman test; **Figure 3D**; **Table 2**). Within area HC CA1, there were more ChAT-YFP cells in stratum lacunosum moleculare (SLM; 2.5 ± 0.2, $p < 0.0001$) and SR (1.9 ± 1.4, $p = 0.017$) than in SO (0.9 ± 0.1). Within CA3, the region most densely populated with ChAT-YFP cells was in SP (13.7 ± 1.0), compared to SO (1.3 ± 0.1, $p < 0.0001$), SR

Table 1 | Regional and laminar distribution of ChAT-tauGFP counts.

Region	GFP+	layer	GFP+	GFP+/NT+	GFP+/NT−
CA1	289	SO	67	3 (0.9%)†	64 (19.9%)*
		SP	42	18 (5.6%)†	24 (7.5%)
		SR	135	110 (34.1%)	25 (7.8%)
		SLM	45	28 (8.7%)†	17 (5.3%)
CA3	222	SO	91	1 (0.3%)†	90 (28.0%)*
		SP	47	12 (3.7%)†	35 (10.9%)
		SR	69	53 (16.4%)§	16 (5.0%)
		SLM	15	14 (4.3%)†	1 (0.3%)
Hilus	28	Hilus	28	13 (4.0%)†	15 (4.7%)
DG	106	SM	30	11 (3.4%)†	19 (5.9%)
		SG	76	60 (18.6%)§	16 (5.0%)

*Values in the table were compiled from 56 hippocampal slices (112 hippocampi total) from 5 ChAT-tauGFP mice. †Denotes $p < 0.05$ compared to CA1 SR layer among GFP+/NT+ group. §Denotes $p < 0.05$ compared to CA3 SO layer among GFP+/NT+ group. *Denotes $p < 0.05$ compared to CA3 SLM layer among GFP+/NT− group. The percentage numbers are of the fraction of GFP+/NT+ or GFP/NT− within the their groups.*

Table 2 | Regional and laminar distribution of ChAT-tauGFP and ChAT-YFP cells.

Region	Layer	ChAT-tauGFP	ChAT-YFP
CA1	SO	0.03 ± 0.02	0.9 ± 0.1*
	SP	0.16 ± 0.04	1.6 ± 0.1*
	SR	0.98 ± 0.10	1.9 ± 1.4*
	SLM	0.25 ± 0.05	2.5 ± 0.2*
CA3	SO	0.01 ± 0.01	1.2 ± 0.1*
	SP	0.11 ± 0.03	13.7 ± 1.0*
	SR	0.47 ± 0.07	4.0 ± 0.3*
	SLM	0.13 ± 0.04	0.4 ± 0.1*
Hilus	Hilus	0.12 ± 0.03	7.6 ± 0.4*
DG	SM	0.98 ± 0.04	2.9 ± 0.2*
	SG	0.54 ± 0.08	11.8 ± 0.7*

*Data in the table are represented as mean cell number ± SEM per hippocampus, from a total of 44 hippocampal slices (88 hippocampi) from ChAT-Rosa mice and a total of 56 hippocampal slices (112 hippocampi) from ChAT-tauGFP mice. Asterisks denote multiple unpaired t-tests *$p < 0.05$.*

(4.0 ± 0.3, $p < 0.0001$), and SLM (0.4 ± 0.1, $p < 0.0001$). This high abundance of ChAT-YFP cells in SP was unique to CA3 and not observed in CA1 SP (1.6 ± 1.4, $p < 0.0001$). By contrast, there were more ChAT-YFP cells in CA1 SLM than CA3 SLM (0.4 ± 0.1, $p < 0.0001$). There were also layer-specific differences within DG. Similar to CA3, the highest density of ChAT-YFP cells was observed in the principal cell layer (11.8 ± 0.7) relative to the stratum moleculare layer of the dentate (2.9 ± 0.2, $p < 0.0001$). The hilus (7.6 ± 0.4) also had abundant ChAT-YFP cells comparable to the CA3 SP and granule cell layer of the DG ($p > 0.05$).

For every HC region, ChAT-YFP cells, which were invariably Neurotrace-positive, were more abundant than Neurotrace-positive ChAT-tauGFP cells ($p < 0.05$, multiple *t*-tests, **Table 2**). When normalized to total cell number, cellular distribution was different between ChAT-tauGFP and ChAT-Rosa mice. The majority of Neurotrace-positive ChAT-tauGFP cells were located in the in CA1 SR, CA3 SR, and DG SG layer (**Figure 3C**, red). In contrast, the majority of ChAT-YFP cells were present in CA3 SP, DG SG layer, and hilus (**Figure 3D**).

Finally, a lateralization difference was detected in the HC of ChAT-Rosa mice. The DG SG layer displayed higher abundance of ChAT-YFP cells in the right (13.2 ± 1.4) than left (8.6 ± 1.0) hemisphere ($p = 0.001$, paired *t*-test, **Figures 3E,F**). A right preference of ChAT-YFP cells was also observed in the hilus (R: 7.3 ± 0.7; L: 5.4 ± 0.5, $p = 0.009$, paired *t*-test, $n = 31$ HC slices from 3 mice). There was no lateralization bias in CA1 or CA3 ($p > 0.05$). On a per mouse basis, there are the same lateralization trends, although without significant difference, in DG SG layer (R: 136.0 ± 21.0; L: 89.3 ± 7.5, $n = 3$) and hilus (R: 75.7 ± 16.8; L: 55.3 ± 4.8, $n = 3$).

ANTI-ChAT IMMUNOREACTIVITY IS MORE READILY DETECTED IN ChAT-YFP CELLS FROM MS-DBB THAN HC

Although ChAT has been detected immunocytochemically in the HC, ChAT expression in rat was reported to be weaker in the HC than in the basal forebrain region (Frotscher et al., 1986, 2000). TSA amplification enabled us to achieve high signal-to-noise ratio of ChAT expression (see Material and Methods; **Figure S1**). In the MS-DBB (**Figures 4A–C**), ChAT expression was readily detected and co-localized strongly in ChAT-YFP cells (**Figures 4A–F**). In contrast, despite meticulously processing HC slices in parallel with positive controls in MS-DBB slices, ChAT labeling was observed in ChAT-YFP cells from only HC SR/LM layer (**Figures 5A–F**), but not in CA3 (**Figures 5G–I**) or DG (**Figures 5J–L**). Consistent with findings in ChAT-EGFP mice (von Engelhardt et al., 2007), ChAT expression was only partially overlapping in cortex and striatum (**Figures S4A–L**).

A SUBSET OF ChAT-YFP CELLS CO-LOCALIZE WITH CALRETININ AND VIP

Cortical ChAT-EGFP cells exhibit a high degree of co-localization with the calcium binding protein calretinin and neuropeptide VIP (Bayraktar et al., 1997; von Engelhardt et al., 2007). We sought to examine whether HC ChAT-YFP cells possess a similar degree of co-localization with calretinin and VIP. We found only partial co-localization (8.2%, 146/1785) of calretinin or VIP (2.0%, 22/1088) with ChAT-YFP (**Figure S5**), with the majority of calretinin-positive ChAT-YFP cells located in CA1 (50.7%, 74/146) and hilus (32.2%, 47/146), and populations sparsely localized to CA3 (15.1%, 22/146) and DG (2.1%, 3/146). In this experiment, some hilar ChAT-YFP neurons were observed to possess spiny proximal dendrites (**Figure S5D1**, open arrow), suggesting that the diffuse YFP labeling of the DG inner molecular layer (**Figure 2A**) could be accounted for by the axons of ChAT-YFP-positive mossy cells (Scharfman and Myers, 2012). VIP-positive ChAT-YFP cells were located in CA1 (81.8%, 18/22) and CA3 (18.2%, 4/22). Therefore, our data suggests that there is limited overlap of ChAT-YFP with calretinin and VIP.

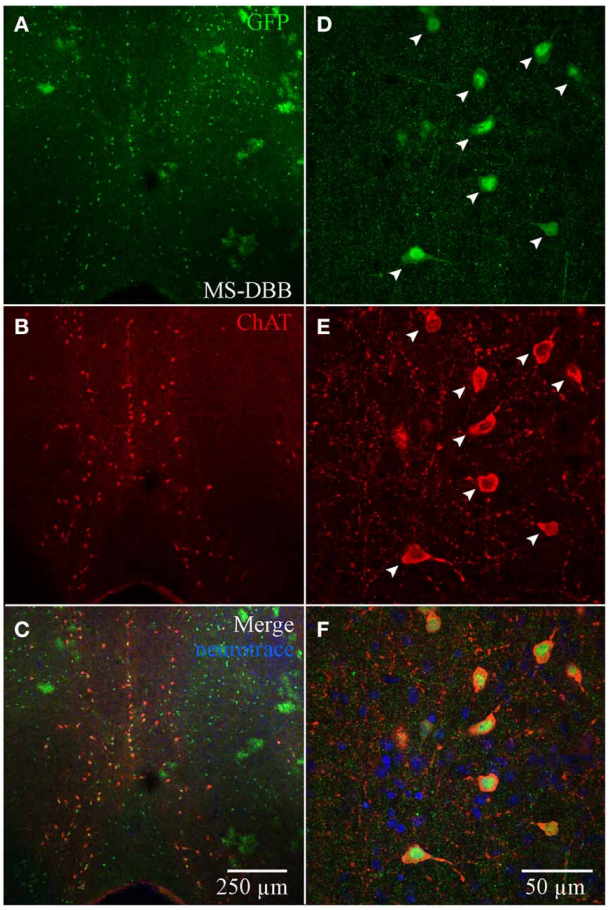

FIGURE 4 | Anti-ChAT labeling in MS-DBB ChAT-YFP cells. (A) YFP, **(B)** anti-ChAT, and **(C)** merged images from the MS-DBB. Higher magnification images **(D–F)** showing co-localization of anti-GFP and anti-ChAT labeling. MS-DBB showing co-localization of YFP and ChAT.

INTRINSIC MEMBRANE PROPERTIES OF HC ChAT-YFP CELLS

Using whole-cell patch clamp recording from ChAT-Rosa mice, we investigated the intrinsic membrane properties of two populations of HC ChAT-YFP cells in CA1 SR/LM and CA3 SP. Representative live images and morphologies of CA1 SR/LM (**Figure 6A**) and CA3 SP (**Figure 6B**) ChAT-YFP cells are shown. As a population, a subset of recorded HC CA1 SR/LM ChAT-YFP cells (5/11, **Table 3**) exhibited spontaneous firing, which was uncommon in CA3 SP ChAT-YFP cells (1/14). Upon injection of a 1 s long, +200 pA depolarizing current step, ChAT-YFP cells in CA1 SR/LM exhibited an irregular firing pattern (**Figure 6A4**), while CA3 SP ChAT-YFP cells exhibited a delayed firing phenotype (**Figure 6B4**). Although AP half-widths were comparable between CA1 SR/LM and CA3 SP ChAT-YFP cells (**Figures 6A5,B5**, **7D**), CA1 SR/LM ChAT-YFP cells had higher R_{in} (**Figure 7A**) and smaller size (**Figure 7B**) than CA3 SP ChAT-YFP cells (**Table 3**). As expected from the differential R_{in} of these populations, CA1 SR/LM ChAT-YFP cells achieved a higher AP frequency than CA3 SP ChAT-YFP cells at small depolarizing current steps ($p < 0.05$ at +100 pA; **Table 3**), whereas CA3 SP ChAT-YFP cells tolerated larger current steps (**Figure 7C**). Therefore, the intrinsic membrane properties are distinct between CA1 SR/LM and CA3 SP ChAT-YFP populations, representing distinct ChAT-YFP subclasses.

The intrinsic membrane properties and appearance of thorny excrescences on the apical dendrites of CA3 ChAT-YFP cells (**Figure 6B3**) led us to compare ChAT-YFP cells to YFP-negative, presumably pyramidal cells, in the CA3 SP (**Figure 6C**). YFP-negative and ChAT-YFP cells in CA3 SP were not significantly different in C_m, R_{in}, and τ_m (**Table 3**). However, as a population, ChAT-YFP cells had a broader AP half-width than CA3 SP YFP-negative cells ($p = 0.013$; **Figures 6B5**, **7D**). Therefore, on the basis of these findings, CA3 SP ChAT-YFP cells could be viewed as a distinct subclass of CA3 pyramidal cells.

Finally, we examined the intrinsic properties of MS-DBB and HC ChAT-YFP cells. MS-DBB ChAT-YFP cells (**Figure 6D**) were comparable to CA1 SR/LM cells in C_m, R_{in}, and τ_m (**Table 3**). In addition, MS-DBB ChAT-YFP cells had broad AP half-widths that were not significantly different than AP half-widths in HC ChAT-YFP cells (**Figures 6D**, **7D**; **Table 3**).

HC ChAT-YFP CELLS UNDERGO CHOLINERGIC NEUROMODULATION

One intriguing hypothesis is that cholinergic interneurons themselves could be targets of cholinergic modulation (Tricoire and Cea-Del Rio, 2007). To investigate whether ChAT-YFP cells underwent cholinergic modulation, we applied ACh to HC ChAT-YFP cells (**Figure 8**). In the continuous presence of the AMPA receptor antagonist DNQX (25 µM), the NMDA receptor antagonist APV (50 µM) and the GABAA receptor antagonist gabazine (5 µM), we applied 1 s long depolarizing current to monitor the AP firing frequency of ChAT-YFP cells from CA1 SR/LM ChAT-YFP (**Figure 8A**) and CA3 SP ChAT-YFP cells (**Figure 8B**) while introducing bias current to maintain the membrane potential at −60 mV. Bath application of ACh (100 µM) increased AP frequency in CA1 SR/LM ChAT-YFP (100–200 pA step, **Figures 8A,B,I**) and CA3 SP ChAT-YFP (400–700 pA step, **Figures 8C,D,I**) cells. In CA1 SR/LM ChAT-YFP cells, the ACh-induced increase in AP frequency (from 13.2 ± 1.8 to 22.8 ± 2.6 Hz, $p = 0.0007$, $n = 11$, two-tailed paired t-test; **Figure 8I**) was accompanied by the elimination of the ADF (from −3.3 ± 0.4 to −0.7 mV ± 0.7 mV, $p = 0.0029$, Wilcoxon matched pairs-signed rank test; **Figure 8J**) and a modest increase in holding current (by −12.1 ± 6.6 pA, $p < 0.001$, Wilcoxon signed rank test; **Figure 8K**). In CA3 SP ChAT-YFP cells, the ACh-induced increase in AP frequency (from 10.1 ± 0.8 to 12.1 ± 1.4 Hz, $p = 0.020$, $n = 11$, Wilcoxon matched pairs signed rank test) was more modest and underwent a similar increase in holding current (by −13.0 ± 6.2 pA, $p < 0.001$, Wilcoxon signed rank test), but the AHP was converted to an ADP (from −2.1 ± 0.6 to 1.0 ± 0.5 mV, $p = 0.002$, Wilcoxon matched pairs signed rank test). Similar to previous studies in HC principal cells (Cole and Nicoll, 1983; Cobb and Davies, 2004), application of ACh increased AP frequency (from 12.1 ± 0.9 Hz to 26.1 ± 3.7 Hz, $p = 0.0044$, $n = 7$, paired t-test; **Figures 8F,I**), generated an ADP (−3.0 ± 1.4 to 0.6 ± 0.6 mV, $p = 0.0003$, paired t-test; **Figure 8J**), and increased holding current (−42.8 ± 9.0 pA, $p = 0.0032$, one sample t-test; **Figure 8K**) in CA3 pyramidal cells (PCs). However, the extent

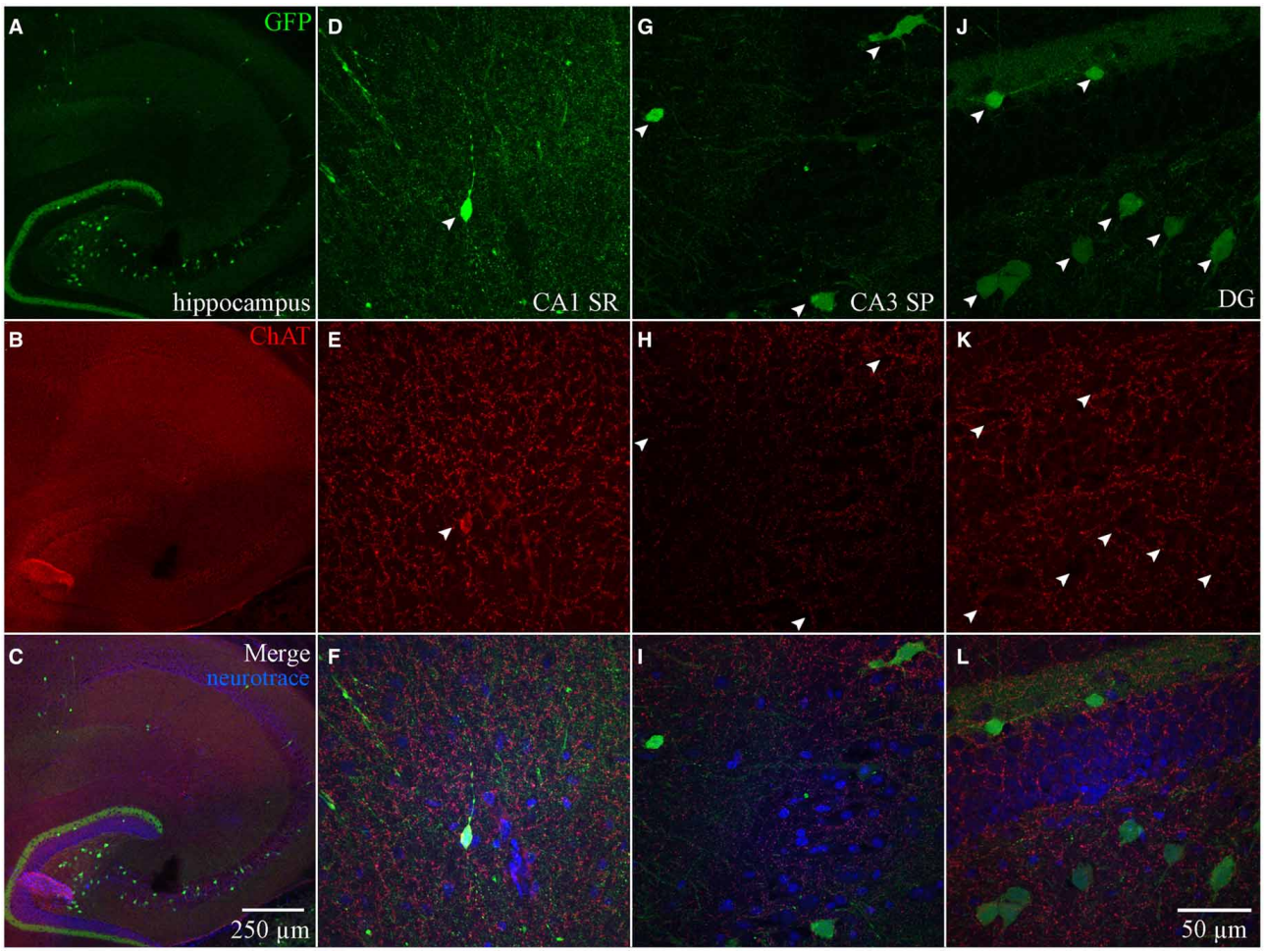

FIGURE 5 | Anti-ChAT labeling in HC ChAT-YFP cells. (A) YFP, **(B)** anti-ChAT, and **(C)** merged images from the HC. Higher magnification images in **(D–F)** CA1 SR, **(G–I)** CA3 SP, and **(J–L)** DG showing that anti-ChAT labeling is not detected in ChAT-YFP cells.

that ACh increased AP frequency was larger in CA1 SR/LM ChAT-YFP and CA3 PCs than CA3 SP ChAT-YFP cells ($p < 0.05$, One-Way ANOVA, **Figure 8L**). Finally, in contrast to HC ChAT-YFP cells, MS-DBB ChAT-YFP cells were not modulated by ACh ($p > 0.05$; **Figures 8D,H–L**). Taken together, ACh resulted in enhanced ChAT-YFP cells in both CA1 and CA3, but to different extents.

OPTOGENETIC STIMULATION OF HC ChAT-CRE NEURONS INDUCES GLUTAMATE RELEASE ONTO CA1 INTERNEURONS

To investigate the neurotransmitter phenotype of HC ChAT-CRE cells, we employed GAD65-GFP/ChAT-CRE mice (see Material and Methods), which enabled optogenetic stimulation of ChAT-CRE cells onto CA1 GAD65-GFP interneurons (López-Bendito et al., 2004; Cea-del Rio et al., 2010; Wierenga et al., 2010). Four weeks after the injection of floxed ChR2-mCherry AAV into HC of GAD65-GFP/ChAT-CRE mice, we performed whole cell recording on GAD65-GFP cells of CA1 SR/SLM (**Figures 9A–C**). In response to 470 nm light flashes (1–5 ms), EPSCs were evoked in 7/17 of recorded CA1 SR/SLM GAD65-GFP cells. Perfusion of AMPA and NMDA receptor blockers DNQX and APV fully blocked the light pulses induced EPSCs in 6/7 cells (**Figures 9D,E**). Consistent with monosynaptic stimulation, the onset of the glutamate EPSC occurred ∼2 ms after onset of light stimulation. Post-hoc confocal analysis revealed colocalization points of ChR2-mCherry-positive presynaptic terminals with the somatodendritc region of recorded GAD65-GFP cells (**Figures 9H,I**), consistent with direct synaptic input from ChAT-CRE cells. In 1/7 cells, optogenetic stimulation elicited an inward polysynaptic current that was resistant to block by DNQX, APV, and mAChR antagonist atropine. Overall, these data indicate that optogenetic stimulation of intrinsic HC ChAT-CRE neurons results in glutamatergic excitation.

DISCUSSION

NEUROCHEMICAL IDENTITY AND CELLULAR DISTRIBUTION OF HC NEURONS VISUALIZED IN ChAT-tauGFP AND ChAT-ROSA MICE

HC cholinergic interneurons were described almost 30 years ago (Frotscher et al., 1986), and, in modern HC interneuron classification schemes, are recognized as one of over 21 distinct HC interneuron subtypes (Klausberger and Somogyi, 2008).

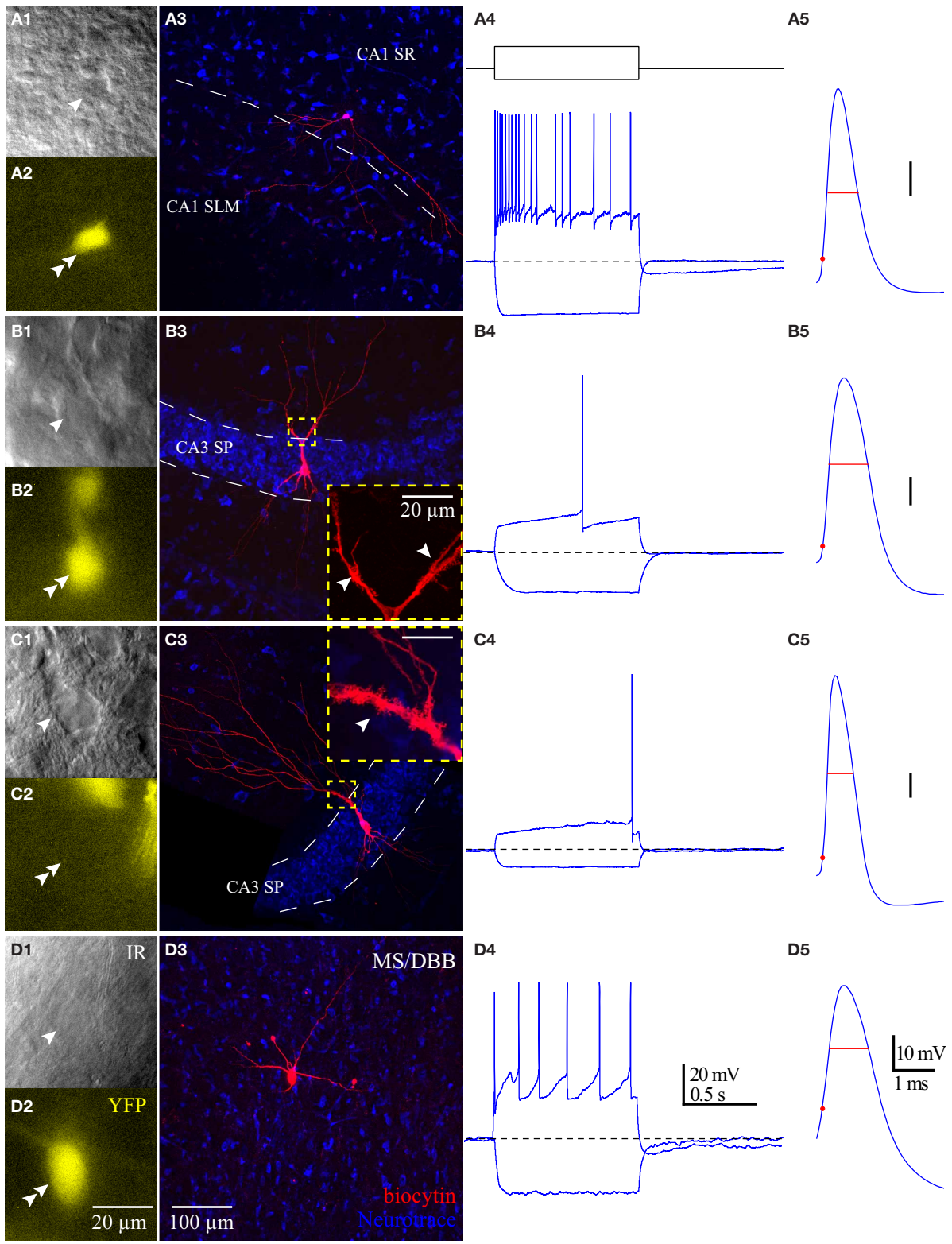

FIGURE 6 | Properties of HC ChAT-YFP cells. Morphology and intrinsic membrane properties of representative **(A)** CA1 SR/LM ChAT-YFP, **(B)** CA3 SP ChAT-YFP, **(C)** CA3 SP YFP-negative, and **(D)** MS/DBB ChAT-YFP cells. For each cell type, representative (1) live IR Dodt contrast images, (2) 505 nm fluorescent images, (3) flat-projected confocal image of the biocytin-filled cell, (4) voltage responses to 1 s long hyperpolarizing (−100 pA) or depolarizing (+200 pA) current steps, and (5) first AP half-width are displayed. Insets in panels **(B3,C3)**: thorny excrescences (white arrows) on the dendrites of **(B3)** CA3 SP ChAT-YFP and **(C3)** CA3 SP YFP-negative cells.

Table 3 | Properties of hippocampal ChAT-YFP neurons.

Property	CA1 ChAT-YFP	CA3 ChAT-YFP	CA3 PC	MS-DBB YFP
R_{in} (MΩ)	236.2 ± 13.8†§*	143.8 ± 12.9§	125.3 ± 11.3	369.8 ± 60.0*
τ_m (ms)	25.3 ± 1.8	38.3 ± 3.2	30.3 ± 2.3	25.4 ± 3.2
C_m (pF)	111.6 ± 7.6†*	267.0 ± 16.9§	243.2 ± 21.6	72.2 ± 4.8*
V_m (mV)	−57.9 ± 3.3	−67.9 ± 2.5	−75.0 ± 1.4	−46.9 ± 5.5
AP half width (μs)	969.6 ± 47.8*	1021.7 ± 38.0*	725.8 ± 26.5	1081.0 ± 42.0*
Sag (SS/peak)	0.95 ± 0.01	0.99 ± 0.01	1.00 ± 0.02	0.98 ± 0.01
Spontaneous firing	5/11	1/12	0/7	5/6
AP frequency (at 100 pA)	10.5 ± 1.9	0	0	4.3 ± 1.1

†*Compared to CA3 SP ChAT-YFP cells; $p < 0.05$.* §*Compared to MSDBB ChAT-YFP cells; $p < 0.05$.* **Compared to CA3 SP YFP-negative cells; $p < 0.05$.*

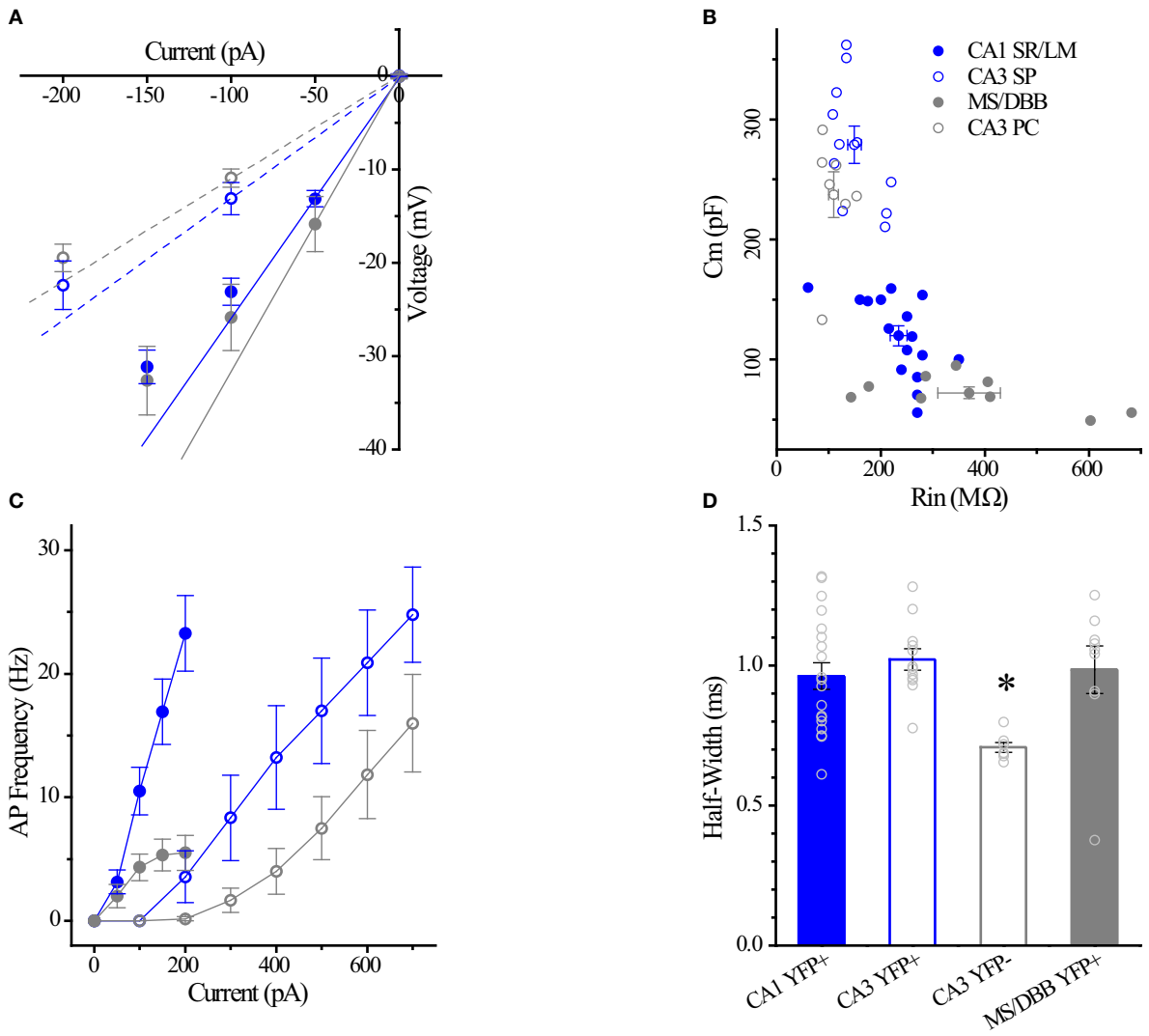

FIGURE 7 | Differences between CA1 and CA3 ChAT-YFP cells. (A) I–V relationship for CA1 ChAT-YFP ($n = 14$, blue filled circle), CA3 ChAT-YFP ($n = 9$, blue open circle), CA3 YFP-negative ($n = 7$, gray open circle), and MSDBB ChAT-YFP ($n = 6$, gray filled circle) cells. Lines denote slopes through 0 pA and (solid) −50 pA or (dotted) −100 pA points illustrating differences in R_{in}. **(B)** Plot of C_m vs. R_{in} for CA1 ChAT-YFP ($n = 20$), CA3 ChAT-YFP ($n = 12$), CA3 YFP-negative ($n = 7$), and MSDBB ChAT-YFP ($n = 9$) cells. **(C)** AP frequency vs. current step for the 4 cell types. **(D)** Bar graph showing narrower half width of CA3 YFP-negative cells than ChAT-YFP cells in CA1 SR/LM, CA3 SP, and MS/DBB (One-Way ANOVA). Asterisk (*) denotes $p < 0.05$ compared to CHAT-YFP cells in CA1, CA3 and MS-DBB.

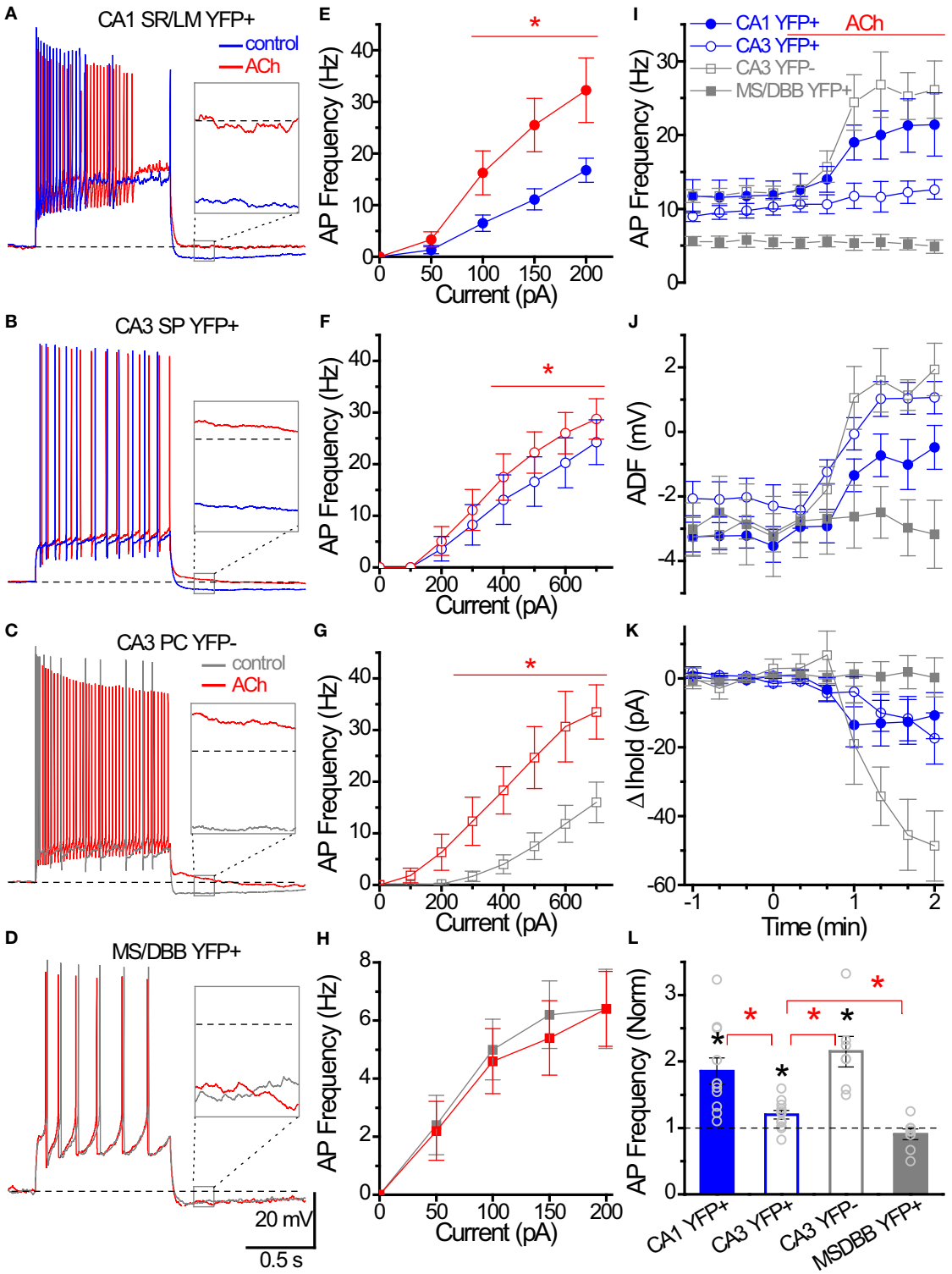

FIGURE 8 | Cholinergic modulation of Hippocampal ChAT-YFP cells. (A–D) Whole-cell voltage responses to the introduction of a 1 s long depolarizing current step from −60 mV in representative cells in (blue/gray) control and (red) 100 μM ACh. Inset, solid box expanded to illustrate the effect of ACh on afterdeflection (ADF) (dotted line indicates −60 mV baseline). **(E–H)** Input-output relationships from AP frequency vs. current step magnitude for CA1 YFP+ (E, close circle $n = 8$), and CA3 YFP+ (F, open circle, $n = 9$), CA3 YFP− (G, open square, $n = 6$) and MSDBB YFP+ (H, close square, $n = 5$) cells. *Denotes $p < 0.05$, Two-Way ANOVA. **(I–K)** Population data for the four group cells summarizing the time course of **(I)** AP frequency, **(J)** afterdeflection, **(K)** relative change in I_{hold} from (blue) control to (red; at time 0) ACh conditions. **(L)** Bar graph showing cholinergic modulation induced changed in normalized AP frequency. Black asterisks denote $p < 0.05$ compared to 1, one sample *t*-test. Red asterisks denote $p < 0.05$ between groups, One-Way ANOVA.

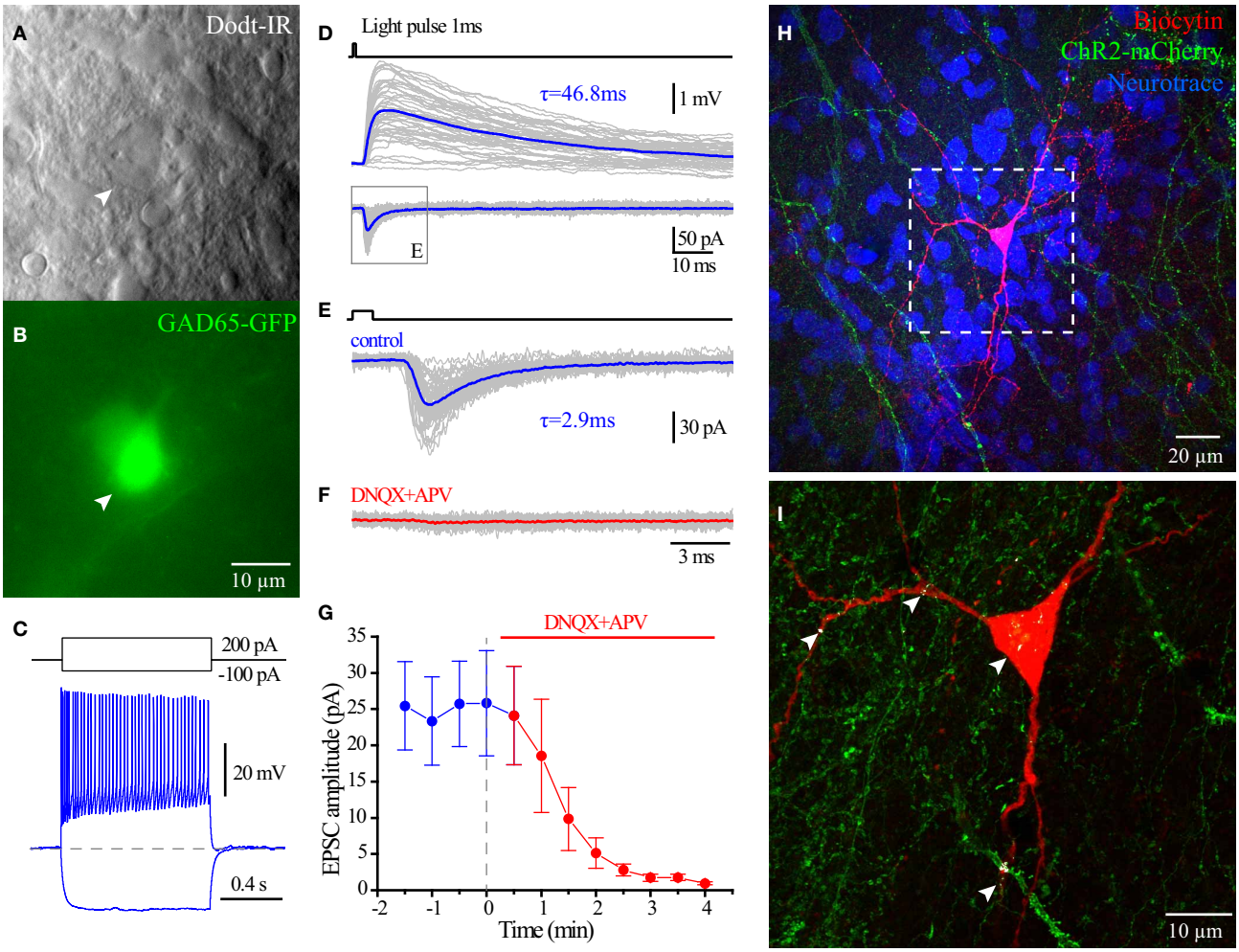

FIGURE 9 | Glutamate release from hippocampal ChAT-CRE cells. Live IR Dodt contrast image **(A)** and green fluorescent image **(B)** of a recorded GFP+ cell in hippocampal CA1 SR/LM layer. **(C)** Voltage responses to 1 s long hyperpolarizing (−100 pA) or depolarizing (200 pA) current steps. **(D)** Averaged light flashes (1 ms, 470 nm) induced EPSCs in acsf (blue) and DNQX+APV (red). **(E)** Population date showing the light induced EPSCs were blocked by ionotropic glutamate receptors blockers (Wilcoxon matched-pairs signed rank test, $n = 6$). **(F)** Flat-projection of a confocal image for the biocytin filled (red) cell with mCherry (green) and Neurotrace (blue). **(G)** White regions showing the co-localization of mCherry (ChAT-CRE-positive synaptic terminals) and biocytin (recorded GAD65-GFP cell).

In a previous study, ChAT-expressing neurons were shown to lack GAD65/67 mRNA (Frotscher et al., 2000). However, in cortex, ChAT-EGFP bipolar neurons immunoreactive for ChAT co-localize strongly with VIP and calretinin (von Engelhardt et al., 2007), which could overlap with GABAergic interneuron subtypes (Chamberland et al., 2010; Chamberland and Topolnik, 2012; Tyan et al., 2014). By contrast, cortical bipolar ChAT-EGFP cells lack GAD67 mRNA (von Engelhardt et al., 2007). Moreover, VIP/calretinin-positive interneurons tended to exhibit an irregular spiking, or stuttering phenotype (Porter et al., 1999; von Engelhardt et al., 2007).

In the present study, we investigated HC neurons that expressed fluorescent proteins under the control of the ChAT promoter (Gong et al., 2007; Grybko et al., 2011), enabling HC ChAT-positive cells to be revealed through transgenic mouse technology. In the initial examination of the HC of ChAT-tauGFP mice, GFP-containing fibers were found to densely innervate all layers of the hippocampus, most of which presumably arose from MS-DBB cholinergic projection neurons (Dutar et al., 1995). Although our use of Neurotrace was originally intended only to define HC layers, upon higher magnification, we noted that some tauGFP-positive structures that were originally counted as cells were actually negative for Neurotrace (**Figure S3**). We then used both Neurotrace and DAPI as cellular markers to unambiguously identify HC neurons in the ChAT-tauGFP population (**Figure 1**; **Table 1**). Consistent with HC ChAT-immunoreactive interneurons in rat (Frotscher et al., 1986, 2000) and ChAT-EGFP neurons in mice (von Engelhardt and colleagues, unpublished observations), we revealed that 59.4% of ChAT-tauGFP structures were labeled with both Neurotrace and DAPI (**Figure 3A**), particularly in CA1 and CA3 SR/SLM regions (**Figure 3C**). Interestingly, 40.6% of ChAT-tauGFP structures, which were

comparable in diameter to the neuronal ChAT-tauGFP population (**Figures 3A,B**), lacked Neurotrace and DAPI (**Figure 3A**). These ChAT-tauGFP structures tended to be found in CA1 and CA3 stratum oriens, which may be a reflection of the density of MS-DBB cholinergic afferents in HC layers. Axon blebs, which can approach the size of ChAT-tauGFP globular structures measured here, are observed only after the axon is severed (Shu et al., 2006; Hu and Shu, 2012). However, the ChAT-tauGFP axon appears to be intact (**Figure 1K**, inset), resembling large, en passant boutons. These globular structures could result from the overexpression of tauGFP in cholinergic axons, and are therefore specific to ChAT-tauGFP mice. Supporting this hypothesis, cell sized ChAT-YFP-positive structures were invariably Neurotrace-positive neurons in ChAT-Rosa mice. In any case, the large diameter of many of these boutons provides a future opportunity to access cholinergic axons electrophysiologically and understand their intrinsic membrane properties, firing activity, and presynaptic modulatory capacity.

The GM24 line of ChAT-CRE mice generally has been used in combination with a ChR2 AAV or with a reporter line to optically stimulate (Witten et al., 2010) and/or visualize cholinergic neurons (Ivanova et al., 2010; Lopes et al., 2012) in various brain regions. However, no study has yet examined YFP-positive cells in the hippocampus of ChAT-Rosa mice. In examining the regional and laminar distribution of ChAT-YFP cells (**Figure 2**), there was some similarity to the original description of ChAT cells in the HC (Frotscher et al., 1986), especially in the location of small cells in the CA1 SR/SLM layer (**Figures 2A,G**). A majority (15/19) of CA1 SR/SLM neurons possessed a stuttering/irregular firing phenotype (**Figure 6**), which is reminiscent of the phenotypes of cortical ChAT-EGFP (von Engelhardt et al., 2007), cortical VIP/calretinin/ChAT (Porter et al., 1998, 1999), and HC VIP/calretinin (Tyan et al., 2014) interneuron subtypes.

In addition to similarities, we noted several differences when comparing the cellular distribution and neurochemical identity of ChAT-YFP cells with previous studies, as well as with the ChAT-tauGFP population also described here. Despite the strong co-localization of ChAT immunoreactivity in MS-DBB ChAT-YFP cells processed in parallel, ChAT immunoreactivity was rarely detected in HC ChAT-YFP cells. There are a number of possibilities that could explain this discrepancy. First, the discrepancy between YFP and anti-ChAT signals could be due to the ectopic expression of CRE in non-ChAT cells (Gong et al., 2007). Second, ChAT immunoreactivity is weaker in the hippocampus (Frotscher et al., 1986) and cortex (von Engelhardt et al., 2007) than in basal forebrain. Driving EGFP or EYFP expression under the control of the ChAT promoter may amplify the detection sensitivity of ChAT-expressing cells above the detection sensitivity of the anti-ChAT antibody. Third, access of the anti-ChAT antibody to the epitope binding site on ChAT may differ depending on the region and/or cell type. Fourth, CRE/loxP recombination may temporally dissociate YFP expression from ChAT expression. Unlike ChAT-EGFP (von Engelhardt et al., 2007) and ChAT-tauGFP (Grybko et al., 2011) mice, where ChAT and GFP expression are expected to be temporally correlated (Erickson et al., 2014), YFP expression in a ChAT-YFP neuron may only indicate that ChAT was transiently expressed during development of the ChAT-YFP neuron. Therefore, YFP may be expressed even if ChAT expression was strongly down-regulated during development.

In a recent study, fetal HC neurons immunopositive for vesicular glutamate transporter 1 (vGluT1) were observed to co-localize with ChAT (Bhargava et al., 2010), consistent with previous observations demonstrating glutamate and ACh co-release in some types of cholinergic neurons (Allen et al., 2006; Ren et al., 2011). However, ChAT immunoreactivity was absent from all but a small subpopulation of vGluT1-positive neonatal cells grown for 13 days in culture (Bhargava et al., 2010). Consistent with the idea that ChAT expression is downregulated in glutamatergic neurons, we found that a subset of CA3 SP neurons express ChAT-YFP (**Figures 2A,F**) but do not exhibit ChAT immunoreactivity (**Figures 5A–C, G–I**). Moreover, CA3 SP neurons were not well represented in ChAT-tauGFP mice (**Figure 3C**). However, optogenetic stimulation induced glutamatergic EPSCs in CA1 HC of ChAT-CRE mice (**Figures 9D–G**), indicating that CRE expression must persist in adult ChAT-CRE mice. ChR2-mCherry-expressing synaptic terminals were observed in CA1 (**Figures 9H,I**), most likely originating from CA3 SP ChAT-CRE cells. Although ChAT-YFP cells in the CA3 area that resemble pyramidal cells are distinct from non-fluorescent pyramidal cells (**Figure 7D**), these observations are consistent with conventional glutamatergic transmission from CA3 ChAT-CRE cells. CA3 ChAT-CRE cells most likely misexpress CRE-recombinase because they have no equivalent in the ChAT-tau GFP mice and exhibit no detectable ChAT immunoreactivity. In hilar ChAT-YFP cells, the presence of thorny excrescences (**Figure S5D**) and the diffuse YFP labeling in the DG inner molecular layer (**Figure S5A**) suggest that at least some of ChAT-YFP cells in hilus are mossy cells (Scharfman and Myers, 2012). Given the above observations, it will be of interest to examine other ChAT-CRE mouse lines for the presence of these cell populations.

Sparse ChAT immunoreactivity and some overlap with calretinin/VIP was observed in some CA1 cells, consistent with a population of Neurotrace-positive CA1 SR/SLM interneurons in ChAT-tauGFP mice (**Figure 3C**). These observations are consistent with the retention of ChAT, albeit at a lower level than in MS-DBB cholinergic neurons, in a small subset of the adult CA1 SR/SLM interneuron population (**Figures 4**, **5**). A subset of CA1 VIP/calretinin interneurons are clearly GABAergic (Chamberland et al., 2010; Chamberland and Topolnik, 2012; Tyan et al., 2014), consistent with original observations made in cortex (Bayraktar et al., 1997; von Engelhardt et al., 2007). However, in situ hybridization data showed lack of GAD65/67 mRNA expression in rat ChAT immunopositive HC interneurons (Frotscher et al., 2000). We attempted to optogentically stimulate this CA1 interneuron population; however, it is likely that the glutamatergic output from CRE-expressing principal cells in CA3 or DG confounded this experimental design. Although it is possible that glutamate is also released (or co-released) from ChAT-CRE cells in CA1 or CA3 SR/SLM, future experiments that more narrowly focus on optogenetic stimulation of this population would unambiguously reveal neurotransmitter phenotype(s) of this interneuron subclass.

CHOLINERGIC MODULATION OF "CHOLINERGIC INTERNEURONS" DURING HC NETWORK OPERATIONS

ACh release is associated with HC-related behaviors (Pepeu and Giovannini, 2004). In the present study, we found that bath application of ACh enhanced the excitability of both HC CA1 SR/SLM and CA3 ChAT-YFP cells. Although we did not differentiate between nicotinic and muscarinic activation, our results clearly demonstrate that the cellular excitability of ChAT-YFP neurons is influenced by cholinergic neuromodulation. The ACh-induced increase in cellular excitability is most likely due to the activation of mAChRs, as suggested by similarities in mAChR-induced changes in intrinsic membrane properties similar to previous studies (Cole and Nicoll, 1983; Lawrence et al., 2006; Cea-del Rio et al., 2010; Dasari and Gulledge, 2011). However, it is possible that a subset of CA1 SR/SLM ChAT-YFP cells are enriched with a high density of nAChRs, as found in calretinin/VIP-positive cells in cortex (Porter et al., 1999). Indeed, a subset of ChAT-tauGFP axons arising from Neurotrace/DAPI-positive ChAT-tauGFP HC interneurons may contribute to the generation of α7 nAChR-mediated EPSCs in CA3 pyramidal cells (Grybko et al., 2011). Nevertheless, these observations raise the possibility that cholinergic interneurons could be excited by the endogenous release of ACh arising from either extrinsic or intrinsic sources.

What are the potential consequences of cholinergic modulation of ChAT-expressing neurons in the HC? Since ACh release from HC ChAT+ cells has not been definitively demonstrated, it remains an unresolved issue, but given the possible developmental downregulation of ChAT expression (Bhargava et al., 2010), ACh release from HC interneurons is likely to play a more prominent role during development than in the adult. Future studies investigating the expression of ChAT immunoreactivity during development of the HC are needed to clarify this issue. It is also possible that these same HC neurons may regress to an earlier stage of development under pathological conditions, and upregulate their synthesis and release of ACh. Evidence for cholinergic dysfunction occurs in epileptic tissue (Romo-Parra et al., 2003), and the abnormal release of ACh may account in part for such dysfunction. Finally, given the partial overlap with VIP and calretinin (**Figure S5**), we cannot rule out possible roles of ACh release from HC ChAT+ cells in neurovascular and/or neurometabolic coupling (Cauli et al., 2004, 2014).

Taken together, our efforts to investigate HC interneurons through transgenic mouse technology have improved our understanding of this heterogeneous class of neurons. However, many questions remain regarding their neurochemical identity, significance to HC function, and potential interaction with MS-DBB cholinergic networks. Next generation transgenic technology may provide additional tools that can be applied to examine distinct subpopulations of HC cholinergic interneurons.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fnsyn.2015.00004/abstract

Figure S1 | Control experiments for anti-ChAT staining. Primary and secondary antibodies for GFP were added in all the experiments. **(A,B)** MS ChAT cells (red) were not revealed in goat anti-ChAT primary only slices. **(C,D)** MS ChAT cells were not revealed in non-primary incubated slices. **(E,F)** ChAT cells were detected in slices incubated with anti-ChAT primary, secondary and TSA.

Figure S2 | Optogenetic stimulation of HC ChAT-CRE cells. (A) Live ChR2-mCherry (590 nm) fluorescence and **(B)** live Dodt-IR contrast image of a ChAT-CRE cell in hippocampus CA1 SR layer. **(C)** Voltage responses upon introduction of (shift-option) ± 200 pA current steps. **(D)** Delivery of 470 nm flashes (blue, 2 ms duration) at 5 Hz for 5 s induced APs in the recorded ChR2-mCherry+ cell. Inset: overlaid AP waveforms.

Figure S3 | Presence of HC ChAT-tauGFP cells and structures in ChAT-tauGFP mice. (A) Flat-projected confocal image displaying (green) ChAT-tauGFP cells in the hippocampus. Cells and layers are counterstained with (blue) Neurotrace 435/455 Blue Fluorescent Nissl Stain. **(B–D)** Magnified views of a ChAT-tauGFP in CA1 SR region. **(E–J)** Large Neurotrace-negative ChAT-tauGFP structures resembling an en passant bouton.

Figure S4 | Detection of ChAT immunoreactivity in cortex and striatum. (A) YFP, **(B)** anti-ChAT, and **(C)** merged images from the cortex. Higher magnification images in **(D–F)**. **(G)** YFP, **(H)** anti-ChAT, and **(I)** merged images from the striatum. Higher magnification images in **(J–L)**. Arrows indicating GFP cells.

Figure S5 | A subset of HC ChAT-YFP cells co-localize with calretinin and VIP. (A) Flat-projected confocal image of the HC from a ChAT-Rosa mouse. Immunoreactivity for ChAT-YFP (green), calretinin (CR, blue), and VIP (red) are shown. **(B–E)** Magnified views of a ChAT-YFP cells in CA1 SR **(B)**, CA3 SP **(C)**, hilus **(D)**, and dentate gyrus **(E)**. Filled arrows denote co-localization of CR and/or VIP with ChAT-YFP cells; the open arrow denotes the thorny excrescences on one ChAT-YFP cell in hilus, suggestive of a mossy cell.

REFERENCES

Allen, T. G. J., Abogadie, F. C., and Brown, D. A. (2006). Simultaneous release of glutamate and acetylcholine from single magnocellular "cholinergic" basal forebrain neurons. *J. Neurosci.* 26, 1588–1595. doi: 10.1523/JNEUROSCI.3979-05.2006

Bayraktar, T., Staiger, J. F., Acsády, L., Cozzari, C., Freund, T. F., and Zilles, K. (1997). Co-localization of vasoactive intestinal polypeptide, gamma-aminobutyric acid and choline acetyltransferase in neocortical interneurons of the adult rat. *Brain Res.* 757, 209–217. doi: 10.1016/S0006-8993(97)00218-7

Bhargava, N., Das, M., Edwards, D., Stancescu, M., Kang, J.-F., and Hickman, J. J. (2010). Coexpression of glutamate vesicular transporter (VGLUT1) and choline acetyltransferase (ChAT) proteins in fetal rat hippocampal neurons in culture. *In Vitro Cell. Dev. Biol. Anim.* 46, 685–692. doi: 10.1007/s11626-010-9324-0

Cauli, B., Tong, X.-K., Rancillac, A., Serluca, N., Lambolez, B., Rossier, J., et al. (2004). Cortical GABA interneurons in neurovascular coupling: relays for subcortical vasoactive pathways. *J. Neurosci.* 24, 8940–8949. doi: 10.1523/JNEUROSCI.3065-04.2004

Cauli, B., Zhou, X., Tricoire, L., Toussay, X., and Staiger, J. F. (2014). Revisiting enigmatic cortical calretinin-expressing interneurons. *Front. Neuroanat.* 8:52. doi: 10.3389/fnana.2014.00052

Cea-del Rio, C. A., Lawrence, J. J., Erdelyi, F., Szabo, G., and McBain, C. J. (2011). Cholinergic modulation amplifies the intrinsic oscillatory properties of CA1 hippocampal cholecystokinin-positive interneurons. *J. Physiol.* 589, 609–627. doi: 10.1113/jphysiol.2010.199422

Cea-del Rio, C. A., Lawrence, J. J., Tricoire, L., Erdelyi, F., Szabo, G., and McBain, C. J. (2010). M3 muscarinic acetylcholine receptor expression confers differential cholinergic modulation to neurochemically distinct hippocampal basket cell subtypes. *J. Neurosci.* 30, 6011–6024. doi: 10.1523/JNEUROSCI.5040-09.2010

Chamberland, S., and Topolnik, L. (2012). Inhibitory control of hippocampal inhibitory neurons. *Front. Neurosci.* 6:165. doi: 10.3389/fnins.2012.00165

Chamberland, S., Salesse, C., Topolnik, D., and Topolnik, L. (2010). Synapse-specific inhibitory control of hippocampal feedback inhibitory circuit. *Front. Cell Neurosci.* 4:130. doi: 10.3389/fncel.2010.00130

Cobb, S. R., and Davies, C. H. (2004). Cholinergic modulation of hippocampal cells and circuits. *J. Physiol.* 562, 81–88. doi: 10.1113/jphysiol.2004.076539

Cole, A. E., and Nicoll, R. A. (1983). Acetylcholine mediates a slow synaptic potential in hippocampal pyramidal cells. *Science* 221, 1299–1301.

Coyle, J. T., Price, D. L., and DeLong, M. R. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* 219, 1184–1190.

Dasari, S., and Gulledge, A. T. (2011). M1 and M4 receptors modulate hippocampal pyramidal neurons. *J. Neurophysiol.* 105, 779–792. doi: 10.1152/jn.00686.2010

Dutar, P., Bassant, M. H., Senut, M. C., and Lamour, Y. (1995). The septohippocampal pathway: structure and function of a central cholinergic system. *Physiol. Rev.* 75, 393–427.

Erickson, C. S., Lee, S. J., Barlow-Anacker, A. J., Druckenbrod, N. R., Epstein, M. L., and Gosain, A. (2014). Appearance of cholinergic myenteric neurons during enteric nervous system development: comparison of different ChAT fluorescent mouse reporter lines. *Neurogastroenterol. Motil.* 26, 874–884. doi: 10.1111/nmo.12343

Freund, T. F., and Buzsáki, G. Y. (1996). Interneurons of the hippocampus. *Hippocampus* 6, 347–470. doi: 10.1002/(SICI)1098-1063(1996)6:4<347::AID-HIPO1>3.0.CO;2-I

Frotscher, M., Schlander, M., and Leranth, C. (1986). Cholinergic neurons in the hippocampus. A combined light- and electron-microscopic immunocytochemical study in the rat. *Cell Tissue Res.* 246, 293–301.

Frotscher, M., Vida, I., and Bender, R. (2000). Evidence for the existence of non-GABAergic, cholinergic interneurons in the rodent hippocampus. *Neuroscience* 96, 27–31. doi: 10.1016/s0306-4522(99)00525-4

Gábriel, R., Wilhelm, M., and Straznicky, C. (1992). Microtubule-associated protein 2 (MAP2)-immunoreactive neurons in the retina of Bufo marinus: colocalisation with tyrosine hydroxylase and serotonin in amacrine cells. *Cell Tissue Res.* 269, 175–182.

Geiger, J. R. P., Bischofberger, J., Vida, I., Fröbe, U., Pfitzinger, S., Weber, H. J., et al. (2002). Patch-clamp recording in brain slices with improved slicer technology. *Pflugers Arch.* 443, 491–501. doi: 10.1007/s00424-001-0735-3

Gong, S., Doughty, M., Harbaugh, C. R., Cummins, A., Hatten, M. E., Heintz, N., et al. (2007). Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J. Neurosci.* 27, 9817–9823. doi: 10.1523/JNEUROSCI.2707-07.2007

Grybko, M. J., Hahm, E.-T., Perrine, W., Parnes, J. A., Chick, W. S., Sharma, G., et al. (2011). A transgenic mouse model reveals fast nicotinic transmission in hippocampal pyramidal neurons. *Eur. J. Neurosci.* 33, 1786–1798. doi: 10.1111/j.1460-9568.2011.07671.x

Hu, W., and Shu, Y. (2012). Axonal bleb recording. *Neurosci. Bull.* 28, 342–350. doi: 10.1007/s12264-012-1247-1

Ivanova, E., Hwang, G. S., and Pan, Z. H. (2010). Characterization of transgenic mouse lines expressing Cre recombinase in the retina. *Neuroscience* 165, 233–243. doi: 10.1016/j.neuroscience.2009.10.021

Klausberger, T., and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* 321, 53–57. doi: 10.1126/science.1149381

Lawrence, J. J., Statland, J. M., Grinspan, Z. M., and McBain, C. J. (2006). Cell type-specific dependence of muscarinic signalling in mouse hippocampal stratum oriens interneurones. *J. Physiol.* 570, 595–610. doi: 10.1113/jphysiol.2005.100875

Lopes, R., Verhey van Wijk, N., Neves, G., and Pachnis, V. (2012). Transcription factor LIM homeobox 7 (Lhx7) maintains subtype identity of cholinergic interneurons in the mammalian striatum. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3119–3124. doi: 10.1073/pnas.1109251109

López-Bendito, G., Sturgess, K., Erdelyi, F., Szabo, G., Molnár, Z., and Paulsen, O. (2004). Preferential origin and layer destination of GAD65-GFP cortical interneurons. *Cereb. Cortex* 14, 1122–1133. doi: 10.1093/cercor/bhh072

Madisen, L., Zwingman, T. A., Sunkin, S. M., Oh, S. W., Zariwala, H. A., Gu, H., et al. (2009). A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat. Neurosci.* 13, 133–140. doi: 10.1038/nn.2467

Micheau, J., and Marighetto, A. (2011). Acetylcholine and memory: a long, complex and chaotic but still living relationship. *Behav. Brain Res.* 221, 424–429. doi: 10.1016/j.bbr.2010.11.052

Pepeu, G., and Giovannini, M. G. (2004). Changes in acetylcholine extracellular levels during cognitive processes. *Learn. Mem.* 11, 21–27. doi: 10.1101/lm.68104

Porter, J. T., Cauli, B., Staiger, J. F., Lambolez, B., Rossier, J., and Audinat, E. (1998). Properties of bipolar VIPergic interneurons and their excitation by pyramidal neurons in the rat neocortex. *Eur. J. Neurosci.* 10, 3617–3628. doi: 10.1046/j.1460-9568.1998.00367.x

Porter, J. T., Cauli, B., Tsuzuki, K., Lambolez, B., Rossier, J., and Audinat, E. (1999). Selective excitation of subtypes of neocortical interneurons by nicotinic receptors. *J. Neurosci.* 19, 5228–5235.

Ren, J., Qin, C., Hu, F., Tan, J., Qiu, L., Zhao, S., et al. (2011). Habenula "cholinergic" neurons co-release glutamate and acetylcholine and activate postsynaptic neurons via distinct transmission modes. *Neuron* 69, 445–452. doi: 10.1016/j.neuron.2010.12.038

Romo-Parra, H., Vivar, C., Maqueda, J., Morales, M. A., and Gutiérrez, R. (2003). Activity-dependent induction of multitransmitter signaling onto pyramidal cells and interneurons of hippocampal area CA3. *J. Neurophysiol.* 89, 3155–3167. doi: 10.1152/jn.00985.2002

Scharfman, H. E., and Myers, C. E. (2012). Hilar mossy cells of the dentate gyrus: a historical perspective. *Front. Neural Circuits* 6:106. doi: 10.3389/fncir.2012.00106

Shu, Y., Hasenstaub, A., Duque, A., Yu, Y., and McCormick, D. A. (2006). Modulation of intracortical synaptic potentials by presynaptic somatic membrane potential. *Nature* 441, 761–765. doi: 10.1038/nature04720

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat. Genet.* 21, 70–71. doi: 10.1038/5007

Srinivas, S., Watanabe, T., Lin, C. S., William, C. M., Tanabe, Y., Jessell, T. M., et al. (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev. Biol.* 1:4. doi: 10.1186/1471-213X-1-4

Teles-Grilo Ruivo, L. M., and Mellor, J. R. (2013). Cholinergic modulation of hippocampal network function. *Front. Synaptic Neurosci.* 5:2. doi: 10.3389/fnsyn.2013.00002

Tesson, L., Heslan, J.-M., Ménoret, S., and Anegon, I. (2002). Rapid and accurate determination of zygosity in transgenic animals by real-time quantitative PCR. *Transgenic Res.* 11, 43–48. doi: 10.1023/A:1013928600442

Tricoire, L., and Cea-Del Rio, C. A. (2007). Illuminating cholinergic microcircuits in the neocortex. *J. Neurosci.* 27, 12119–12120. doi: 10.1523/JNEUROSCI.3856-07.2007

Tyan, L., Chamberland, S., Magnin, E., Camiré, O., Francavilla, R., David, L. S., et al. (2014). Dendritic inhibition provided by interneuron-specific cells controls the firing rate and timing of the hippocampal feedback inhibitory circuitry. *J. Neurosci.* 34, 4534–4547. doi: 10.1523/JNEUROSCI.3813-13.2014

von Engelhardt, J., Eliava, M., Meyer, A. H., Rozov, A., and Monyer, H. (2007). Functional characterization of intrinsic cholinergic interneurons in the cortex. *J. Neurosci.* 27, 5633–5642. doi: 10.1523/JNEUROSCI.4647-06.2007

Wierenga, C. J., Müllner, F. E., Rinke, I., Keck, T., Stein, V., and Bonhoeffer, T. (2010). Molecular and electrophysiological characterization of GFP-expressing

CA1 interneurons in GAD65-GFP mice. *PLoS ONE* 5:e15915. doi: 10.1371/journal.pone.0015915

Witten, I. B., Lin, S.-C., Brodsky, M., Prakash, R., Diester, I., Anikeeva, P., et al. (2010). Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science* 330, 1677–1681. doi: 10.1126/science.1193771

Yi, F., Ball, J., Stoll, K. E., Satpute, V. C., Mitchell, S. M., Pauli, J. L., et al. (2014). Direct excitation of parvalbumin-positive interneurons by M1 muscarinic acetylcholine receptors: roles in cellular excitability, inhibitory transmission and cognition. *J. Physiol.* 592, 3463–3494. doi: 10.1113/jphysiol.2014.275453

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MINI REVIEW
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Distribution and effects of the muscarinic receptor subtypes in the primary visual cortex

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Muscarinic cholinergic receptors modulate the activity and plasticity of the visual cortex. Muscarinic receptors are divided into five subtypes that are not homogeneously distributed throughout the cortical layers and cells types. This distribution results in complex action of the muscarinic receptors in the integration of visual stimuli. Selective activation of the different subtypes can either strengthen or weaken cortical connectivity (e.g., thalamocortical vs. corticocortical), i.e., it can influence the processing of certain stimuli over others. Moreover, muscarinic receptors differentially modulate some functional properties of neurons during experience-dependent activity and cognitive processes and they contribute to the fine-tuning of visual processing. These functions are involved in the mechanisms of attention, maturation and learning in the visual cortex. This minireview describes the anatomo-functional aspects of muscarinic modulation of the primary visual cortex's (V1) microcircuitry.

Keywords: acetylcholine, attention, basal forebrain, cholinergic system, GABAergic interneurons, muscarinic transmission, visual cortex, visual learning

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Introduction

Acetylcholine (ACh) is released in the primary visual cortex (V1) by visual stimulation, especially by novel stimuli (Collier and Mitchell, 1966; Laplante et al., 2005) and attentional demand (Herrero et al., 2008). The cholinergic innervation of the cortex originates from the basal forebrain neurons through topographical projections. Specifically, V1 receives cholinergic projections from the horizontal limb of the diagonal band of Broca (Gaykema et al., 1990; Laplante et al., 2005). In V1, ACh modulates the responses of cortical neurons to visual or cortico-cortical inputs through two receptor families, the metabotropic muscarinic receptors (mAChRs) and the ionotropic nicotinic receptors (nAChRs; Prusky et al., 1987; Volpicelli and Levey, 2004; Disney et al., 2007; Thiele, 2013). These receptors are located on axons originating from thalamic, cortical or basalocortical fibers as well as on pyramidal excitatory neurons and inhibitory GABAergic interneurons (Zilles et al., 1989; Mrzljak et al., 1993; Hashimoto et al., 1994; Thiele, 2013). They are found in each level of the V1 cortical circuitry, i.e., the recipient layer of the thalamic projections, in layer IV neurons and their lateral projections, and throughout the vertical intracortical connections that convey the information to supragranular (I, II/III) and infragranular (V, VI) layers (Burkhalter, 1989; Van Hooser, 2007).

Abbreviations: ACh, acetylcholine; GABA, γ-Aminobutyric acid; KO, knock-out; mAChRs, muscarinic acetylcholine receptors; M1, M2, M3, M4, M5, muscarinic receptor subtypes 1–5; nAChRs, nicotinic acetylcholine receptors; NMDAR, N-Methyl-D-aspartate receptor; V1, primary visual cortex.

The V1 microcircuitry, whose connectivity is organized vertically and horizontally, provides an anatomical substrate for the receptive field—binocularity (Dräger and Olsen, 1980; Grieve, 2005) or ocular dominance (LeVay et al., 1978; Cynader et al., 1987)—and for the selective properties of the neurons—orientation (Grinvald et al., 1986), direction (Shmuel and Grinvald, 1996; DeAngelis et al., 1999) and contrast preference (Levitt and Lund, 1997), for example. Each functional property of the neuron results from the sum and diversity of the connections it receives and might be adapted according to the strength of the inputs received. The strength of the neuronal response further determines the transmission and processing of the stimulus in higher cognitive cortical areas. V1 is thus the first cortical step of the integration of complex visual stimuli. Its modulation by ACh is then important for the selection of specific stimuli from the visual field and the elaboration of fine visual conscious perception.

In this mini review, we discuss how muscarinic transmission plays a key role in neuronal transmission, synaptic strength and the interaction between excitatory and inhibitory neurons. These mechanisms lead to the reinforcement of particular neuronal connections and contribute to the processes of memory, perceptual learning and attention but also to the maturation and the fine-tuning of the visual cortex.

Muscarinic Receptors' Organization in the Primary Visual Cortex

In the neonatal and adult cortices, the five subtypes of mAChRs (M1–M5) are present in both pre- and postsynaptic positions (Wess, 2003; Krnjević, 2004). The terms pre- and postsynaptic are used here to identify the neuronal location of the receptors even though the cholinergic system acts in the cerebral cortex mostly by diffuse transmission rather than synaptic transmission (Umbriaco et al., 1994; Descarries et al., 1997) except in layer V, where the synaptic density on cholinergic terminals is particularly rich (Avendano et al., 1996; Turrini et al., 2001). Depending on the species, the density of each subtype of mAChR differs across the cortical layers (I–VI; Gu, 2003). The species-selective immunocytochemical detection of the different subtypes of mAChRs may, however, vary due to the poor specificity of the antibodies, especially in rodents (Jositsch et al., 2009). Many studies have thus used binding or mRNA expression of the mAChRs to localize them within the cortical microcircuitry. In the rodent's visual cortex, the subtypes M1 and M2 predominate. In humans (and primates), the subtypes M1, M2 and M4 prevail (Flynn et al., 1995).

The M1, M3 and M5 subtypes are mainly post-synaptic and lead to an increase in the intracellular Ca^{2+} concentration by activating phospholipase C (PLC; **Figure 1A**). These receptors are coupled with Gαq/11 G-proteins. In the cerebral cortex, the M1 subtype, the main excitatory mAChR subtype (Levey et al., 1991; Caulfield and Birdsall, 1998; Lucas-Meunier et al., 2003; Wess, 2003; Krnjević, 2004; Thiele, 2013), appears to be present mainly in layers II/III and VI, but it is found in all the cortical layers (Levey et al., 1991; Aubert et al., 1996; Vaucher et al., 2002; Roberts et al., 2005). In rats, M1 mAChRs represent almost 40% of the total mAChRs (Levey et al., 1991), and in the human occipital cortex, they represent nearly 35% (Flynn et al., 1995). This subtype is found essentially on the cell bodies and dendrites of postsynaptic pyramidal cells (Mrzljak et al., 1993; Gu, 2003; Gulledge et al., 2009; **Figure 2A**). However, in the primate's visual cortex, the M1 mAChR seems to be largely expressed on GABAergic interneurons (Disney et al., 2006). M1 is also found on the cortico-cortical fibers, where it plays an inhibitory role by reducing excitatory transmission across horizontal as well as long-range cortico-cortical connections (Amar et al., 2010). The M3 subtype is located on the rat intracortical cell bodies and dendrites at a postsynaptic level, but it is virtually not detected in V1 by immunocytochemistry (Levey et al., 1994). In spite of this, the M3 receptor appears to be involved in several functions of the rodent's V1 (see other sections), and it is expressed in GABAergic interneurons, where it enhances the transmission of γ-Aminobutyric acid (GABA; Amar et al., 2010). The M5 subtype is found on endothelial cells and only small number is found in the rodent's

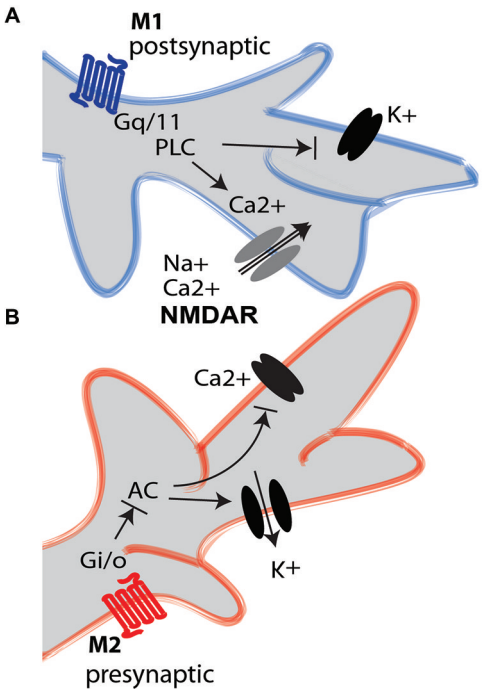
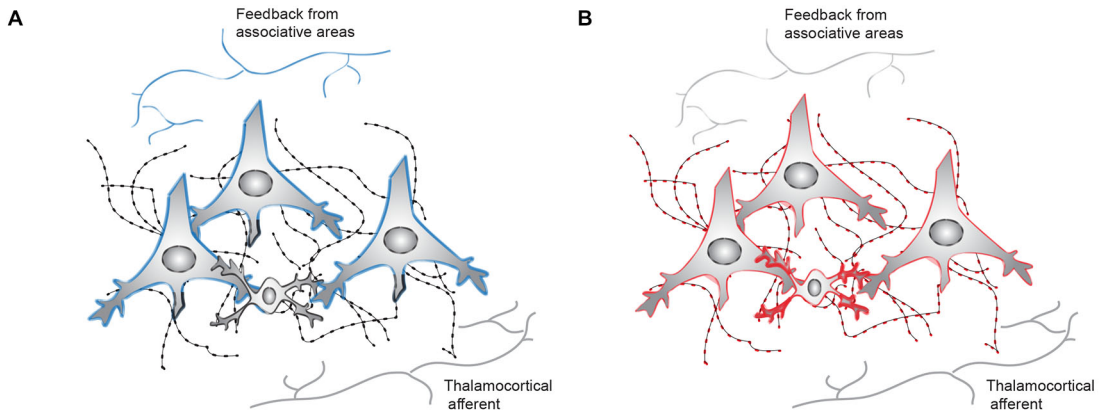


FIGURE 1 | M1 and M2 mAChRs intracellular mediation. (A) The activation of the M1 excitatory mAChR (blue) triggers the $G_{q/11}$ G-protein, which activates phospholipase C (PLC). This induces depolarization of the neuronal element by closing different K^+ channels, including voltage-gated channels and leaky channels, and by activating calcium channels that increase the intracellular concentration of Ca^{2+} from the intracellular stores. The M1 receptor induces long-term potentiation-like effects in glutamatergic neurons through interaction with NMDA receptors (NMDARs). The M1 receptors are mainly postsynaptic, although they are also found on some glutamatergic axon terminals. **(B)** The activation of the M2 inhibitory mAChR (red) triggers the $G_{i/o}$ G-coupled protein, which inhibits adenylate cyclase (AC). This closes the Ca^{2+} voltage-gated channel and opens the K^+ channel to hyperpolarize the neuron. The M2 receptors are mainly presynaptic, although they are also found on some GABAergic interneurons.



M1 mAChR	M2 mAChR
Main excitatory mAChR subtype (Levey and al., 1991; Caulfield and Birdsall, 1998; Lucas-Meunier et al., 2003; Wess, 2003; Krnjevic, 2004; Thiele, 2013)	Cholinergic inhibitory autoreceptor – decreases ACh release (Mrzljak et al., 1993)
Present in all cortical layers (Levey et al., 1991; Aubert et al., 1996)	Present in layers IV and V (Zilles et al., 1989)
Present on cell bodies and dendrites of pyramidal cells (Mrzljak et al., 1993; Gu, 2003; Disney and Aoki, 2008; Gulledge et al., 2009)	Present on cell bodies and terminals of GABAergic neurons (Salgado et al., 2007)
Present on corticocortical fibres (Gil et al., 1997; Amar et al., 2010)	Present on basalocortical fibres (Mrzljak et al., 1993)
Increases the neuronal optimal spatial frequency (Groleau et al., 2014)	Fine tunes neuronal connectivity (Groleau et al., 2014)
Enhances glutamatergic drive except in the layer V (Gulledge et al., 2009; Amar et al., 2010)	Modulates the incortical GABAergic inhibitory drive (Salgado et al., 2007)
Influences contrast sensitivity (Groleau et al., 2014; Bhattacharyya et al., 2012)	Increases cortical sensitivity (Sarter et al., 2005; Salgado et al., 2007)
Involved in the plasticity of the developing visual cortex (Gu and Singer, 1993; Kirkwood et al., 1995)	Involved in the plasticity of the developing visual cortex (Gu and Singer, 1993)
Involved in long-term potentiation and long-term depression (Greuel et al., 1988; Kirkwood et al., 1995, 1999; Gu, 2003; Origlia et al., 2006)	Involved in long-term potentiation (Origlia et al., 2006)

non selective mAChRs modulation
Modulates neuron selective properties (orientation, direction, receptive field) (see text for references)
Facilitates or suppresses intracortical neurons response (Gil et al., 1997)
Suppresses the horizontal spread of excitation in V1 (Kimura et al., 1999) by inhibiting lateral connections
Contributes to attentional mechanisms (Herrero et al., 2008; Klinkenberg and Blokland, 2010)
Influences gamma band activity (Rodriguez et al., 2004; Bartos et al., 2007; Sohal et al., 2009)
Improves visual perception (Kang et al., 2014)
Increases GABAergic neuron activity following basal forebrain stimulation (Dotigny et al, 2008; Alitto and Dan, 2012)

FIGURE 2 | Distribution of the mAChRs on the inhibitory and excitatory cells of the cortical microcircuitry and their associated functions. Integration of the information within V1 is mediated through the vertical and horizontal connections between excitatory neurons (large cells) and inhibitory interneurons (small cells). The cortical connections originating from associative areas are represented on the top, and the thalamocortical afferents are represented on the bottom. The cholinergic fibers and their varicosities (swellings) are represented on the back. **(A)** The M1 receptor (blue) is present on the cell bodies and dendrites of pyramidal cells in V1 as well as on the long range cortical connections from associative areas. **(B)** The M2 mAChR (red) is present on the inhibitory interneurons in V1 and also on cholinergic fibers and some pyramidal cells. The thicker the colored line is, the higher the expression of the receptor is. Principal functions of these specific receptors or of the sum of all mAChRs—demonstrated by non-selective agonists or antagonists—are represented in the left (M1 mAChR, blue), right (M2 mAChR, red) and bottom (undifferentiated action of mAChRs) lines.

(Elhusseiny and Hamel, 2000) and human's visual cortex (Flynn et al., 1995). The M5 subtype has a major function in cortical perfusion.

The M2 and M4 subtypes are found mostly at the presynaptic level, extending the opening of potassium channels by reducing the intracellular concentration of cAMP (**Figure 1B**). They are coupled to $G\alpha_{i/o}$ G-protein, inhibiting adenylyl cyclase (Caulfield and Birdsall, 1998; Wess, 2003). These subtypes appear to have an inhibitory function. Among the presynaptic receptors in the rodent and human visual cortex, the M2 receptor is very abundant and the M4 subtype is less prevalent (Flynn et al., 1995; Zhang et al., 2002). The M2 subtype is mainly found in layer IV (thalamic recipient) and layer V in the rat's V1 (Zilles et al., 1989), but its distribution in the cortical layers, however, varies depending on the species (Gu, 2003). Its expression is up to 36% of the total mAChRs in the primate's V1 (Flynn et al., 1995). At the cholinergic terminals, the M2 subtype is the main inhibitory autoreceptor (Mrzljak et al., 1993; **Figure 2B**) and it decreases the release of ACh, thereby controlling extracellular levels of ACh by negative feedback (Rouse et al., 1999; Douglas et al., 2001; Bymaster et al., 2003). On GABAergic terminals, M2 activation inhibits the

release of GABA (Salgado et al., 2007). Although predominantly presynaptic, M2 and M4 receptors are also present on the cell bodies of GABAergic interneurons in layers II/III and IV (Volpicelli and Levey, 2004)—representing 29% of the GABAergic cells in the primate (Disney and Aoki, 2008)—and on pyramidal cells (Mash and Potter, 1986; Kimura and Baughman, 1997), where its activation inhibits excitatory conductance (Amar et al., 2010).

Muscarinic Influence on Visual Processing in V1

The action of ACh on both pre- and postsynaptic mAChRs results in improved sensory coding of novel and trained visual stimuli (Kang et al., 2014). This change in neuron properties is due to improved neuronal sensitivity resulting from a change in membrane conductance, synaptic strength or connectivity with adjacent neurons and long-range cortical projections. The M1 and M3 subunits seem to have a strong influence on neuronal sensitivity because the optimal spatial frequency of the neuronal population is decreased and the contrast sensitivity is increased in M1/M3-KO mice (Groleau et al., 2014).

ACh has been shown to influence the response of V1 neurons in terms of intensity (Bröcher et al., 1992; Lewandowski et al., 1993; Gil et al., 1997; Kimura et al., 1999; Kirkwood et al., 1999; Kuczewski et al., 2005; Levy et al., 2006; Thiel, 2007; Dotigny et al., 2008; Kang and Vaucher, 2009; Pinto et al., 2013; Soma et al., 2013a,b,c), preferred responses (Murphy and Sillito, 1991; Roberts et al., 2005; Thiel, 2007) and receptive field properties (Herrero et al., 2008; Thiel and Fink, 2008). ACh executes an action by controlling the gain of the neuron response (Soma et al., 2012, 2013a). For example, ACh increases the gain of the visual response to contrast (Bhattacharyya et al., 2013; Soma et al., 2013a) or orientation selectivity (Zinke et al., 2006). These effects might be due to the facilitation of the depolarization of glutamatergic neurons in response to visual input (**Figures 1**, **2**) due to the increased concentration of Ca^{2+} associated with NMDA receptor-gated conductance (Kirkwood et al., 1999) or the reduction of membrane K+ conductance (Thiele, 2013), both potentiated by the muscarinic receptors. The M1 mAChR also amplifies the spiny stellate cell/pyramidal cell response through a postsynaptic intracellular pathway (Gu, 2003), but inhibition through the M4 mAChR has also been observed on spiny neurons in the somatosensory cortex (Eggermann and Feldmeyer, 2009). M2 receptor activation of GABAergic perisomatic terminals (**Figures 1**, **2**) inhibits the release of GABA, causing an increase in the cortical sensitivity of glutamatergic neurons (Sarter and Parikh, 2005; Sarter et al., 2005; Salgado et al., 2007). The M2 subtype, which is largely found on GABAergic cells in rodents, plays a strong role in the modulation of the intracortical GABAergic inhibitory drive.

The amplification of the neuronal response to a certain stimulus could also be due to the depression of the neural response of adjacent neurons that have distinct receptive field and selective properties. By acting on horizontal connections, ACh might thereby modulate the weight of a selective stimulus. In humans, an increase in extracellular ACh levels following the administration of donepezil (an inhibitor of the cholinesterase inhibitor) reduces the horizontal spread of the excitatory response following visual stimulation. This could result from a reduction in the size of the excitatory receptive field by ACh due to the depression of the lateral connectivity (Silver et al., 2008). The reduction of the spread of lateral excitation (Kimura et al., 1999) and neuron depression (Kimura and Baughman, 1997; Soma et al., 2013b) following ACh administration is also shown in rodents. It is, however, possible that the cholinergic system not only inhibits the lateral competition but also strengthens the connectivity for a trained orientation, thereby increasing the number of responding neurons to this trained orientation (Kang et al., 2014). In primates, it has been suggested that the lateral connections between similarly tuned neurons are reinforced by cholinergic stimulation (Ramalingam et al., 2013). Such a change increases the cortical response (Frenkel et al., 2006), enhances the sensitivity of trained visual stimulus (Matthews et al., 1999) and thus facilitates the discrimination from the background (Jehee et al., 2012).

An alternate action of the mAChRs in the increase of the neuron sensitivity of the afferent visual inputs is the increase in the long-term responsiveness of the neuron, leading to an acquired change of its functional property. The action mechanism of ACh strongly resembles long-term potentiation (Gu, 2003; Kang and Vaucher, 2009; Rodriguez et al., 2010; Kang et al., 2014) and heterosynaptic facilitation. When repetitive visual stimulation of sub-optimal orientation is paired with the application of ACh, the responses of neurons become stronger and more long-lasting at the expense of a diminishing response to the previous optimal orientation (Greuel et al., 1988; Kang et al., 2014). Moreover, coupling visual stimulation with cholinergic stimulation induces long-lasting increases in cortical responsiveness and improved visual acuity (Dringenberg et al., 2007; Kang and Vaucher, 2009; Kang et al., 2014) relative to NMDA-dependent mechanisms. The joint action of ACh on both GABAergic and glutamatergic neurons also compromises the excitation-inhibition balance (Amar et al., 2010). This would induce cortical plasticity (Arckens et al., 2000; Hensch and Fagiolini, 2005; Benali et al., 2008; Mainardi et al., 2009; Sale et al., 2010).

Muscarinic Influence on the Development and Maturation of the Visual Cortex

The above muscarinic contribution to the tuning of the receptive field and preferred properties of V1 neurons has a potent role in the maturation and fine-tuning of the visual cortex. The retinotopic organization of V1 is established during embryogenesis, and the properties of the neurons are acquired and refined during the post-natal period with visual experience, especially during the critical period. The critical period is thus an important time in the formation of synapses and pruning (Consonni et al., 2009) and for synaptic plasticity, which strengthens and stabilizes the neural connections.

It has been shown that the cholinergic system is essential during embryogenesis, although the amount of M1, M2 and M3 receptors is very small at the end of the rat prenatal period compared with the adult animal. The cholinergic innervation in V1 is settled at the end of the first postnatal week, and a robust cholinergic staining is visible at P8 (Mechawar and Descarries, 2001). It is similar to the adult cholinergic innervation of the cortex at the end of the second postnatal week (Mechawar and Descarries, 2001). The cholinergic receptors are present in the cortex before the beginning of the critical period, which starts at the end of the third postnatal week (Fagiolini et al., 1994). Between weeks 3 and 5, M1 and M3 levels reach the levels found in the mature animal, while it is not until week 5 that the M2 receptor level reaches that found in the adult (Aubert et al., 1996). Thus, the level of muscarinic expression fits well with the acquisition of the functional properties of the V1 neurons and the establishment of the functional maps. In agreement with a role of the mAChRs in the maturation of the visual cortex rather than development by itself, we recently showed that the gross retinotopic map was virtually unaffected by diverse mAChR subtypes' deletion (Groleau et al., 2014). However, there was altered neuronal connectivity in adult M2/M4-KO mice as visualized using intrinsic signal optical imaging. In these animals, the spatial representation of the visual field was not smooth as it was in control mice, but rather it was stepwise, suggesting a lack of fine-tuning of the retinotopic map. M1/M3 deletion resulted in an alteration of the neurons' sensitivity. Therefore, different mAChRs or combinations thereof can modulate visual properties during the establishment of visual functions (Groleau et al., 2014).

In rodents, a basal forebrain lesion during the critical period transiently affects the ocular dominance of the visual cortical neurons, i.e., the preference response of the neuron to input of one eye over the other. In basal forebrain lesioned animals, an altered ocular dominance toward the contralateral eye is observed. However, at the end of the critical period, a cholinergic deafferentation does not alter ocular dominance (Siciliano et al., 1997). Immunolesion of the cholinergic fibers affects the mRNA expression of the M1 and M2 mAChR subtypes as measured by RT-PCR in young animals (Kuczewski et al., 2005), suggesting the involvement of these mAChR subtypes in the plasticity of the developing visual cortex. At the receptor level, the M1 subtype, but not the M2 subtype, is involved in ocular dominance because its blockade prevents the shift of ocular dominance (Gu and Singer, 1993).

The stabilization of the neuronal connections during maturation happens through synaptic plasticity, i.e., long-term potentiation and long-term depression. In the cortex, long-term potentiation is strongly active during the critical period and experience-dependent plasticity (Crair and Malenka, 1995; Kirkwood et al., 1995). The involvement of mAChRs in critical period plasticity has been demonstrated through *in vitro* electrical stimulation. Long-term depression is dependent on the M1 receptor in layers II/III of the V1 in young rats (3–4 weeks). In adults, long-term depression also depends on the M3 receptor in addition to the M1 subtype (McCoy and McMahon, 2010). When the visual cortex was stimulated through a 100 Hz tetanic stimulation, long-term potentiation was recorded in the cortex of young M1/M3-KO, but not in M2/M4-KO, mice. Conversely, low frequency stimulation produced expected long-term depression in M2/M4-KO mice while long-term potentiation was recorded in M1/M3-KO mice. Thus, it appears that various subtypes of mAChRs regulate distinct forms of long-term synaptic plasticity (Origlia et al., 2006).

Muscarinic Influence on Visual Cognition

In adults, the effect of ACh on neuron sensitivity and the long-lasting enhancement of neuronal responses contribute to the processes of attention and perceptual learning. Indeed, the intensity of the response of V1 cells to a particular stimulus as well as the number of cells responding to the stimulus determine the weight for further processing of this stimulus in higher-level cortical areas, i.e., enhanced or depressed visual processing. In learning and experience-dependent acquisition of new visual abilities, the response selectivities of V1 neurons are changed (Froemke et al., 2007), as are neural connections, with an increased number of synaptic contacts or the formation of new neurons (Majewska and Sur, 2003; Hofer et al., 2009; Yamahachi et al., 2009). The synapse strength of V1 neurons is adjusted by long-term potentiation or depression, which is dependent on N-Methyl-D-aspartate receptor (NMDAR; Quinlan et al., 2004; de Marchena et al., 2008; Kang and Vaucher, 2009) and induces a persistent increase of cortical responsiveness to a particular stimulus. The synchronization of a large number of neurons firing rises to macroscopic oscillations, which change cortical activity.

Oscillation in gamma frequency is suggested to reflect cognitive activity, such as sensory perception (Cardin et al., 2009), attention (Fries, 2009) and learning (Paik and Glaser, 2010; Headley and Weinberger, 2011). Previous studies have demonstrated that cholinergic stimulation could increase gamma band activity (Rodriguez et al., 2004), and this can enhance visual encoding (Goard and Dan, 2009) or contrast sensitivity (Bhattacharyya et al., 2013). Specifically, the muscarinic influence on gamma band activity might be due to its action on GABAergic cells, which are also involved in gamma oscillations (Bartos et al., 2007; Sohal et al., 2009).

A number of studies have shown that lesion or blockade of the cholinergic system with antagonist injection in the primary sensory cortex could significantly reduce attentional task performance (Klinkenberg and Blokland, 2010). Different studies have shown that ACh could increase either pre- or postsynaptic responses via mAChR (Gil et al., 1997; Oldford and Castro-Alamancos, 2003). Such variation enables the cholinergic system to amplify relevant information at the expense of unreliable information, which is consistent with the function of attention (Briggs et al., 2013). A voluntary focus on a stimulus observed in top down attention originates from long range cortico-cortical connections from associative areas and the prefrontal cortex compared with bottom up attention reaching layer IV from

thalamic afferents. Bottom up attention does not seem to be altered by the cholinergic system (Rokem and Silver, 2010), but sustained attention is altered by it. For example, cholinergic-dependent visual attention also results in modulating the size of the cortical receptive field. Focused attention within the receptive field will result in a decrease of its size, whereas attention paid right next to the receptive field will result in an increase in its size (Anton-Erxleben et al., 2009). Scopolamine, a non-specific mAChR antagonist, has been shown to disrupt the attentional mechanism at various levels (Klinkenberg and Blokland, 2010). Similarly, in V1, voluntary visual attention is suppressed by the blockade of mAChR (Herrero et al., 2008).

Compared with attention, which emphasizes the upcoming information, perceptual learning is a long-term process that improves behavioral performance after repetitive training. Recent studies have demonstrated that cholinergic innervation in V1 facilitates perceptual learning in rodents (Kang et al., 2014) and in humans (Rokem and Silver, 2010). Cholinergic activation during a visual task seems to increase the cortical response, resulting in an enhancement of visual capacity. An increase in the cortical response to the trained stimulus suggests an increase in the number of neurons encoding stimulus properties (Frenkel et al., 2006) and the efficiency of the neuronal transmission between neurons (Gilbert and Li, 2012). mAChR-induced long-term modulation could thus change the efficiency of selective neuronal networks for this trained stimulus through the modulation of lateral connectivity and the enhancement of some feed-forward inputs. For example, a visual stimulus with the preferred orientation presented outside of the classic receptive field normally suppresses the neuronal visual response. However, after a perceptual learning task, the neuronal response can be enhanced (Kapadia et al., 2000) by this stimulus. Overall, a long-term increase in cortical neurons' activation could be due to mAChR strengthening the lateral connectivity between similarly tuned neurons, thereby changing the orientation index or the receptive field size.

Conclusion

Muscarinic transmission influences visual processing by facilitating or depressing neuronal responses to specific stimuli and by modulating lateral connections' strength and neuronal synchronization. This effect is primarily mediated through M1 and M2 mAChRs, the predominant muscarinic subtypes in V1, at least in rodents. These effects result in fine-tuning of the neuronal and network properties during maturation, attention and perceptual learning.

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References

Amar, M., Lucas-Meunier, E., Baux, G., and Fossier, P. (2010). Blockade of different muscarinic receptor subtypes changes the equilibrium between excitation and inhibition in rat visual cortex. *Neuroscience* 169, 1610–1620. doi: 10.1016/j.neuroscience.2010.06.019

Anton-Erxleben, K., Stephan, V. M., and Treue, S. (2009). Attention reshapes center-surround receptive field structure in macaque cortical area MT. *Cereb. Cortex* 19, 2466–2478. doi: 10.1093/cercor/bhp002

Arckens, L., Schweigart, G., Qu, Y., Wouters, G., Pow, D. V., Vandesande, F., et al. (2000). Cooperative changes in GABA, glutamate and activity levels: the missing link in cortical plasticity. *Eur. J. Neurosci.* 12, 4222–4232. doi: 10.1046/j.0953-816x.2000.01328.x

Aubert, I., Cécyre, D., Gauthier, S., and Quirion, R. (1996). Comparative ontogenic profile of cholinergic markers, including nicotinic and muscarinic receptors, in the rat brain. *J. Comp. Neurol.* 369, 31–55. doi: 10.1002/(sici)1096-9861(19960520)369:1<31::aid-cne3>3.0.co;2-l

Avendano, C., Umbriaco, D., Dykes, R. W., and Descarries, L. (1996). Acetylcholine innervation of sensory and motor neocortical areas in adult cat: a choline acetyltransferase immunohistochemical study. *J. Chem. Neuroanat.* 11, 113–130. doi: 10.1016/0891-0618(96)00132-9

Bartos, M., Vida, I., and Jonas, P. (2007). Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat. Rev. Neurosci.* 8, 45–56. doi: 10.1038/nrn2044

Benali, A., Weiler, E., Benali, Y., Dinse, H. R., and Eysel, U. T. (2008). Excitation and inhibition jointly regulate cortical reorganization in adult rats. *J. Neurosci.* 28, 12284–12293. doi: 10.1523/jneurosci.1952-08.2008

Bhattacharyya, A., Veit, J., Kretz, R., Bondar, I., and Rainer, G. (2013). Basal forebrain activation controls contrast sensitivity in primary visual cortex. *BMC Neurosci.* 14:55. doi: 10.1186/1471-2202-14-55

Briggs, F., Mangun, G. R., and Usrey, W. M. (2013). Attention enhances synaptic efficacy and the signal-to-noise ratio in neural circuits. *Nature* 499, 476–480. doi: 10.1038/nature12276

Bröcher, S., Artola, A., and Singer, W. (1992). Agonists of cholinergic and noradrenergic receptors facilitate synergistically the induction of long-term potentiation in slices of rat visual cortex. *Brain Res.* 573, 27–36. doi: 10.1016/0006-8993(92)90110-u

Burkhalter, A. (1989). Intrinsic connections of rat primary visual cortex: laminar organization of axonal projections. *J. Comp. Neurol.* 279, 171–186. doi: 10.1002/cne.902790202

Bymaster, F. P., McKinzie, D. L., Felder, C. C., and Wess, J. (2003). Use of M1–M5 muscarinic receptor knockout mice as novel tools to delineate the physiological roles of the muscarinic cholinergic system. *Neurochem. Res.* 28, 437–442. doi: 10.1023/A:1022844517200

Cardin, J. A., Carlén, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., et al. (2009). Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 459, 663–667. doi: 10.1038/nature08002

Caulfield, M. P., and Birdsall, N. J. (1998). International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50, 279–290.

Collier, B., and Mitchell, J. F. (1966). The central release of acetylcholine during stimulation of the visual pathway. *J. Physiol.* 184, 239–254. doi: 10.1113/jphysiol.1966.sp007913

Consonni, S., Leone, S., Becchetti, A., and Amadeo, A. (2009). Developmental and neurochemical features of cholinergic neurons in the murine cerebral cortex. *BMC Neurosci.* 10:18. doi: 10.1186/1471-2202-10-18

Crair, M. C., and Malenka, R. C. (1995). A critical period for long-term potentiation at thalamocortical synapses. *Nature* 375, 325–328. doi: 10.1038/375325a0

Cynader, M. S., Swindale, N. V., and Matsubara, J. A. (1987). Functional topography in cat area 18. *J. Neurosci.* 7, 1401–1413.

DeAngelis, G. C., Ghose, G. M., Ohzawa, I., and Freeman, R. D. (1999). Functional micro-organization of primary visual cortex: receptive field analysis of nearby neurons. *J. Neurosci.* 19, 4046–4064.

de Marchena, J., Roberts, A. C., Middlebrooks, P. G., Valakh, V., Yashiro, K., Wilfley, L. R., et al. (2008). NMDA receptor antagonists reveal age-dependent differences in the properties of visual cortical plasticity. *J. Neurophysiol.* 100, 1936–1948. doi: 10.1152/jn.90290.2008

Descarries, L., Gisiger, V., and Steriade, M. (1997). Diffuse transmission by acetylcholine in the CNS. *Prog. Neurobiol.* 53, 603–625. doi: 10.1016/s0301-0082(97)00050-6

Disney, A. A., and Aoki, C. (2008). Muscarinic acetylcholine receptors in macaque V1 are most frequently expressed by parvalbumin-immunoreactive neurons. *J. Comp. Neurol.* 507, 1748–1762. doi: 10.1002/cne.21616

Disney, A. A., Aoki, C., and Hawken, M. J. (2007). Gain modulation by nicotine in macaque v1. *Neuron* 56, 701–713. doi: 10.1016/j.neuron.2007.09.034

Disney, A. A., Domakonda, K. V., and Aoki, C. (2006). Differential expression of muscarinic acetylcholine receptors across excitatory and inhibitory cells in visual cortical areas V1 and V2 of the macaque monkey. *J. Comp. Neurol.* 499, 49–63. doi: 10.1002/cne.21096

Dotigny, F., Ben Amor, A. Y., Burke, M., and Vaucher, E. (2008). Neuromodulatory role of acetylcholine in visually-induced cortical activation: behavioral and neuroanatomical correlates. *Neuroscience* 154, 1607–1618. doi: 10.1016/j.neuroscience.2008.04.030

Douglas, C. L., Baghdoyan, H. A., and Lydic, R. (2001). M2 muscarinic autoreceptors modulate acetylcholine release in prefrontal cortex of C57BL/6J mouse. *J. Pharmacol. Exp. Ther.* 299, 960–966.

Dräger, U. C., and Olsen, J. F. (1980). Origins of crossed and uncrossed retinal projections in pigmented and albino mice. *J. Comp. Neurol.* 191, 383–412. doi: 10.1002/cne.901910306

Dringenberg, H. C., Hamze, B., Wilson, A., Speechley, W., and Kuo, M. C. (2007). Heterosynaptic facilitation of in vivo thalamocortical long-term potentiation in the adult rat visual cortex by acetylcholine. *Cereb. Cortex* 17, 839–848. doi: 10.1093/cercor/bhk038

Eggermann, E., and Feldmeyer, D. (2009). Cholinergic filtering in the recurrent excitatory microcircuit of cortical layer 4. *Proc. Natl. Acad. Sci. U S A* 106, 11753–11758. doi: 10.1073/pnas.0810062106

Elhusseiny, A., and Hamel, E. (2000). Muscarinic–but not nicotinic–acetylcholine receptors mediate a nitric oxide-dependent dilation in brain cortical arterioles: a possible role for the M5 receptor subtype. *J. Cereb. Blood Flow Metab.* 20, 298–305. doi: 10.1097/00004647-200002000-00011

Fagiolini, M., Pizzorusso, T., Berardi, N., Domenici, L., and Maffei, L. (1994). Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. *Vision Res.* 34, 709–720. doi: 10.1016/0042-6989(94)90210-0

Flynn, D. D., Ferrari-Dileo, G., Mash, D. C., and Levey, A. I. (1995). Differential regulation of molecular subtypes of muscarinic receptors in Alzheimer's disease. *J. Neurochem.* 64, 1888–1891. doi: 10.1046/j.1471-4159.1995.64041888.x

Frenkel, M. Y., Sawtell, N. B., Diogo, A. C., Yoon, B., Neve, R. L., and Bear, M. F. (2006). Instructive effect of visual experience in mouse visual cortex. *Neuron* 51, 339–349. doi: 10.1016/j.neuron.2006.06.026

Fries, P. (2009). Neuronal gamma-band synchronization as a fundamental process in cortical computation. *Annu. Rev. Neurosci.* 32, 209–224. doi: 10.1146/annurev.neuro.051508.135603

Froemke, R. C., Merzenich, M. M., and Schreiner, C. E. (2007). A synaptic memory trace for cortical receptive field plasticity. *Nature* 450, 425–429. doi: 10.1038/nature06289

Gaykema, R. P., Luiten, P. G., Nyakas, C., and Traber, J. (1990). Cortical projection patterns of the medial septum-diagonal band complex. *J. Comp. Neurol.* 293, 103–124. doi: 10.1002/cne.902930109

Gil, Z., Connors, B. W., and Amitai, Y. (1997). Differential regulation of neocortical synapses by neuromodulators and activity. *Neuron* 19, 679–686. doi: 10.1016/s0896-6273(00)80380-3

Gilbert, C. D., and Li, W. (2012). Adult visual cortical plasticity. *Neuron* 75, 250–264. doi: 10.1016/j.neuron.2012.06.030

Goard, M., and Dan, Y. (2009). Basal forebrain activation enhances cortical coding of natural scenes. *Nat. Neurosci.* 12, 1444–1449. doi: 10.1038/nn.2402

Greuel, J. M., Luhmann, H. J., and Singer, W. (1988). Pharmacological induction of use-dependent receptive field modifications in the visual cortex. *Science* 242, 74–77. doi: 10.1126/science.2902687

Grieve, K. L. (2005). Binocular visual responses in cells of the rat dLGN. *J. Physiol.* 566, 119–124. doi: 10.1113/jphysiol.2005.090878

Grinvald, A., Lieke, E., Frostig, R. D., Gilbert, C. D., and Wiesel, T. N. (1986). Functional architecture of cortex revealed by optical imaging of intrinsic signals. *Nature* 324, 361–364. doi: 10.1038/324361a0

Groleau, M., Nguyen, H. N., Vanni, M. P., Huppé-Gourgues, F., Casanova, C., and Vaucher, E. (2014). Impaired functional organization in the visual cortex of muscarinic receptor knock-out mice. *Neuroimage* 98, 233–242. doi: 10.1016/j.neuroimage.2014.05.016

Gu, Q. (2003). Contribution of acetylcholine to visual cortex plasticity. *Neurobiol. Learn. Mem.* 80, 291–301. doi: 10.1016/s1074-7427(03)00073-x

Gu, Q., and Singer, W. (1993). Effects of intracortical infusion of anticholinergic drugs on neuronal plasticity in kitten striate cortex. *Eur. J. Neurosci.* 5, 475–485. doi: 10.1111/j.1460-9568.1993.tb00514.x

Gulledge, A. T., Bucci, D. J., Zhang, S. S., Matsui, M., and Yeh, H. H. (2009). M1 receptors mediate cholinergic modulation of excitability in neocortical pyramidal neurons. *J. Neurosci.* 29, 9888–9902. doi: 10.1523/jneurosci.1366-09.2009

Hashimoto, T., Shu, H., and Kuriyama, K. (1994). Muscarinic M1 receptor mediated inhibition of GABA release from rat cerebral cortex. *Neurochem. Int.* 24, 389–394. doi: 10.1016/0197-0186(94)90117-1

Headley, D. B., and Weinberger, N. M. (2011). Gamma-band activation predicts both associative memory and cortical plasticity. *J. Neurosci.* 31, 12748–12758. doi: 10.1523/jneurosci.2528-11.2011

Hensch, T. K., and Fagiolini, M. (2005). Excitatory-inhibitory balance and critical period plasticity in developing visual cortex. *Prog. Brain Res.* 147, 115–124. doi: 10.1016/s0079-6123(04)47009-5

Herrero, J. L., Roberts, M. J., Delicato, L. S., Gieselmann, M. A., Dayan, P., and Thiele, A. (2008). Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. *Nature* 454, 1110–1114. doi: 10.1038/nature07141

Hofer, S. B., Mrsic-Flogel, T. D., Bonhoeffer, T., and Hübener, M. (2009). Experience leaves a lasting structural trace in cortical circuits. *Nature* 457, 313–317. doi: 10.1038/nature07487

Jehee, J. F., Ling, S., Swisher, J. D., Van Bergen, R. S., and Tong, F. (2012). Perceptual learning selectively refines orientation representations in early visual cortex. *J. Neurosci.* 32, 16747–16753a. doi: 10.1523/jneurosci.6112-11.2012

Jositsch, G., Papadakis, T., Haberberger, R. V., Wolff, M., Wess, J., and Kummer, W. (2009). Suitability of muscarinic acetylcholine receptor antibodies for immunohistochemistry evaluated on tissue sections of receptor gene-deficient mice. *Naunyn Schmiedebergs Arch. Pharmacol.* 379, 389–395. doi: 10.1007/s00210-008-0365-9

Kang, J. I., Huppé-Gourgues, F., and Vaucher, E. (2014). Boosting visual cortex function and plasticity with acetylcholine to enhance visual perception. *Front. Syst. Neurosci.* 8:172. doi: 10.3389/fnsys.2014.00172

Kang, J. I., and Vaucher, E. (2009). Cholinergic pairing with visual activation results in long-term enhancement of visual evoked potentials. *PLoS One* 4:e5995. doi: 10.1371/journal.pone.0005995

Kapadia, M. K., Westheimer, G., and Gilbert, C. D. (2000). Spatial distribution of contextual interactions in primary visual cortex and in visual perception. *J. Neurophysiol.* 84, 2048–2062.

Kimura, F., and Baughman, R. W. (1997). Distinct muscarinic receptor subtypes suppress excitatory and inhibitory synaptic responses in cortical neurons. *J. Neurophysiol.* 77, 709–716.

Kimura, F., Fukuda, M., and Tsumoto, T. (1999). Acetylcholine suppresses the spread of excitation in the visual cortex revealed by optical recording: possible differential effect depending on the source of input. *Eur. J. Neurosci.* 11, 3597–3609. doi: 10.1046/j.1460-9568.1999.00779.x

Kirkwood, A., Lee, H. K., and Bear, M. F. (1995). Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. *Nature* 375, 328–331. doi: 10.1038/375328a0

Kirkwood, A., Rozas, C., Kirkwood, J., Perez, F., and Bear, M. F. (1999). Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. *J. Neurosci.* 19, 1599–1609.

Klinkenberg, I., and Blokland, A. (2010). The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. *Neurosci. Biobehav. Rev.* 34, 1307–1350. doi: 10.1016/j.neubiorev.2010.04.001

Krnjević, K. (2004). Synaptic mechanisms modulated by acetylcholine in cerebral cortex. *Prog. Brain Res.* 145, 81–93. doi: 10.1016/s0079-6123(03)45005-x

Kuczewski, N., Aztiria, E., Leanza, G., and Domenici, L. (2005). Selective cholinergic immunolesioning affects synaptic plasticity in developing visual cortex. *Eur. J. Neurosci.* 21, 1807–1814. doi: 10.1111/j.1460-9568.2005.04014.x

Laplante, F., Morin, Y., Quirion, R., and Vaucher, E. (2005). Acetylcholine release is elicited in the visual cortex, but not in the prefrontal cortex, by patterned visual stimulation: a dual in vivo microdialysis study with functional correlates in the rat brain. *Neuroscience* 132, 501–510. doi: 10.1016/j.neuroscience.2004.11.059

LeVay, S., Stryker, M. P., and Shatz, C. J. (1978). Ocular dominance columns and their development in layer IV of the cat's visual cortex: a quantitative study. *J. Comp. Neurol.* 179, 223–244. doi: 10.1002/cne.901790113

Levey, A. I., Edmunds, S. M., Heilman, C. J., Desmond, T. J., and Frey, K. A. (1994). Localization of muscarinic m3 receptor protein and M3 receptor binding in rat brain. *Neuroscience* 63, 207–221. doi: 10.1016/0306-4522(94)90017-5

Levey, A. I., Kitt, C. A., Simonds, W. F., Price, D. L., and Brann, M. R. (1991). Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J. Neurosci.* 11, 3218–3226.

Levitt, J. B., and Lund, J. S. (1997). Contrast dependence of contextual effects in primate visual cortex. *Nature* 387, 73–76. doi: 10.1038/387073a0

Levy, R. B., Reyes, A. D., and Aoki, C. (2006). Nicotinic and muscarinic reduction of unitary excitatory postsynaptic potentials in sensory cortex; dual intracellular recording in vitro. *J. Neurophysiol.* 95, 2155–2166. doi: 10.1152/jn.00603.2005

Lewandowski, M. H., Müller, C. M., and Singer, W. (1993). Reticular facilitation of cat visual cortical responses is mediated by nicotinic and muscarinic cholinergic mechanisms. *Exp. Brain Res.* 96, 1–7. doi: 10.1007/bf00230433

Lucas-Meunier, E., Fossier, P., Baux, G., and Amar, M. (2003). Cholinergic modulation of the cortical neuronal network. *Pflugers Arch.* 446, 17–29.

Mainardi, M., Landi, S., Berardi, N., Maffei, L., and Pizzorusso, T. (2009). Reduced responsiveness to long-term monocular deprivation of parvalbumin neurons assessed by c-Fos staining in rat visual cortex. *PLoS One* 4:e4342. doi: 10.1371/journal.pone.0004342

Majewska, A., and Sur, M. (2003). Motility of dendritic spines in visual cortex in vivo: changes during the critical period and effects of visual deprivation. *Proc. Natl. Acad. Sci. U S A* 100, 16024–16029. doi: 10.1073/pnas.2636949100

Mash, D. C., and Potter, L. T. (1986). Autoradiographic localization of M1 and M2 muscarine receptors in the rat brain. *Neuroscience* 19, 551–564. doi: 10.1016/0306-4522(86)90280-0

Matthews, N., Liu, Z., Geesaman, B. J., and Qian, N. (1999). Perceptual learning on orientation and direction discrimination. *Vision Res.* 39, 3692–3701. doi: 10.1016/s0042-6989(99)00069-3

McCoy, P. A., and McMahon, L. L. (2010). Sympathetic sprouting in visual cortex stimulated by cholinergic denervation rescues expression of two forms of long-term depression at layer 2/3 synapses. *Neuroscience* 168, 591–604. doi: 10.1016/j.neuroscience.2010.04.027

Mechawar, N., and Descarries, L. (2001). The cholinergic innervation develops early and rapidly in the rat cerebral cortex: a quantitative immunocytochemical study. *Neuroscience* 108, 555–567. doi: 10.1016/s0306-4522(01)00389-x

Mrzljak, L., Levey, A. I., and Goldman-Rakic, P. S. (1993). Association of m1 and m2 muscarinic receptor proteins with asymmetric synapses in the primate cerebral cortex: morphological evidence for cholinergic modulation of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U S A* 90, 5194–5198. doi: 10.1073/pnas.90.11.5194

Murphy, P. C., and Sillito, A. M. (1991). Cholinergic enhancement of direction selectivity in the visual cortex of the cat. *Neuroscience* 40, 13–20. doi: 10.1016/0306-4522(91)90170-s

Oldford, E., and Castro-Alamancos, M. A. (2003). Input-specific effects of acetylcholine on sensory and intracortical evoked responses in the "barrel cortex" in vivo. *Neuroscience* 117, 769–778. doi: 10.1016/s0306-4522(02)00663-2

Origlia, N., Kuczewski, N., Aztiria, E., Gautam, D., Wess, J., and Domenici, L. (2006). Muscarinic acetylcholine receptor knockout mice show distinct synaptic plasticity impairments in the visual cortex. *J. Physiol.* 577, 829–840. doi: 10.1113/jphysiol.2006.117119

Paik, S. B., and Glaser, D. A. (2010). Synaptic plasticity controls sensory responses through frequency-dependent gamma oscillation resonance. *PLoS Comput. Biol.* 6:e1000927. doi: 10.1371/journal.pcbi.1000927

Pinto, L., Goard, M. J., Estandian, D., Xu, M., Kwan, A. C., Lee, S. H., et al. (2013). Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat. Neurosci.* 16, 1857–1863. doi: 10.1038/nn.3552

Prusky, G. T., Shaw, C., and Cynader, M. S. (1987). Nicotine receptors are located on lateral geniculate nucleus terminals in cat visual cortex. *Brain Res.* 412, 131–138. doi: 10.1016/0006-8993(87)91447-8

Quinlan, E. M., Lebel, D., Brosh, I., and Barkai, E. (2004). A molecular mechanism for stabilization of learning-induced synaptic modifications. *Neuron* 41, 185–192. doi: 10.1016/s0896-6273(03)00874-2

Ramalingam, N., McManus, J. N., Li, W., and Gilbert, C. D. (2013). Top-down modulation of lateral interactions in visual cortex. *J. Neurosci.* 33, 1773–1789. doi: 10.1523/jneurosci.3825-12.2013

Roberts, M. J., Zinke, W., Guo, K., Robertson, R., McDonald, J. S., and Thiele, A. (2005). Acetylcholine dynamically controls spatial integration in marmoset primary visual cortex. *J. Neurophysiol.* 93, 2062–2072. doi: 10.1152/jn.00911.2004

Rodriguez, R., Kallenbach, U., Singer, W., and Munk, M. H. (2004). Short- and long-term effects of cholinergic modulation on gamma oscillations and response synchronization in the visual cortex. *J. Neurosci.* 24, 10369–10378. doi: 10.1523/jneurosci.1839-04.2004

Rodriguez, R., Kallenbach, U., Singer, W., and Munk, M. H. (2010). Stabilization of visual responses through cholinergic activation. *Neuroscience* 165, 944–954. doi: 10.1016/j.neuroscience.2009.10.059

Rokem, A., and Silver, M. A. (2010). Cholinergic enhancement augments magnitude and specificity of visual perceptual learning in healthy humans. *Curr. Biol.* 20, 1723–1728. doi: 10.1016/j.cub.2010.08.027

Rouse, S. T., Marino, M. J., Potter, L. T., Conn, P. J., and Levey, A. I. (1999). Muscarinic receptor subtypes involved in hippocampal circuits. *Life Sci.* 64, 501–509. doi: 10.1016/s0024-3205(98)00594-3

Sale, A., Berardi, N., Spolidoro, M., Baroncelli, L., and Maffei, L. (2010). GABAergic inhibition in visual cortical plasticity. *Front. Cell. Neurosci.* 4:10. doi: 10.3389/fncel.2010.00010

Salgado, H., Bellay, T., Nichols, J. A., Bose, M., Martinolich, L., Perrotti, L., et al. (2007). Muscarinic M2 and M1 receptors reduce GABA release by Ca2+ channel modulation through activation of PI3K/Ca2+ -independent and PLC/Ca2+ -dependent PKC. *J. Neurophysiol.* 98, 952–965. doi: 10.1152/jn.00060.2007

Sarter, M., Hasselmo, M. E., Bruno, J. P., and Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res. Brain Res. Rev.* 48, 98–111. doi: 10.1016/j.brainresrev.2004.08.006

Sarter, M., and Parikh, V. (2005). Choline transporters, cholinergic transmission and cognition. *Nat. Rev. Neurosci.* 6, 48–56. doi: 10.1038/nrn1588

Shmuel, A., and Grinvald, A. (1996). Functional organization for direction of motion and its relationship to orientation maps in cat area 18. *J. Neurosci.* 16, 6945–6964.

Siciliano, R., Fontanesi, G., Casamenti, F., Berardi, N., Bagnoli, P., and Domenici, L. (1997). Postnatal development of functional properties of visual cortical cells in rats with excitotoxic lesions of basal forebrain cholinergic neurons. *Vis. Neurosci.* 14, 111–123. doi: 10.1017/s0952523800008816

Silver, M. A., Shenhav, A., and D'esposito, M. (2008). Cholinergic enhancement reduces spatial spread of visual responses in human early visual cortex. *Neuron* 60, 904–914. doi: 10.1016/j.neuron.2008.09.038

Sohal, V. S., Zhang, F., Yizhar, O., and Deisseroth, K. (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* 459, 698–702. doi: 10.1038/nature07991

Soma, S., Shimegi, S., Osaki, H., and Sato, H. (2012). Cholinergic modulation of response gain in the primary visual cortex of the macaque. *J. Neurophysiol.* 107, 283–291. doi: 10.1152/jn.00330.2011

Soma, S., Shimegi, S., Suematsu, N., and Sato, H. (2013a). Cholinergic modulation of response gain in the rat primary visual cortex. *Sci. Rep.* 3:1138. doi: 10.1038/srep01138

Soma, S., Shimegi, S., Suematsu, N., Tamura, H., and Sato, H. (2013b). Modulation-specific and laminar-dependent effects of acetylcholine on visual responses in the rat primary visual cortex. *PLoS One* 8:e68430. doi: 10.1371/journal.pone.0068430

Soma, S., Suematsu, N., and Shimegi, S. (2013c). Cholinesterase inhibitor, donepezil, improves visual contrast detectability in freely behaving rats. *Behav. Brain Res.* 256, 362–367. doi: 10.1016/j.bbr.2013.08.022

Thiel, C. M. (2007). Pharmacological modulation of learning-induced plasticity in human auditory cortex. *Restor. Neurol. Neurosci.* 25, 435–443.

Thiel, C. M., and Fink, G. R. (2008). Effects of the cholinergic agonist nicotine on reorienting of visual spatial attention and top-down attentional control. *Neuroscience* 152, 381–390. doi: 10.1016/j.neuroscience.2007.10.061

Thiele, A. (2013). Muscarinic signaling in the brain. *Annu. Rev. Neurosci.* 36, 271–294. doi: 10.1146/annurev-neuro-062012-170433

Turrini, P., Casu, M. A., Wong, T. P., De Koninck, Y., Ribeiro-Da-Silva, A., and Cuello, A. C. (2001). Cholinergic nerve terminals establish classical synapses in the rat cerebral cortex: synaptic pattern and age-related atrophy. *Neuroscience* 105, 277–285. doi: 10.1016/s0306-4522(01)00172-5

Umbriaco, D., Watkins, K. C., Descarries, L., Cozzari, C., and Hartman, B. K. (1994). Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections. *J. Comp. Neurol.* 348, 351–373. doi: 10.1002/cne.903480304

Van Hooser, S. D. (2007). Similarity and diversity in visual cortex: is there a unifying theory of cortical computation? *Neuroscientist* 13, 639–656. doi: 10.1177/1073858407306597

Vaucher, E., Reymond, I., Najaffe, R., Kar, S., Quirion, R., Miller, M. M., et al. (2002). Estrogen effects on object memory and cholinergic receptors in young and old female mice. *Neurobiol. Aging* 23, 87–95. doi: 10.1016/s0197-4580(01)00250-0

Volpicelli, L. A., and Levey, A. I. (2004). Muscarinic acetylcholine receptor subtypes in cerebral cortex and hippocampus. *Prog. Brain Res.* 145, 59–66. doi: 10.1016/s0079-6123(03)45003-6

Wess, J. (2003). Novel insights into muscarinic acetylcholine receptor function using gene targeting technology. *Trends Pharmacol. Sci.* 24, 414–420. doi: 10.1016/s0165-6147(03)00195-0

Yamahachi, H., Marik, S. A., McManus, J. N., Denk, W., and Gilbert, C. D. (2009). Rapid axonal sprouting and pruning accompany functional reorganization in primary visual cortex. *Neuron* 64, 719–729. doi: 10.1016/j.neuron.2009.11.026

Zhang, W., Basile, A. S., Gomeza, J., Volpicelli, L. A., Levey, A. I., and Wess, J. (2002). Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. *J. Neurosci.* 22, 1709–1717.

Zilles, K., Schröder, H., Schröder, U., Horvath, E., Werner, L., Luiten, P. G., et al. (1989). Distribution of cholinergic receptors in the rat and human neocortex. *EXS* 57, 212–228. doi: 10.1007/978-3-0348-9138-7_22

Zinke, W., Roberts, M. J., Guo, K., McDonald, J. S., Robertson, R., and Thiele, A. (2006). Cholinergic modulation of response properties and orientation tuning of neurons in primary visual cortex of anaesthetized marmoset monkeys. *Eur. J. Neurosci.* 24, 314–328. doi: 10.1111/j.1460-9568.2006.04882.x

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Muscarinic cholinergic receptors modulate inhibitory synaptic rhythms in hippocampus and neocortex

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Activation of muscarinic acetylcholine (ACh) receptors (mAChRs) powerfully affects many neuronal properties as well as numerous cognitive behaviors. Small neuronal circuits constitute an intermediate level of organization between neurons and behaviors, and mAChRs affect interactions among cells that compose these circuits. Circuit activity is often assessed by extracellular recordings of the local field potentials (LFPs), which are analogous to *in vivo* EEGs, generated by coordinated neuronal interactions. Coherent forms of physiologically relevant circuit activity manifest themselves as rhythmic oscillations in the LFPs. Frequencies of rhythmic oscillations that are most closely associated with animal behavior are in the range of 4–80 Hz, which is subdivided into theta (4–14 Hz), beta (15–29 Hz) and gamma (30–80 Hz) bands. Activation of mAChRs triggers rhythmic oscillations in these bands in the hippocampus and neocortex. Inhibitory responses mediated by GABAergic interneurons constitute a prominent feature of these oscillations, and indeed, appear to be their major underlying factor in many cases. An important issue is which interneurons are involved in rhythm generation. Besides affecting cellular and network properties directly, mAChRs can cause the mobilization of endogenous cannabinoids (endocannabinoids, eCBs) that, by acting on the principal cannabinoid receptor of the brain, CB1R, regulate the release of certain neurotransmitters, including GABA. CB1Rs are heavily expressed on only a subset of interneurons and, at lower density, on glutamatergic neurons. *Exogenous* cannabinoids typically disrupt oscillations in the theta (θ) and gamma (γ) ranges, which probably contributes to the behavioral effects of these drugs. It is important to understand how neuronal circuit activity is affected by mAChR-driven eCBs, as this information will provide deeper insight into the actions of ACh itself, as well as into the effects of eCBs and exogenous cannabinoids in animal behavior. After covering some basic aspects of the mAChR system, this review will focus on recent findings concerning the mechanisms and circuitry that generate θ and γ rhythms in hippocampus and neocortex. The ability of optogenetic methods to probe the many roles of ACh in rhythm generation is highlighted.

Keywords: optogenetics, GABA, interneuron, endocannabinoid, opioid, theta, cholecystokinin, parvalbumin

INTRODUCTION

The numerous effects that acetylcholine (ACh) has in the nervous system are mediated by both muscarinic (mAChR) and nicotinic (nAChR) receptors. Initially, attention focused on the mAChRs, following the classical experiments of Otto Loewi that showed that chemical transmission at synapses in the heart was mediated by ACh acting at mAChRs. With the recognition that nAChRs are also present in the brain and are directly relevant to the understanding of, e.g., the addictive potency of nicotine and its importance in schizophrenia, an enormous effort has gone into investigating the nAChRs in the central nervous system, although work continued on the molecular structure and pharmacology of the mAChRs. In addition, electrophysiological studies have provided a wealth of data on their cellular actions, the ion channels that they control, and their downstream biochemical mechanisms. Yet, despite these efforts, there remain important gaps in our knowledge of how the mAChRs affect neuronal circuits. Neuronal oscillations are among the most prominent and readily detected signs of neuronal circuit behavior. Certain oscillations, particularly those in the theta (θ, 4–14 Hz) and gamma (γ, 30–80 Hz) frequency ranges, are widely believed to be essential for the performance of various behavioral and cognitive functions. A general cholinergic agonist, carbachol (CCh), is often used to induce these rhythms which are mediated by mAChRs in hippocampus and neocortex in model experimental systems. However, a full understanding of the cellular and

molecular mechanisms of rhythm generation has not been achieved. GABAergic inhibitory interneurons are key elements in rhythm generation, and mAChRs affect their behavior in many ways.

This review will highlight some new results on the generation of oscillations in hippocampus and neocortex; useful reviews of earlier work (e.g., Lawrence, 2008) have appeared. We will discuss the types of mAChRs, their influence on the some of the main interneuron subtypes and to lesser extent on principal cells. An emerging but still under-investigated theme is the ability of certain mAChRs to stimulate the synthesis and release of endogenous cannabinoids (endocannabinoids, eCBs), the natural ligands for the cannabinoid receptors (CB1Rs) in the brain (see diagram in **Figure 1**). In many regions, certain interneurons are heavily invested with CB1Rs while other interneurons have none; glutamatergic neurons often have far lower densities (up to 30 times lower) of the CB1Rs than do the interneurons. The great majority of CB1Rs are located on or near synaptic nerve terminals where their activation by exogenous cannabinoids and eCBs inhibit transmitter release. There has been little concerted effort to understand the implications of the mAChR-eCB link on complex nervous system activity, however. In view of the potency of mAChRs to mobilize eCBs, and thereby indirectly alter neuronal activity, it will be of great interest to work out the details and functional implications of this association. A key issue that has been difficult to explore with conventional methods concerns endogenously released ACh. The bulk of all experimental work on mAChRs and their physiological effects has been carried out either with gross tissue stimulation delivered by extracellular electrodes or perfusion with pharmacological agents. These methods lack specificity and selectivity of action, and the conclusions they permit are accordingly limited. We will discuss recent experiments in which optogenetic techniques have been used to probe the workings of the ACh system in unprecedented detail.

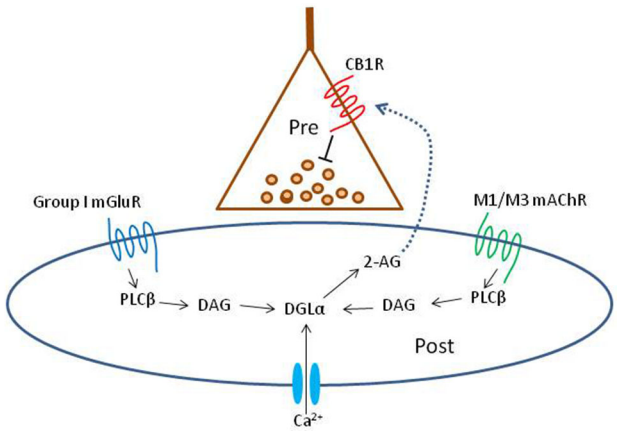

FIGURE 1 | Schematic summary diagram of the endocannabinoid system. A presynaptic nerve terminal is shown synapsing on a postsynaptic cell. Agonist binding of either group I mGluRs or M1 or M3 mAChRs activates phospholipase Cβ (PLCβ) in the postsynaptic cell. The product of the reaction catalyzed by PLCβ, diacylglycerol (DAG), is metabolized by the enzyme diacylglycerol lipase α (DGLα) to form the endocannabinoid, 2-arachidonyl glycerol (2-AG). DGLα can also be activated by Ca^{2+} influx via a PLC-independent mechanism. 2-AG is membrane permeant and gains access through an unknown mechanism to the cannabinoid receptor, CB1R, on the presynaptic nerve terminal. Binding of CB1R by 2-AG inhibits transmitter release, mainly by inhibiting Ca^{2+} influx into the terminal, although other mechanisms can be involved. This is the simplest conventional model of the system and various details are controversial or have been omitted for the sake of simplicity; see a comprehensive review, e.g., Kano et al. (2009), for more information.

CENTRAL CHOLINERGIC PROJECTION NEURONS ASSOCIATED WITH mAChR-INDUCED RHYTHM GENERATION

Almost all of the ACh in the hippocampus and neocortex comes from distal axons of cholinergic projection neurons that are highly concentrated in the basal forebrain (Lewis and Shute, 1967; see Woolf, 1991; van der Zee and Luiten, 1999; for review). This collection of nuclei includes the medial septum (MS) and lateral septum, the horizontal and vertical limb of the diagonal band of Broca (DBB), and the nucleus basalis magnocellularis (NBM). In rodents, cholinergic neurons in the MS/DBB project to olfactory-related structures, cingulate cortex, retrospenial cortex, medial prefrontal cortex, hippocampus and parahippocampus (Gaykema et al., 1989). The cholinergic projection from the MS/DBB to the hippocampus via the fornix/fimbria system is quite large, and selective ablation of the cholinergic cells in the MS/DBB or transection of the fimbria-fornix leads to a virtual loss of ACh fibers in the hippocampus (Lee et al., 1994; Naumann et al., 1994). Cholinergic neurons in the NBM project to the entire cortical mantle, with laminar projection patterns varying with cortical area. NBM also projects to the amygdala and olfactory bulb (see Woolf, 1991; van der Zee and Luiten, 1999 for reviews).

An important anatomical feature of the ACh system in the brain is that cholinergic fibers only rarely (e.g., 3% in hippocampus and neocortex, Yamasaki et al., 2010) make classical morphologically defined synapses onto their target neurons (one-to-one, or "wired transmission", Zoli et al., 1999). Rather, large vesicle-filled varicosities appear along the axons, and ACh is released into the local environment (Vizi and Kiss, 1998), where it diffuses in a paracrine-like way to receptors on target neurons and glia. This has been referred to as "volume conduction" (Zoli et al., 1999) and may be especially relevant for understanding mAChR-mediated effects, as they tend to have slow kinetics themselves and may involve the release of other modulators for which rapid kinetics is also not a key feature. A correlational EM study for the localization of M1 mAChRs and presynaptic synaptic specializations, including the presynaptic active-zone protein, bassoon, along cortical and hippocampal pyramidal cell (PC) dendrites, found that, unlike glutamate terminals and AMPA receptors, there was no close relationship between a cholinergic varicosity (identified by either the choline transporter, CHT1, or choline acetyl transferase, ChAT) near a dendrite and postsynaptic clusters of M1 receptors (Yamasaki et al., 2010). These findings appear to be consistent with the volume transmission mode. Sarter et al. (2009) question the relevance of the distinction between wired and volume transmission, since both can mediate responses with very slow kinetics, but admit that the difference may still be significant in the spatial domain—proximity of the release site

to the receptors—which is the one most relevant to the present discussion.

DISTRIBUTION, ACTION, AND CELLULAR LOCALIZATION OF mAChR SUBTYPES IN HIPPOCAMPUS AND NEOCORTEX

Muscarinic acetylcholine receptors (mAChRs) are G-protein coupled receptors of the Class A, rhodopsin-like family, with ACh being the main endogenous agonist (the ACh precursor, choline, reportedly induces γ activity in an atropine-sensitive way at 2–5 mM, Fischer et al., 2014). mAChRs are widely distributed throughout the central nervous system. The five subtypes, designated M1-5 (Bonner, 1989; Caulfield and Birdsall, 1998) can be divided into two broad groups based on their primary coupling to G-proteins. M1, M3 and M5 receptors (M1-class) are preferentially coupled to $G_{q/11}$ proteins and activate phospholipase C, which initiates the IP3—diacylglycerol (DAG) cascade leading to intracellular Ca^{2+} mobilization, and activation of protein kinase C and mitogen-activated protein kinase (MAPK) pathways. M2 and M4 receptors (M2-class) couple to the pertussis-toxin sensitive $G_{i/o}$ proteins and inhibit adenylyl cyclase activity.

Although widespread in the brain, there is considerable regional variability in the distribution of mAChR subtypes. Throughout the brain, M1 is the most abundant subtype and M5 the least. In the hippocampus and neocortex, M1 is present at high levels; M3 is present at moderate levels (though generally low elsewhere). M4 is very high almost everywhere in the brain, while M2 is found at much lower densities. M5 mRNA is relatively sparse except in hippocampal CA1 PCs and some scattered subcortical nuclei. M1-class receptors are often located on somato-dendritic regions of neurons, and their activation leads to membrane depolarization and increases in cellular excitability by enhancing the mixed-cation Na^+/K^+ current (I_h), Ca^{2+} -dependent, non-selective cation current (e.g., Fisahn et al., 2002), and by inhibiting certain potassium channels, such as Kv7 (M-current), K_{sAHP}, and K ("leak" channels) (e.g., Brown and Adams, 1980; Cole and Nicoll, 1983; Halliwell, 1990; Cobb and Davies, 2005; Lawrence et al., 2006b; Broicher et al., 2008). M2-class receptors frequently reside on presynaptic axonal terminals (although there are exceptions), and agonist binding can activate Kir3 potassium channels and inhibit some voltage-gated Ca^{2+} channels (especially $Ca_{v2.2}$), which in turn hyperpolarizes the neuron or inhibits transmitter release (Hájos et al., 1998; Brown, 2010) (It should be noted that cholinergic axon terminals, including the axons of the MS/DBB fibers, generally express mAChRs, often M2-types, that probably act as presynaptic autoreceptors and regulate ACh outflow; this review will not cover this topic and interested readers can consult, e.g., Vizi and Kiss, 1998; Zoli et al., 1999 for reviews). While the exact cellular location and functional role of each subtype has not been fully elucidated, some correlations between different forms of cholinergic neuromodulation and the neurochemical identities of distinct neuron classes have been established in both hippocampus and neocortex.

mAChRs ON PYRAMIDAL CELLS AND INTERNEURONS IN HIPPOCAMPUS AND NEOCORTEX

In the hippocampal CA1 region, PCs provide excitatory output to other cortical and subcortical areas, and carry information about spatial location and episodic memories (e.g., Eichenbaum, 2013). The functions of PCs are supported by local inhibitory circuits comprising more than 20 types of GABAergic interneurons (Freund and Katona, 2007; Klausberger and Somogyi, 2008; Whittington et al., 2011). The majority of these interneurons are morphologically and neurochemically distinct (Klausberger and Somogyi, 2008), yet their detailed functions have not been worked out. One broad distinction is based on whether the interneurons participate in feedback or feedforward inhibition; another is whether they synapse on dendritic or somatic regions of their target cells. There is some overlap between these classifications, with feedforward inhibition often mediated by dendritic targeting interneurons, and feedback inhibition mediated by perisomatic (i.e., including the soma and proximal dendrites 50–100 μm away) regions. However, there are many exceptions to this generalization (Bartos et al., 2011) and we will focus on the dendritic vs. perisomatic targeting distinction, which seems to be quite general across many brain regions.

Perisomatic targeting interneurons include two non-overlapping classes of basket cells (BCs): the parvalbumin-expressing (PV+), fast-spiking interneurons, and cholecystokinin-expressing (CCK+) regular-spiking interneurons (Freund and Katona, 2007; Bartos and Elgueta, 2012). A third type, the PV+ axo-axonic interneurons (often referred to as "chandelier cells" especially in the neocortex, e.g., Povysheva et al., 2013), innervates only the initial segments of PC axons; we will not discuss axo-axonic cells in detail.

The CCK+ and PV+ BCs differ in a number of fundamental features (Freund and Katona, 2007; Bartos and Elgueta, 2012). In addition to differences in firing patterns—non-accommodating, γ-synchronized action potentials in PV+ BCs; accommodating, poorly γ-synchronized action potentials in CCK+ BCs—one other distinction is that CCK+ BCs express the main receptor for the cannabinoids, CB1R, while PV+ BCs express the mu-opioid (μOR) receptor (Drake and Milner, 2002), which responds to certain opioids. Traditional anatomical and physiological evidence suggests that these two receptor populations have virtually no overlap (recent evidence that suggests this conclusion should be modified will be discussed below). These distinctions between PV+ and CCK+ BCs hold for both hippocampus and cortex, but not everywhere. For example, in the striatum PV+ BCs express CB1Rs (Kano et al., 2009). Additional differences between PV+ and CCK+ BCs can be found in their complement of mAChRs, as discussed below.

A well-studied representative of the dendritic targeting class of interneurons is the somatostatin (SOM)- and mGluR1a-expressing interneuron. Hippocampal interneurons in this class have their somata in the stratum oriens and the great bulk of their axonal arbor in the stratum lacunosum-moleculare, and are referred to as oriens-lacunosum moleculare (O-LM) cells. O-LM cells target the dendrites rather than perisomatic regions of PCs and have different phase preferences for firing within θ rhythm oscillations than do the BCs (or axo-axonic cells). Therefore O-LM cells play distinctive functional roles in regulating both PC excitability and temporal patterning of PC activity (Buzsáki, 2002; Klausberger and Somogyi, 2008; Bartos et al., 2011).

These classes of interneurons are not unique to the CA1 region; most other regions of the hippocampus and the neocortex have similar inhibitory configurations (Lund and Lewis, 1993; Curley and Lewis, 2012). The BCs in the neocortex have essentially the same properties as in the hippocampus, as do the axo-axonic cells (Curley and Lewis, 2012), although it appears the cortical axo-axonic cells have not received as much attention as the hippocampal ones have. In the cortex, the Martinotti cells are also SOM+ /mGluR1α+, and target PC dendrites, and seem generally analogous to the O-LM cells of the hippocampus. Therefore, understanding how mAChR activation regulates these interneurons and consequently the dynamics of hippocampal network activity may be applicable to the neocortex.

mAChRs are widely distributed on the principal cells of the hippocampus and neocortex. PCs in CA1-CA3 and granule cells in dentate gyrus all have abundant postsynaptic expression of M1 receptors, and weaker expression of M3 receptors (Levey et al., 1995). Activation of M1 or M3 usually increases cellular excitability. Therefore, the most dramatic direct effect of either exogenously applied cholinergic agonists or endogenously released ACh on PCs is a pronounced membrane potential depolarization and decrease in membrane conductance (Dodd et al., 1981; Cole and Nicoll, 1983; Pitler and Alger, 1990). This response, together with a decrease of the afterhyperpolarization (AHP; Cole and Nicoll, 1983) and the activation of a persistent, voltage-dependent sodium current (Yamada-Hanff and Bean, 2013), often results in sustained action potential firing (Cobb and Davies, 2005), particularly in hippocampal CA3, where the PCs form a strong recurrent intercollateral network. Neocortical PCs are similarly affected by muscarinic agonists (McCormick and Prince, 1986; Haj-Dahmane and Andrade, 1999).

For the inhibitory interneurons, the major muscarinic response is also depolarization, but with a less prominent associated change in cell input resistance. Generalizations are somewhat difficult to make however, given the diversity of mAChR subtypes, interneurons, and the specific distributions of mAChRs along the cells. For example, M1 is predominantly expressed on PCs but found in very low abundance, if at all, on GAD67-expressing interneurons, including O-LM cells (Yamasaki et al., 2010). These factors make the muscarinic modulation of interneurons much more complicated than that of the PCs. Besides the depolarizing effects of mAChR activation, some interneurons exhibit pure hyperpolarizations or biphasic responses, in which an initial hyperpolarization is followed by a secondary depolarizing phase (McQuiston and Madison, 1999a; Widmer et al., 2006; Bell et al., 2013); the hyperpolarizing responses are attributable to activation of M4 receptors, which activate inwardly rectifying K^+ channels (Bell et al., 2013).

Both CCK+ and PV+ BCs are depolarized by mAChR activation, but some CCK+ Schaffer collateral-associated (SCA) cells also express M2 and M4 receptors to some extent and show biphasic responses when a cholinergic agonist is applied (Cea-del Rio et al., 2010, 2011). CCK+ BCs and SCA cells have strong expression of both M1 and M3 mAChRs, while PV+ BCs and axo-axonic cells express only M1 receptors in their somato-dendritic regions (Cea-del Rio et al., 2010, 2011). Therefore, CCK+ cells are more sensitive to ACh stimulation. More importantly, M3 receptor activation controls the mAChR-mediated increase in firing frequency, and both M1 and M3 mAChR activation is required for the full conversion of the spike AHP into a spike afterdepolarization. mAChR activation increases action potential duration and frequency and reduces spike adaptation in CCK+ cells, as in O-LM cells (Lawrence et al., 2006a), but not in PV+ cells (Cea-del Rio et al., 2010). On the other hand, the outputs of both types of interneurons are also modulated by mAChRs. PV+ cells express M2 receptors on their presynaptic axon terminals; activation of these receptors directly inhibits Ca^{2+} channels and suppresses GABA release (Hájos et al., 1998; Fukudome et al., 2004). ACh and muscarinic agonists also inhibit GABA release from CCK+ cells, but rather than directly activating presynaptic mAChRs, postsynaptic mAChRs on PCs reduce GABA release via an indirect retrograde signaling mechanism, as discussed in the section on eCBs and mAChRs, below.

The O-LM cells express both M1 and M3 receptors (Lawrence et al., 2006a) and generate large depolarizing responses upon mAChR activation (Kawaguchi, 1997; Widmer et al., 2006). Besides direct depolarization by inhibition of M-current, M1 or M3 activation also greatly accelerates action potential firing rate and generates a prominent suprathreshold afterdepolarization in these cells (Lawrence et al., 2006a,b).

ACh GENERATION AND MODULATION OF OSCILLATIONS

IN VIVO OSCILLATIONS IN HIPPOCAMPUS AND NEOCORTEX

Rhythmic fluctuations in cell membrane potentials produce field potential oscillations. Depending on how many cells are synchronously involved, the oscillations can coordinate neuronal activity both locally and across brain regions and are considered to be essential for various cognitive functions. The two most prominent oscillations in the hippocampus are in the θ and γ ranges, which are often concatenated such that γ activity is observed "riding" on a θ carrier wave. ACh can have either a causal or modulatory role in these oscillations, most notably in the θ band, and the mechanisms by which ACh influences them are controversial. It is generally agreed that mAChRs play a more prominent role than nAChRs in rhythm generation.

θ-frequency firing is a basic operational mode of the hippocampus, and is proposed to underlie the formation of episodic memories and spatial maps of the environment (Buzsáki, 2005). θ can be detected in all layers of the CA1 hippocampus, although its amplitude and phase change with depth, with a current source located in s. pyramidale and a current sink near the border of s. radiatum and s.l.m. θ rhythms can modulate plasticity, particularly at the CA3-CA1 Schaffer collateral pathway. For instance, LTP is optimally induced if a train of electrical stimuli coincides with the peak of the θ rhythm, and stimulation given at θ frequency ("theta burst") is optimal for the induction of LTP in CA1 neurons (Larson and Lynch, 1986).

Output from the MS/DBB is necessary for generating hippocampal θ-frequency rhythms *in vivo*, and lesioning the septum abolishes these rhythms and decreases the rate of learning by rats on a spatial maze task (Winson, 1978). However, the mechanisms of θ generation are not homogeneous, and differ depending on the behavior or state of an animal. "Type 1" θ occurs during active, exploratory behavior and is relatively insensitive to the

mAChR antagonist atropine. This does not mean, however, that ACh has no role in Type I θ or associated behaviors. The mAChR antagonist, scopolamine, reduces the positive correlation between hippocampal θ and maze-running speed, and also diminishes the normally sharp spatial tuning of "grid" cells in the entorhinal cortex that provide a coordinate system for spatial navigation and memory formation (Newman et al., 2014). It is not known if scopalamine's effects can be attributed to disruptions of the network oscillations, although this seems likely. In contrast to Type I θ, "Type II" θ occurs under urethane anesthesia and during REM sleep, and is abolished by atropine or selective immunolesioning of the septal cholinergic neurons (Stewart and Fox, 1989). Type II is often referred to as "atropine sensitive" θ. Injection of CCh, physostigmine, or muscarine into the hippocampus of an awake cat elicits θ rhythms in the EEG that can also be blocked by atropine, but not by the broad spectrum nAChR antagonist, mecamylamine (Konopacki and Goebiewski, 1992), again suggesting that nicotinic signaling does not play a major role in ACh associated rhythms. The causal role of ACh in Type II θ is not without controversy; ACh release appears to lag behind Type II θ onset during urethane anesthesia (Zhang et al., 2010). MS/DBB cholinergic neurons fire in a manner that is phase-locked to the hippocampal θ rhythm *in vivo* (Brazhnik and Fox, 1997), although given the slow kinetics of mAChR activation and the bulk or volume transmission that probably characterizes most ACh release, the cholinergic cells are unlikely to be true pacemakers for θ rhythms.

Higher frequency γ oscillations in the hippocampus may act in concert with θ oscillations to encode and retrieve memory traces (Bragin et al., 1995; Csicsvari et al., 2003). γ and θ can occur concurrently, particularly in deeper hippocampal layers, and γ is strongest during periods of θ (Bragin et al., 1995; Buzsáki, 2005). A cross-frequency correlation (CFC) analysis showed that the degree of θ–γ coupling in CA3 *in vivo* increased during a context learning task in rats, and the strength of the coupling was directly correlated with the increase in performance accuracy (Tort et al., 2009). Their interaction provides a mechanism for the temporal ordering of individual episodic events (θ) and the reconstruction of different facets of a memory (γ). The latter, the so-called "binding phenomenon", occurs when disparate cortical areas encoding different facets of a memory, such as the shape, color, and texture of an object, must be activated simultaneously in order to form a coherent representation of the object (Singer and Gray, 1995). However, the hypothesis that neural synchrony through coherent γ oscillation solves the binding problem is controversial. γ rhythms have also been proposed to provide the exact temporal framework for spike-timing-dependent plasticity to occur, as θ oscillations would be too slow for the rapid and precise coordination required (Axmacher et al., 2006).

GABA inhibition is widely agreed to be a major factor in the generation of γ (Whittington and Traub, 2003; Whittington et al., 2011). Nevertheless, the details of the connection between endogenous ACh in hippocampus are less clear for γ than for θ rhythms. Atropine reduces hippocampal γ power in awake, behaving animals (Leung, 1985; Hentschke et al., 2007), and reduces θ–γ coupling (Hentschke et al., 2007). *In vivo*, however, hippocampal γ is abolished by lesioning the entorhinal cortex (Buzsáki, 2002, 2005), suggesting a requirement for glutamatergic, but not cholinergic, inputs in the generation of γ. After this ablation a somewhat slower γ appears in CA3 and CA1, suggesting that, under some conditions, the hippocampus can generate a form of γ without the extrinsic glutamatergic inputs from the entorhinal cortex. One hypothesis is that ACh could trigger the intrinsic γ oscillations: γ can be pharmacologically-induced by CCh in hippocampal slices (Fisahn et al., 1998; Traub et al., 2003). Muscarine-induced γ rhythm in the CA3 region *in vitro* depends on the activation of M1 mAChRs in PCs, and is absent in M1 mAChR$^{-/-}$ mice. This M1-dependent γ is produced by modulation of the mixed-cation Na^+/K^+ current and the Ca^{2+} -dependent non-selective cation current, but does not involve modulation of the M-current (Fisahn et al., 2002).

Although ACh-induced oscillations are prominent in the hippocampus, other brain regions can generate them locally as well, especially the neocortex. Stimulus-evoked γ activity in visual cortex is blocked by intracortical infusion of atropine (Rodriguez et al., 2004), for example. Unlike the MS/DBB cholinergic projection, which drives primarily the lower frequency θ oscillations in the hippocampus, the NBM is believed to underlie cortical "activation", or a decrease in lower frequency synchronized EEG activity accompanied by an increase in local γ frequency. Such a mechanism may underlie, among other things, selective attention (review by Wang, 2010). The discharge rate of NBM cholinergic neurons is much higher during cortical activation, ACh release in the cortex is higher, and lesions of NBM decrease both cortical ACh release and cortical activation (Dringenberg and Vanderwolf, 1998). Despite a lack of direct projections to the hippocampus, activity in the NBM does affect hippocampal activity, and lesions of the NBM can modulate event-related oscillations in the delta (δ, 0.1–3 Hz), θ, β (15–29 Hz), and γ frequency ranges in dorsal hippocampus, as well as in the amygdala and pre-frontal cortex (PFC). Cholinergic neurons in the MS/DBB and NBM could modulate oscillations between and within brain regions, respectively—excitotoxic lesions of MS/DBB decrease γ frequency event-related oscillations in frontal cortex, and reduce phase locking between frontal cortex and hippocampus in the θ band (Sanchez-Alavez et al., 2014). Similar lesions of NBM cause increases in frontal cortex δ and θ, decreases in γ, and reductions in phase-locking between frontal cortex and hippocampus in the γ band. The NBM mediates increases in cortical δ activity during stress in the PFC (a direct target) and retrosplenial cortex (an indirect target; Knox and Berntson, 2008).

IN VITRO MODELS OF ACh-GENERATED OSCILLATIONS IN HIPPOCAMPUS AND NEOCORTEX

Whether ACh plays a causal or modulatory role in rhythm generation *in vivo* is controversial. *In vitro*, cholinergic agonists or released ACh generate rhythmic cell firing, synaptic currents, and local field potentials (LFPs). These effects have been reported most frequently in hippocampal slices, but also occur in neocortical slices. Thus, the brain slice preparation has been an invaluable tool for studying the mechanisms by which ACh can generate oscillations at multiple levels, especially when considering the "inverse problem" of the LFP (Buzsáki et al.,

2012), i.e., the task of inferring microscopic variables (e.g., synaptic or cellular components) from macroscopic data (e.g., a current source density analysis). Solving the "forward problem" i.e., identifying the synaptic or non-synaptic events generating the LFP by correlating them with the LFP, may be a prerequisite for solving the inverse problem.

Application of CCh to hippocampal slices induces θ frequency membrane potential oscillations and firing in a majority of cells in CA1, CA3, and DG (Bland et al., 1988). The mixed ACh agonist CCh induces oscillations in the LFP (Hájos et al., 2004), membrane potentials or firing patterns (Williams and Kauer, 1997), and rhythmic inhibitory post-synaptic responses in CA1 PCs (Reich et al., 2005). It has been proposed that ACh-generated rhythms are initiated in CA3 and transmitted into CA1 via the Schaffer collaterals (Williams and Kauer, 1997; Fisahn et al., 1998; Buzsáki, 2002), although using a novel slicing procedure, Pietersen et al. (2014) report evidence for intrinsic γ generation in CA1. *In vivo* Type II (atropine sensitive) θ might additionally require rhythmic inhibition onto interneurons from septal GABAergic afferents (Stewart and Fox, 1990; Tóth et al., 1997; Buzsáki, 2002). These models cannot explain all of the data however, since θ-frequency rhythmic sIPSP/Cs in CA1 PCs can be induced by CCh application to slices in the presence of iGluR antagonists (**Figure 2C**; Reich et al., 2005; Karson et al., 2008) or in small isolated sections of CA1 (Reich et al., 2005).

Rhythmic inhibition is thought to be essential for the generation of hippocampal oscillations, including ACh-mediated oscillations (Stewart and Fox, 1990; Buzsáki, 2002; Mann and Paulsen, 2007; Klausberger and Somogyi, 2008). Electrical stimulation of cholinergic afferents in hippocampal slices increases the frequency of spontaneous IPSPs in CA1 PCs (Pitler and Alger, 1992b). Stimulation of single hippocampal BCs at θ frequency produces unitary IPSPs in synaptically connected PCs that are sufficient to entrain the PC firing (Cobb et al., 1995). As noted earlier, most interneuron types in CA1 are modulated by ACh (McQuiston and Madison, 1999a,b; Widmer et al., 2006). Hippocampal interneurons are very heterogeneous, and different interneuron classes will fire in a distinct pattern (or not at all) within a given type of oscillation (Klausberger et al., 2003).

The two populations of perisomatic-targeting interneurons, PV+ and CCK+ BCs, are activated by ACh (Karson et al., 2009; Cea-del Rio et al., 2010), and have been functionally implicated in fast and slow oscillations, respectively, generated by cholinergic agonists (Reich et al., 2005; Gulyás et al., 2010). Fast, iGluR-mediated excitatory stimulation of PV+ cells produces IPSPs that contribute to atropine-insensitive, but not atropine-sensitive θ rhythms (Korotkova et al., 2010). Exogenous muscarinic agonists or endogenous ACh activate PV+ cells (Cea-del Rio et al., 2010). At the same time, M2 mAChR activation suppresses GABA release from PV+ terminals, but does not entirely eliminate it (Hájos et al., 1998; Gulyás et al., 2010). Application of CCh, or release of endogenous ACh, generates θ frequency IPSP/Cs in CA1 PCs which can be disrupted by exogenous or endogenous cannabinoids, suggesting that ACh generates θ in CA1 by activating CCK+ BCs (discussed below, and cf Cea-del Rio et al., 2012). In addition to the perisomatic-targeting interneurons, the dendritic targeting O-LM cells exhibit rhythmic membrane potential oscillations in response to CCh application, even in the absence of fast glutamatergic signaling (Chapman and Lacaille, 1999; Lawrence, 2008).

In CA3, CCh application generates γ-frequency oscillations (Hájos et al., 2004; Oren et al., 2006, 2010). The γ LFPs are greatly suppressed by a μOR agonist (μORs are predominantly located on PV+ terminals, where their activation suppresses GABA release), and PV+ BCs show the highest degree of phase modulation by the LFP (Gulyás et al., 2010). *In vitro*, morphine also suppresses CA3 γ rhythms that arise from tetanic stimulation of the s. oriens, and this effect is blocked by the μOR antagonist cyprodime (Whittington et al., 1998). Thus, it would appear that in CA3, unlike in CA1, ACh generates oscillations by selectively activating the PV+ network.

Whereas θ and γ are the two prominent ACh-generated oscillations in hippocampus, several different frequency ranges have been observed in neocortical slices. Unlike hippocampal slices, where atropine-sensitive θ or γ oscillations can be reliably elicited by CCh alone, oscillations in neocortical slices are frequently generated by applying kainate, or GABA-A receptor antagonists along with CCh. For example, CCh application generates β oscillations in rat PFC (van Aerde et al., 2009). The combination of CCh plus kainate also elicits β oscillations in primary motor cortical slices that are unaffected by AMPA blockers but prevented by GABA-A or a gap junction blocker (carbenoxolone) (Yamawaki et al., 2008). CCh generates β activity in an intact preparation of newborn rat neocortex that is dependent on mAChRs and AMPA/kainate receptors, but not GABA-A receptors (Kilb and Luhmann, 2003). CCh plus kainate elicits γ in somatosensory and visual cortex slices (Oke et al., 2010). Both CCh and bicuculline application are required to generate oscillations in neonatal rat cortical slices (Lukatch and MacIver, 1997). CCh and bicuculline also generate 3–22 Hz LFP activity in slices of occipital lobe, including "spiral" waves (Huang and Hsu, 2010). Thus, the differences in frequency ranges and pharmacology of ACh generated oscillations in neocortical slices might reflect the different circuitry activated by ACh in various cortical regions.

eCBs AND mAChRs

Depolarization-induced suppression of inhibition (DSI) is profound, reversible disinhibition of principal cells that was initially described in the hippocampus as a transient suppression of sIPSPs or sIPSCs that followed a brief, 1 or 2 s, depolarizing current injection into a CA1 PC (Pitler and Alger, 1992a). A great deal of evidence showed that DSI was mediated by a retrograde signal process, i.e., as a result of Ca^{2+} entry into a PC, a chemical messenger was released and traveled backwards across the synaptic cleft, and by activating an initially unidentified G-protein coupled receptor on certain GABAergic nerve terminals, temporarily prevented GABA release (Alger, 2002, for review). CCh markedly increased IPSP frequency by activating mAChRs on hippocampal interneurons (Pitler and Alger, 1992b) and in addition enhanced and prolonged DSI (Pitler and Alger, 1992a). nAChRs were found to have no role in enhancing DSI (Martin and Alger, 1999): nicotine did not mimic the effects of CCh and a broad spectrum nAChR-antagonist, mecamylamine, did not antagonize them. In contrast, the CCh-effects on DSI were abolished by

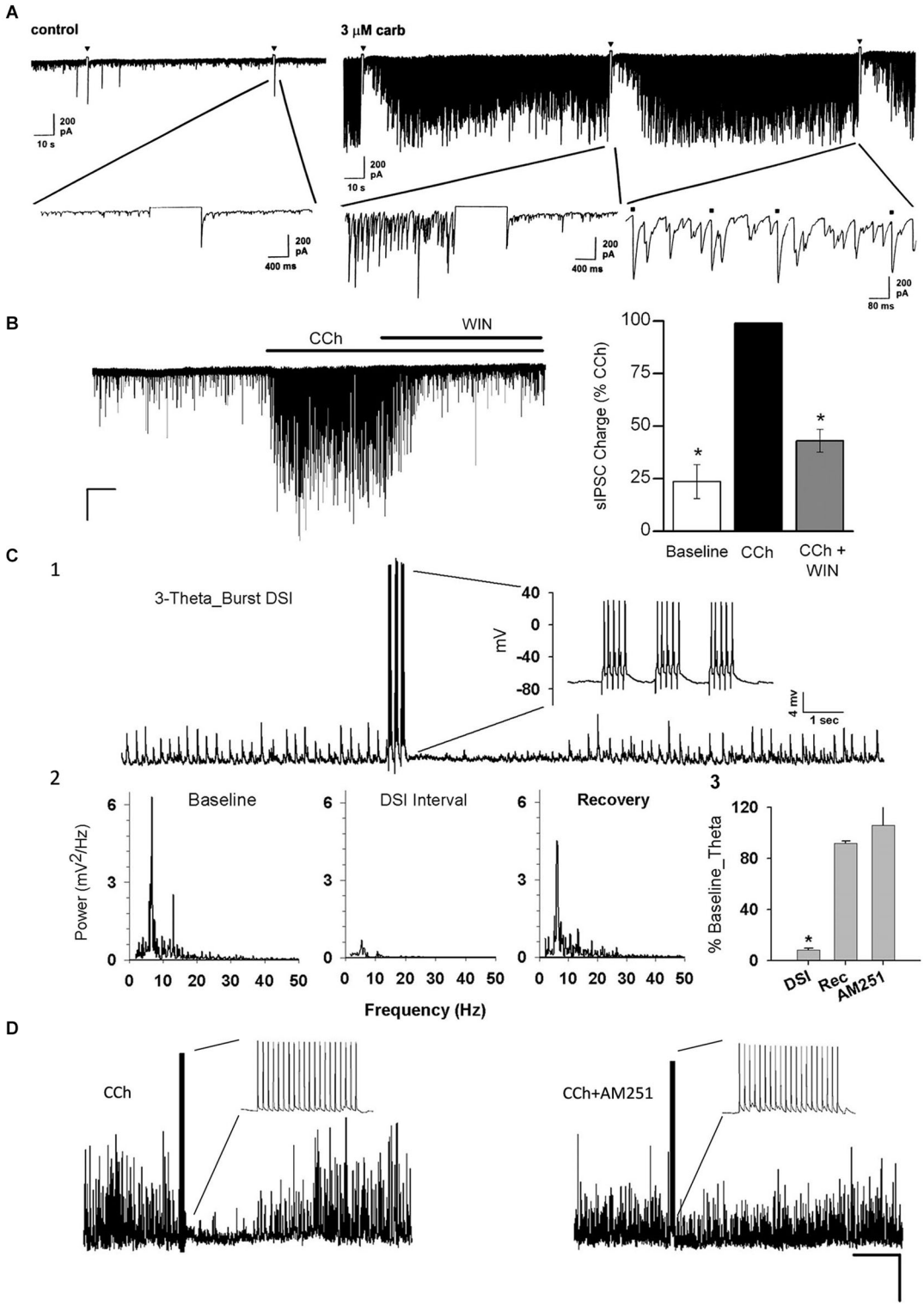

FIGURE 2 | Carbachol (CCh) elicits persistent occurrence of large, rhythmic inhibitory synaptic responses in hippocampus and neocortex. **(A)** Representative whole-cell recording from a Sprague/Dawley rat CA1 pyramidal cell (PC) in control saline (iGluR antagonists present in experiments shown in all panels) *in vitro* and a 1-s depolarizing voltage-step (downward triangles) has no effect on the small spontaneous IPSCs visible on the baseline. Bath application of 3 μM CCh induces a persistent barrage of large IPSCs that are transiently interrupted by the periods of DSI that occur after the voltage steps (From Martin and Alger, 1999; with permission). **(B)** Representative trace from a layer II/III neocortical PC from a Swiss CD-1 mouse slice. A large barrage of IPSCs occurs after CCh (5 μM) is added to the bath, and the IPSCs are suppressed by further addition of the CB1R agonist WIN55212-2 (5 μM). From Trettel et al. (2004) with permission.

(*Continued*)

FIGURE 2 | Continued
(C1) Representative sharp electrode recording of large, rhythmic IPSPs induced by 5 μM CCh in a rat CA1 PC in a hippocampal slice. Brief bursts of action potentials induced by depolarizing current injections induced a period of DSI. **(C2)** Power spectral analyses of the IPSPs before, during and after DSI. Note peak power in the theta frequency range. **(C3)** Group data showing "relative theta power" (integral of power from 4–14 Hz/total power from 2–50 Hz) from experiments as in **C1**, **C2**. DSI strongly suppressed the theta power (which recovered fully following DSI). DSI was abolished by the CB1R antagonist, AM251 (3 μM). From Reich et al. (2005) with permission. **(D)** DSI of 5 μM, CCh-induced IPSPs produced by an action potential train in a layer II/III PC in a mouse neocortical slice. DSI was abolished by 5 μM AM251. From Trettel et al. (2004) with permission.

atropine and other mAChR antagonists, and were mimicked by selective mAChR agonists, such as Oxo-M. A battery of mAChR antagonists, including pirenzepine, 4-DAMP, and AFDX-116, led to the conclusion that either the M1 or M3, but not the M2 receptor, were responsible for inducing persistent action potential firing of the interneurons that were most highly sensitive to DSI (Martin and Alger, 1999; cf Trettel et al., 2004), i.e., the firing of these interneurons produced GABAergic IPSCs that were readily suppressed by DSI (**Figure 2**). The results explained the great sensitivity of CCh-induced sIPSCs to DSI, but provided no insight into the actual mechanism of DSI itself.

In 2001 the retrograde messenger for DSI was reported to be an eCB, and the GPCR-coupled receptor on the interneuron terminals was the cannabinoid receptor, CB1R (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). There are two major eCBs, anandamide and 2-arachidonyl glycerol (2-AG), and 2-AG was demonstrated to be the main signaling eCB (see Kano et al., 2009 for review and **Figure 1** DSI). It was soon found that a mGluR agonist, (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (ACPD) or the selective group I mGluR agonist, (S)-3,5-dihydroxyphenylglycine (DHPG), markedly increased DSI (Varma et al., 2001). At low concentrations, DHPG enhanced DSI without affecting the IPSCs directly, while at higher concentrations DHPG directly suppressed the IPSCs as well. Most significantly, the three phenomena, DSI, the enhancement of DSI by mGluRs, and the direct suppression of IPSCs by high concentrations of an mGluR agonist were all abolished by a CB1R antagonist, and absent in the $CB1R^{-/-}$ mouse. The explanation was that mGluRs on PCs either enhanced the mobilization of eCBs by DSI, or directly caused eCB mobilization from these cells, and the eCBs crossed the synaptic cleft and inhibited the IPSCs by activating the CB1Rs on the interneuron terminals (**Figure 1**; "mobilized" is the preferred term because the processes of eCB synthesis and release cannot be distinguished electrophysiologically and are not inextricably linked, see Alger and Kim, 2011, for review). eCBs are also retrograde signals at excitatory synapses (Kreitzer and Regher, 2001) and mGluRs mobilize eCBs there as well (Maejima et al., 2001). Thus eCBs are not only produced by high levels of Ca^{2+} in principal cells, but are intermediaries in modulating synaptic transmitter release by glutamate, and hence were likely to have a broad range of actions.

Both group I mGluRs and M1-class mAChRs are GPCRs that are coupled to $G_{q/11}$ type G-proteins. Kim et al. (2002) found that activating mAChRs with low μM concentrations of CCh markedly enhanced DSI without affecting the IPSCs directly, but at higher concentrations directly suppressed them. For concentrations up to ∼5 μM CCh, the suppressive effects on the IPSCs were entirely reversed by a CB1R antagonist, demonstrating that, like the type I mGluRs, mAChRs could mobilize eCBs. Above 5 μM, a portion of the CCh-induced IPSC suppression could not be prevented by CB1R antagonists, suggesting that a distinct, eCB-independent form of synaptic depression also occurred. Significantly, bath-application of the AChE inhibitor, physostigmine, in the absence of other treatments, induced an atropine- and CB1R-dependent suppression of IPSCs, indicating that the low, tonic levels of ACh present in hippocampal slices were sufficient to induce persistent mobilization of eCBs (Kim et al., 2002). In accordance with this suggestion, Colgin et al. (2003) found that an AChE inhibitor depressed fEPSPs in the dentate gyrus and CA1 of hippocampal slices. This effect was absent when, prior to the *in vitro* experiments, the fimbria/fornix was lesioned and allowed to deteriorate. Most importantly, the effect was abolished by atropine and a CB1R antagonist, but unaffected by an M2 mAChR inhibitor, clearly arguing that ACh from cholinergic afferents could suppress glutamate transmission heterosynaptically via mAChR-induced, eCB release. Presumably *in vivo* release of ACh can have the same ability to regulate synaptic transmission indirectly by stimulating the release of eCBs. It is important to note that, in addition to glutamate and GABA, eCBs may also directly regulate the release of ACh itself (Gifford and Ashby, 1996; Kathmann et al., 2001; Tzavara et al., 2003; Degroot et al., 2006), although detailed physiological mechanisms of these effects have yet to be worked out.

Neither the mAChR-dependent increase of DSI, nor the direct mobilization of eCBs by mAChR activation was associated with any change in $[Ca^{2+}]_i$ (Kim et al., 2002), suggesting that the GPCR-dependent pathway of eCB mobilization and the Ca^{2+}-dependent pathways were independent. Indeed, the ability of mAChRs to mobilize eCBs was occluded when $GTP_\gamma S$, a generalized activator of G-proteins, was infused into the cells, but unaffected when intracellular Ca^{2+} was chelated by high concentrations of intracellular BAPTA (Kim et al., 2002). Hence, mAChR-dependent eCB mobilization is independent of changes in $[Ca^{2+}]_i$ but entirely dependent on G-protein activation, whereas, conversely, DSI is totally dependent on a rise in $[Ca^{2+}]_i$ and unaffected by $GTP_\gamma S$. Thus the two pathways for eCB mobilization are independent, but, importantly, can interact, as shown by the enhancement of DSI (Ca^{2+}-dependent pathway) by co-activation of a GPCR pathway (Varma et al., 2001; Kim et al., 2002).

The findings on mAChRs and eCBs were confirmed and extended in paired recordings from principal cells and interneurons in tissue-cultured primary hippocampal neurons (Ohno-Shosaku et al., 2003). Ohno-Shosaku et al. (2003) observed no real change in the ability of CCh (or Oxo-M) to mobilize eCBs in tissue cultured cells from knock out mice with either $M1^{-/-}$ or $M3^{-/-}$ mAChRs eliminated, but a virtual elimination of the eCB-related effects in the combined $M1^{-/-}/M3^{-/-}$ line. This demonstrated involvement of both M1 and M3 mAChRs, and suggested that activation of either receptor alone could produce

enough eCBs for maximal suppression of IPSCs. In the dorsal striatum, tonic activity of the cholinergic interneurons leads to a persistent enhancement of DSI in the medium spiny neurons, which is blocked by the M1 antagonist, pirenzepine, and is absent in the $M1^{-/-}$ mouse, and hence is also mediated via M1 mAChRs (Narushima et al., 2007).

Although these issues have not been dissected as thoroughly in the neocortex as in the hippocampus, the apparently identical observations of IPSC frequency enhancement by CCh and eCB-dependent DSI in neocortical slices (**Figure 2**; cf. Fortin et al., 2004; Trettel et al., 2004; Yoshino et al., 2011) makes it likely that the association between M1/M3 receptors and eCBs holds there as well. Fukudome et al. (2004) showed, also in paired principal cell-interneuron recordings in hippocampal tissue-culture, that the *eCB-independent*, CCh-induced suppression of GABA release was mediated by M2 receptors, as it was mimicked by the M2 preferring agonist, gallamine, and absent in tissue from the $M2^{-/-}$ mouse. Importantly, the M2-mediated suppression occurred in those interneurons that were not sensitive to suppression by eCBs, and vice versa, interneurons from which GABA release was suppressed by eCBs were insensitive to suppression by gallamine. It is therefore likely that the interneurons from which GABA release is inhibited indirectly by M1/M3 (i.e., eCB-sensitive) actions and those inhibited by M2 mAChRs are of different classes. Undoubtedly, the former represented the CCK+/CB1R+ interneurons and the latter the CCK−/CB1R− interneurons, probably the PV+ cells, although the cells were not immunologically identified. The results from slices (Martin and Alger, 1999; Kim et al., 2002) and tissue-culture (Ohno-Shosaku et al., 2003; Fukudome et al., 2004) are in substantial agreement in identifying the M1/M3 receptors as the likely stimulants of the eCB mobilization, while M2 receptors mediate an eCB-independent form of presynaptic inhibition.

Studies with tissue from phospholipase C beta (PLC_β) isoform-specific, knock-out mice, $PLC_{\beta 1}^{-/-}$ and $PLC_{\beta 4}^{-/-}$ showed that PLC_β is an essential element in the G-protein signaling pathway between mAChRs, or mGluRs, and eCBs in hippocampus and cerebellum, with the different isoforms being predominant in different brain structures (Hashimotodani et al., 2005; Maejima et al., 2005); in the absence of PLC_β neither of these GPCRs can mobilize eCBs. Another key observation was that DSI is independent of PLC_β (Hashimotodani et al., 2005), which confirms that the Ca^{2+} -dependent and GPCR-dependent forms of eCB mobilization utilize distinct biochemical pathways, although 2-AG is the eCB produced by both of them. PLC_β is activated by M1/M3 mAChRs and requires Ca^{2+} for its enzymatic activity, hence it is proposed that PLC_β acts as a coincidence-detector (Hashimotodani et al., 2005) that can integrate the actions of Ca^{2+} and G-proteins and thereby explain the ability of mAChRs to enhance DSI. Some challenges to this straightforward story exist (Edwards et al., 2006, 2008) and future work should provide more mechanistic detail. Nevertheless, PLC is a part of the molecular cascade that produces 2-AG, though not anandamide, and so the involvement of PLC also confirmed that 2-AG was the major eCB produced by mAChRs. 2-AG is produced by the enzyme diacylglycerol lipase alpha (DGL_α) and the absence of this enzyme in $DGL_\alpha^{-/-}$ mice prevents the mAChRs from mobilizing eCBs (Tanimura et al., 2010; Yoshino et al., 2011). DSI is also absent in $DGL\alpha^{-/-}$ mice (Gao et al., 2010; Tanimura et al., 2010; Yoshino et al., 2011), unlike the case with $PLC_\beta^{-/-}$ mice in which DSI is unaffected. Evidently, if diacylglycerol is the common precursor for the production of 2-AG, two independent pathways supply diacylglycerol to DGL_α for the production of 2-AG.

The summary picture is that M1 and M3 mAChRs mobilize the eCB 2-AG via a molecular pathway involving PLC_β and DGL_α.

OPTOGENETIC STUDIES

Release of endogenous ACh via bulk tissue electrical stimulation activates interneurons in hippocampus (Pitler and Alger, 1992b; Widmer et al., 2006), and drives inhibitory oscillations in CA1 PCs (Martin and Alger, 1999). However, bulk stimulation of tissue can also affect non-cholinergic fibers and glia, and complete pharmacological isolation of ACh responses is often not possible. The advent of optogenetics has allowed for stimulation or silencing of specific neuron populations in slice preparations as well as *in vivo*, and the cholinergic system was one of the first targeted for optogenetic manipulation (see Fenno et al., 2011, for review). Expression of the light-activated non-selective cation channel, Channelrhodopsin2 (ChR2, Boyden et al., 2005), in cholinergic neurons allows for specific stimulation of cholinergic cells or fibers and release of endogenous ACh in slice preparations. ChR2 is commonly delivered *in vivo* to target nuclei via an adeno-associated virus (AAV) vector, which has a high tropism for neural tissue and can result in expression levels exceeding 90% in target cells (**Figure 3**). To ensure specificity of ChR2 expression in cholinergic cells, the cre-loxP system has been utilized: the vector constructs carry a double-floxed inverted (FLEXED) ChR2 sequence (Atasoy et al., 2008), and the vectors are injected into the brains of ChAT-Cre mice, which express cre recombinase only in cholinergic cells. Injection of AAV-ChR2 into cholinergic nuclei results in expression of ChR2 in distal axon terminals of projection neurons in ∼2–5 weeks (**Figure 3A**; cf Gu and Yakel, 2011; Nagode et al., 2011, 2014; Tang et al., 2011; Kalmbach et al., 2012; Kalmbach and Waters, 2014), allowing for stimulation of ACh release in slice preparations which do not retain the cholinergic cell bodies (e.g., the hippocampus). The variability in expression time may have to do with differences in viral serotype used (AAV2/1—2/9 have all been used), viral titer (typically higher than 10^{12} genome copies/ml), or ChR2 variant. In general the AAVs appear to have equal tropism for all cells, although selective transformation of inhibitory cells in the cortex was reported with low titer levels (e.g., Nathanson et al., 2009) and specific tropism for cholinergic neurons has apparently not been reported. AAV2/1, 2/5, and 2/9 have all been used successfully with the cre/lox strategy in the Chat-Cre mice. The ChR2-variant, ChIEF, which has faster kinetics, hence greater suitability for high frequency stimulation, and increased steady-state photocurrent, than does the more commonly used H134R ChR2, has also been targeted to septal cholinergic cells and used to stimulate ACh release in hippocampal slices (Bell et al., 2011, 2013). ChIEF appears to show superior expression and transport to plasma membranes when compared to H134R ChR2 (Mattis et al., 2011),

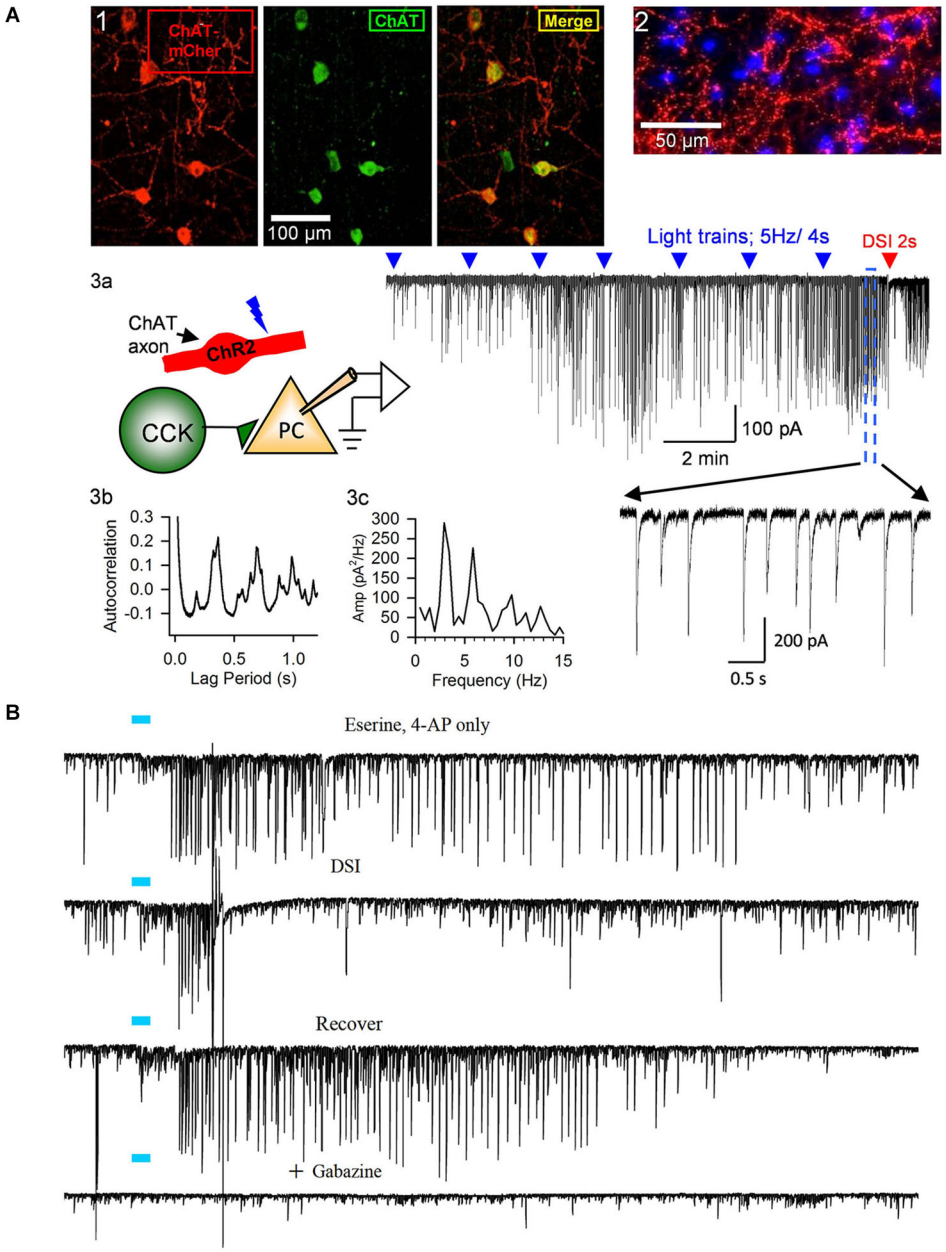

FIGURE 3 | Release of ACh by light-stimulation of ChR2 in ChAT-expressing axons induces bursts of rhythmic IPSCs in the CA1 region of hippocampal slices. (A1) Examples of ChAT-positive cells in MS/DBB expressing ChR2+mCherry following viral injection of AAV (see text) into a ChAT-Cre mouse (from Nagode et al., 2011, with permission), and **(A2)** ChR2+mCherry axons plus DAPi staining showing cholinergic axons in proximity to cells in CA1. Details of procedures are found in Nagode et al. (2011). **(A3)** Diagram of experimental setup; light-stimulation of ChR2-expressing axons in CA1 release ACh onto CCK+ interneurons that fire trains of action potentials and thereby induce IPSPs in CA1 PCs. Sample trace to the right shows trains of blue-light pulses (blue triangles) given at 2 min intervals gradually come to induce prolonged bursts of GABAergic IPSCs (downward deflections in the presence of iGluR blockers to prevent EPSC occurrence in experiments shown in this panel; cf. expanded portion, below) in a PC. A 2-s voltage step was given to the PC near the end of the trace (red arrow) to induce DSI, the transient interruption of the IPSCs. **(A3b,c)** Autocorrelation function and power spectrum of data from this cell illustrate the rhythmic nature of the ACh-induced IPSCs. Neither physostigmine nor 4-AP were used in this experiment. **(B)** Top trace, light pulse (blue bar) delivered to ChR2-expressing axons in a slice from a ChAT-Cre, AAV-injected mouse induced a burst of large IPSCs in a CA1 PC; second trace, the burst of IPSCs was interrupted during the period of DSI produced by a brief depolarization of the PC; third trace, recovery of the IPSC burst after the DSI trial; fourth trace, application of the GABA-A receptor antagonist, gabazine, blocks all light-induced activity, confirming their identity as IPSCs. Physostigmine, 1 µM, and 4-AP, 20 µM, were present in the bathing solution. Results are typical of numerous experiments. **(A2, B)** from D.A. Nagode Ph.D. thesis at http://archive.hshsl.umaryland.edu/handle/10713/2315. **(A3a–c)** is a typical result (c.f. Nagode et al., 2011).

although whether this translates into advantages of ChIEF over H134R ChR2 for release of ACh from terminals is not yet known.

In addition to the viral strategy, transgenic mouse lines constitutively expressing ChR2 have been developed. The most widely-used strain is the ChAT-ChR2-EYFP mouse (Strain 014546; Jackson Laboratories; e.g., Ren et al., 2011; Pinto et al., 2013). *In vivo* stimulation of basal forebrain ACh neurons, or their axon terminals in visual cortex, has also been achieved using this mouse. Pinto et al. (2013) found that ChR2 activation of basal forebrain cell bodies or axon terminals desynchronized cortical activity and enhanced visual discrimination. On the other hand, crossing Chat-Cre mice with the inhibitory halorhodopsin or archerhodopsin-expressing mice to silence basal forebrain cholinergic neurons synchronized cortical activity and decreased visual discrimination. However, the ChAT-ChR2-EYFP strain exhibits some deficits in attention and working and spatial memory, due to increased copy number and expression of VAChT (Kolisnyk et al., 2013), which should be considered before using them, especially for behavioral studies. Indeed, because efficiency of optogenetic activators or silencers is low, the high protein expression required to affect neuronal activity, especially during brain development, might permanently alter brain circuitry and therefore behavior. The expression of opsins in ChAT-Cre mice also varies across brain regions. The viral transduction method in adults, though more invasive, may be more advantageous than constitutively expressing mouse models in some cases.

It must be noted that in some parts of the nervous system ACh is reportedly co-localized with other neurotransmitters, including glutamate (e.g., Allen et al., 2006; Lamotte d'Incamps and Ascher, 2008) and GABA (Bayraktar et al., 1997, but see Chédotal et al., 1994), and furthermore that they may be co-released with ACh (e.g., Allen et al., 2006; Lamotte d'Incamps and Ascher, 2008; Ren et al., 2011). In principle co-release of glutamate by ChR2-induced depolarization could confound studies of optogenetic ACh release. Although we have seen no evidence for this in our experiments (Nagode et al., 2011, 2014), the possibility should be explored. Therefore, even precise cellular targeting of light-activated molecules to ChAT-expressing cells may not absolutely guarantee that light stimulation will cause the release of only ACh, or conversely, that any light-induced biological effects can unambiguously be attributed to ACh *a priori*. This will probably be true whether the opsins are expressed in the target cells virally or transgenically. Additional pharmacological or perhaps molecular biological controls would have to be taken to identify the active agent. For the use of pharmacological tools, *in vitro* slice preparations will undoubtedly be most effective. Of course, from the point of view of behavioral relevance, *in vivo* preparations will be most desirable. Thus, it seems that combinations of *in vivo* and *in vitro* approaches will be required to achieve definitive conclusions regarding axonally released transmitter actions, even using optogenetic techniques, for the forseeable future.

For the study of ACh effects in slice preparations, particularly oscillations or other network phenomena, generating sufficient ACh release is a significant concern. ChR2 expression in axon terminals is usually weaker than in somata, and axons from the basal nuclei are unavoidably severed by the slicing. If the "bulk transmission" hypothesis is correct (Vizi and Kiss, 1998), large amounts of ACh release might be required to activate receptors on target cells, especially since acetylcholinesterase (AChE) hydrolyzes ACh very efficiently. It is nevertheless possible to stimulate long bursts of DSI-sensitive IPSCs that closely mimic those induced by mAChR agonists (e.g., **Figures 3**, **4**). While these IPSCs can be elicited by light-induced ACh release in slices in normal recording saline (**Figure 3A**), we (Tang et al., 2011; Nagode et al., 2011, 2014) often use physostigmine to inhibit AChE, and a low concentration of the K-channel blocker, 4-AP, to enhance ChR2-induced ACh release (as has been done by others, e.g., Petreanu et al., 2007; Hull et al., 2009). This greatly increases the occurrence of ChR2-induced rhythmic activity without otherwise altering the IPSCs (**Figures 2B** and **3**; cf Nagode et al., 2011). Such pharmacological enhancement is not necessary for evoking post-synaptic ACh currents (Bell et al., 2011, 2013) or nAChR-dependent plasticity of EPSCs in CA1 PCs (Gu and Yakel, 2011), suggesting that less ACh is required to generate single cell firing than sustained network activity.

An important new finding (Nagode et al., 2014) was that light-induced ACh release triggers IPSCs that are sensitive to DSI, and that most of them are also sensitive to μOR agonists even when the output of PV+ cells (which express the great majority of hippocampal μORs) has been abolished (**Figure 4B**). Dual CB1R/μOR sensitivity has been reported (e.g., Neu et al., 2007; Glickfeld et al., 2008) but it was surprising to encounter it so frequently in the optogenetic experiments. The explanation for this observation is not understood, but may imply that axonally released ACh has an unexpectedly strong tendency to activate dually sensitive interneurons, which could be important for understanding cannabinoid/opioid interactions *in vivo*. It will be of great interest to explore the effects of silencing septal cholinergic neurons with halorhodopsin or archerhodopsin *in vivo* during ongoing hippocampal θ oscillations. The unique ability to release ACh from cholinergic axons optogenetically probably made this discovery possible.

While dramatic effects of optogentically released ACh are on the induction of θ rhythm frequency oscillations of IPSPs via activation of mAChRs, a pulse of released ACh also elicits a burst of IPSCs by activating nAChRs and a highly novel mechanism involving T-type Ca^{2+} channel activation and Ca^{2+} stores (Tang et al., 2011). These events occur even in the presence of TTX, strongly suggesting that the nAChRs are on the GABAergic nerve terminals. Moreover, since there are no morphologically defined synapses along these axons, this appears to be a direct example of a non-synaptic effect of axonally released ACh. Non-synaptic stimulation of GABA release can also be produced by optogenetic stimulation of striatal cholinergic interneurons. Interestingly, the same ionic mechanism as proposed in hippocampus (Tang et al., 2011) appears to operate in striatum (Nelson et al., 2014).

mAChRs, eCBs, AND NEURONAL NETWORK OSCILLATIONS IN HIPPOCAMPUS

mAChR-activation induces the firing of CCK+/CB1R+ cells and IPSCs from these cells are a major factor in inhibitory θ rhythms. However, there is extremely good evidence that the M1 and M3 mAChRs are also very effective in stimulating the mobilization

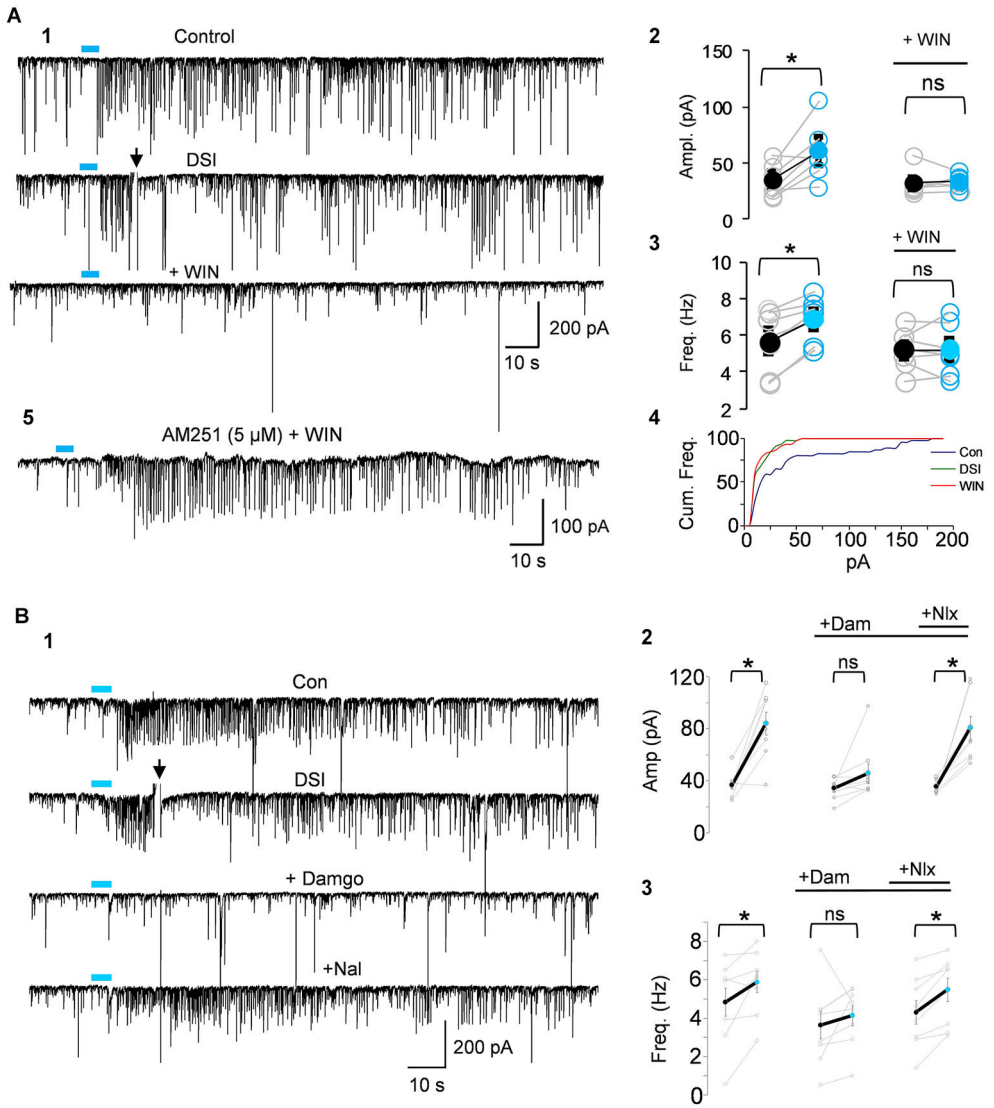

FIGURE 4 | IPSCs triggered by light-induced ACh release arise from CB1R+ interneurons. (A1) Top two traces as in **Panel 3B**, the CB1R agonist, WIN55212-2 (WIN) was then applied to the bathing solution of the same cell, and prevented the ability of ACh to induce repetitive IPSCs. **(A2–A4)** Group data showing that the increases in IPSC amplitudes **(A2)** or frequency **(A3)** or cumulative frequency **(A4)** induced by light in control solution (left graphs in **A2,A3**), were occluded in WIN-treated slices; i.e., that they arose from CB1R+ interneurons. **(A5)** Shows that pretreatment with the CB1R antagonist, AM251, prevented the ability of WIN to suppress the ACh-induced IPSCs. **(B1)** DSI-sensitive IPSCs induced by ACh release can also be reversibly suppressed by the μOR agonist, DAMGO, and recover when the μOR antagonist, naloxone, is applied. **(B2,3)** Show group data for experiments such as in **(A)**. Physostigmine, 1 μM, and 4-AP, 5–20 μM, were present in the bathing solution. Figures taken from D. Nagode Ph.D. Thesis, at http://archive.hshsl.umaryland.edu/handle/10713/2315.

of eCBs. One might expect that the GABAergic output from CCK+/CB1R+ cells would be rapidly eliminated by the eCBs, and indeed it has been proposed that CCh-generated eCBs silence the CB1R+ cells during rhythm generation in CA3 (Gulyás et al., 2010; Holderith et al., 2011). Nevertheless, on the contrary, the CCh-induced IPSCs are highly susceptible to inhibition by DSI (e.g., Pitler and Alger, 1992b; Alger et al., 1996; Martin and Alger, 1999; Wilson and Nicoll, 2001; Kim et al., 2002; Hampson et al., 2003; Fortin et al., 2004; Trettel et al., 2004; Yoshino et al., 2011). Thus the CB1R+ interneurons are not entirely silenced by the mAChR-induced eCBs. As noted, studies from DGL$\alpha^{-/-}$ mice, which are incapable of generating the major eCB, 2-AG, confirm that both mAChR-dependent eCB effects and DSI are mediated by 2-AG (Tanimura et al., 2010; Yoshino et al., 2011), so differences in eCB identity cannot account for the continued sensitivity of CCh-induced θ IPSCs to DSI.

An entirely different mechanism was described by Makara et al. (2007) who reported that, in the presence of CCh, the eCB-system becomes dependent on nitric oxide (NO) production. When mAChRs were activated DSI could be

prevented by inhibitors of NO synthesis or NO scavengers. NO scavengers injected into the postsynaptic PCs prevented the action of NO and soluble cGMP, a proposed intracellular target of NO, was selectively located in presynaptic CB1R+ nerve terminals. Hence the picture was that NO was released as a retrograde signal from the PCs, affected cGMP in the presynaptic CB1R+ terminals and acted in concert with eCBs to inhibit GABA release. It was proposed that NO acted at a step downstream of CB1R, although activation of CB1R via the synthetic CB1R agonist, WIN55212-2, was not affected. It was unclear why eCB-mediated actions were immune to NO in the absence of CCh. To our knowledge, these provocative observations have not been replicated, hence although mAChRs do generate NO at neuromuscular synapses (Malomouzh et al., 2007; Newman et al., 2007), even interacting with eCBs at other synapses (e.g., via M3 activation at vertebrate neuromuscular synapses, Newman et al., 2007), a role for NO in mAChR actions in hippocampus remains conceivable but undefined.

Resolution of the puzzle that mAChRs both mobilize eCBs and stimulate the activity of eCB-sensitive interneurons could well involve a mechanism that modulates presynaptic CB1R actions and partially offsets their depressive effects on GABA release. Several candidates exist (**Figure 5**), including: (1) K^+ channel antagonists—blocking K^+ channels pharmacologically can completely abolish DSI (Alger et al., 1996; Morishita et al., 1998; Diana and Marty, 2003), probably because voltage-gated Ca^{2+} channel opening and intra-terminal $[Ca^{2+}]_i$ increase when K^+ channels are blocked (Varma et al., 2002). ACh-induced blockade of presynaptic K^+ channels (or other factors, e.g., retrograde release of arachidonic acid; Carta et al., 2014) might thus overcome CB1R-induced depression during ACh action. (2) Direct effects on the transmitter release machinery via application of N-ethylmaleimide (NEM), which blocks pertussis toxin-sensitive G-proteins, increases GABA release and reverses DSI through an unknown mechanism (Morishita et al., 1997). (3) The firing frequency of the interneuron—the degree of eCB-mediated suppression of GABA release decreases as the firing of the interneuron increases (Losonczy et al., 2004; Földy et al., 2007). Since CCh stimulates interneuron firing (Pitler and Alger, 1992a; Martin and Alger, 1999; Cea-del Rio et al., 2010; Gulyás et al., 2010), the net effect of eCBs on persistently occurring IPSCs will represent a balance between inhibition and excitation of interneuronal output.

If indeed mAChR-released eCBs suppress, but do not abolish, CCK+/CB1R+ interneuron output, then a CB1R antagonist should increase the IPSCs coming from these cells. That is, we predict that action of mAChRs on the interneurons will cause them to fire and release GABA, while the CB1R antagonist will prevent the eCBs generated by the PCs from simultaneously retarding the occurrence of the IPSCs. Thus a given ACh stimulus should give rise to more IPSCs in the presence of CB1R antagonism than it normally would. While concerted effort will be required to test this hypothesis in detail, we have observed (Nagode et al., 2011) that indeed the CB1R antagonist AM251 increases the number of IPSCs triggered by optogenetically released ACh (**Figure 6**). This example shows that the number and mean amplitude of the IPSCs triggered by ChR2-induced ACh release are increased after AM251 was applied. This suggests that eCBs generated by mAChRs can influence the IPSC rhythms. It will also be important to determine whether similar influences can be detected on atropine-sensitive, inhibitory rhythms *in vivo*, as this would suggest that mAChR-induced eCBs could be involved in regulation of persistent, behaviorally significant rhythms.

Finally, CB1R+ interneurons are electrically interconnected (Galarreta et al., 2004) and eCBs can indirectly strengthen electrical synapses (Pereda, 2014). Additionally, weakening of inhibition between electrically connected interneurons (as might occur during enhanced eCB release), also strengthens electrical coupling (Iball and Ali, 2011). Strengthening of electrical coupling will enhance the synchrony of firing within such networks. Thus complex interactions among chemical and electrical synapses and eCBs could help to rationalize the role of mAChRs in θ inhibitory oscillations. Unraveling the details of the modulation of eCB actions initiated by the cholinergic system will be an important task for the future.

mAChR DRIVEN eCB RELEASE AND ELECTRICAL SYNAPTIC CONNECTIONS SHARPEN DISTINCTIONS AMONG INTERNEURON CIRCUITS AND TUNE INTERNEURONAL OSCILLATIONS

The two major BC interneuron subtypes, the CCK+ (regular-spiking, RS) and the PV+ (fast-spiking, FS) cells are sharply segregated by their divergent properties (Freund and Katona, 2007), including their complements of mAChRs. These cellular and molecular differences imply that the two cell types are activated under different circumstances, by different neurotransmitters and modulators, and cause different effects on their target cells. Another factor is critical to ensuring that the cells within each group do not act in isolation, but participate in coordinated circuit based activity. As noted, electrical synaptic coupling often exists among like cells in hippocampus and neocortex (Galarreta and Hestrin, 1999; Gibson et al., 1999), although some interneurons are electrically coupled to cells of different classes (e.g., Krook-Magnuson et al., 2011). Generally, PV+ cells are electrically coupled to other PV+ cells, but not to PCs or other types of interneurons, including the CCK+ cells. CCK+ cells are electrically coupled to other CCK+ cells (Galarreta et al., 2004), but not to PCs or other types of interneurons, including the PV+ cells. The steady-state electrotonic coupling (coupling coefficient) among the cells averages from 4–7%, meaning that, e.g., a 10 mV change in membrane potential in one cell changes the potential in the coupled cell from 0.4–0.7 mV. This is enough to induce a cell to fire if it is near threshold. Injection of two random noise signals (Galarreta and Hestrin, 1999) or small sinusoidal voltages (Gibson et al., 1999) into two electrically coupled interneurons can cause both of the cells to fire synchronously when either of them reaches threshold. Thus the PV+ cells would tend to fire together as a circuit and the CCK+ cells would also tend to fire as an independent circuit. Interneuron circuit-wide activity will powerfully influence PC population activity.

The existence of electrical gap junctions between the cells is also noteworthy because it confers susceptibility to modulation by various regulatory factors. The strength of gap junctional

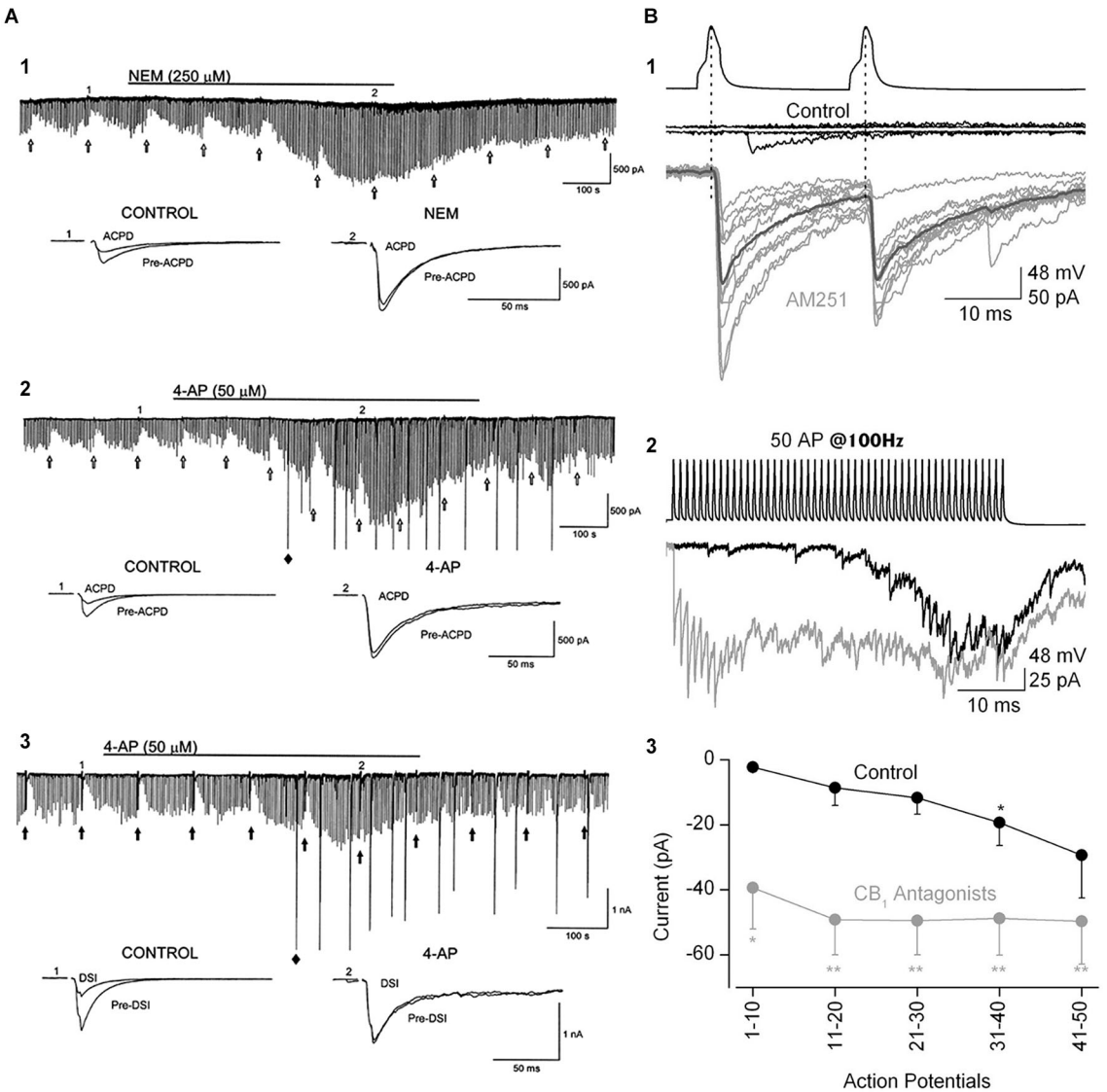

FIGURE 5 | The ability of eCBs to inhibit GABA release can be modulated by manipulations that increase transmitter release. (A1) Bath-application N-ethylmaleimide, an organic compound that affects G-proteins, ion channels and other biochemical processes, increases GABA release and abolishes the GPCR-dependent, eCB-mediated depression of IPSCs, as well as DSI (not shown; cf. Morishita et al., 1997). **(A2,A3)** The K^+ channel blocker, 4-AP, increases IPSCs and abolishes mGluR-dependent, eCB-mediated IPSC suppression and DSI. From Morishita et al. (1998) with permission. **(B)** Paired recording from mossy-fiber associated (MFA), CCK+ interneurons and CA3 PCs. **(B1)** Top two sets of traces show two presynaptic action potentials in the interneuron and the absence of a response in the PC in control saline. After addition of the CB1R antagonist, AM251, the interneuron action potentials reliably elicit large unitary IPSCs. **(B2)** A train of 50 interneuron action potentials initially produces only a few sparse IPSCs in the PC towards the end of the train in control solution (black trace). In the presence of AM251 (gray trace) the IPSCs are detected from the first action potential and occur throughout the train. **(B3)** Group data showing the difference in the IPSC currents, integrated within 100-ms time windows, in control and CB1R antagonist conditions. The conclusion is that a tonic, eCB mediated suppression of GABA release can be overcome by vigorous stimulation of interneuron activity. From Losonczy et al. (2004) with permission.

transmission is dependent on the input (leak) resistance of the target cells, and is frequency dependent, decreasing as the frequency of the voltage deflections through the junctional channels increases. When the leak resistance is low the coupling among cells is also low, because the currents, instead of passing via the gap junction into the coupled cells and depolarizing them, are shunted through the leak resistance to the extracellular space. When the leak resistance is high, the strength of electrical coupling increases. Interestingly the PV+ and CCK+ interneurons that are electrically coupled to each other are frequently also chemically coupled; that is, the target postsynaptic cells receive both electrical and chemical synaptic transmission from their upstream presynaptic partners (Galarreta and Hestrin, 1999; Gibson et al., 1999; Ali, 2007; Iball and Ali, 2011). The release

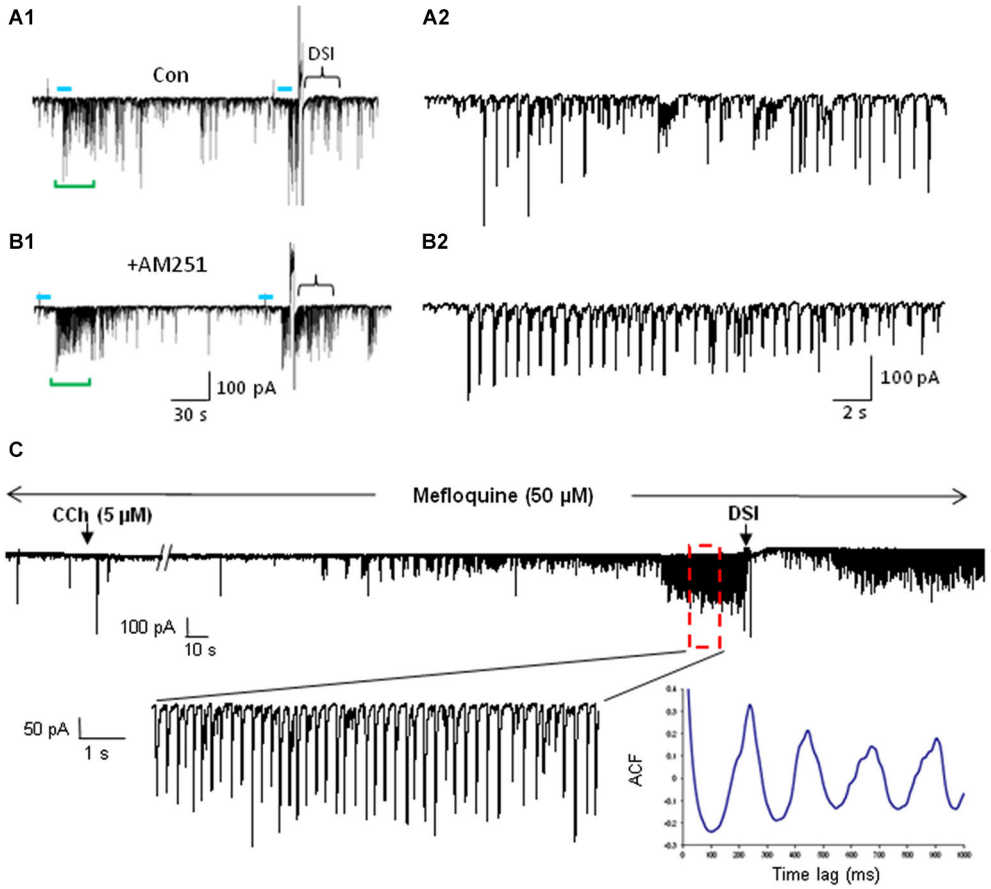

FIGURE 6 | mAChR-induced IPSCs are regulated by eCBs but do not depend on electrical coupling for their occurrence. **(A)** Bath application of the CB1R antagonist AM251 increases the occurrence of IPSCs evoked by optogenetic release of ACh (CA1 PC recording). Blue bars indicate the period of light stimulation (5 Hz). Traces in **(A2)** and **(B2)** depict an expansion of ∼29 s of the traces (green brackets) in **(A1)** and **(B1)** beginning just before the onset of L-IPSC activity. The increase in number and average amplitude of the L-IPSCs caused by AM251 indicates that they had been partially suppressed by the eCBs mobilized by ACh. Bracket at the end of **(A1)** indicates approximate period of DSI after a voltage step given to the pyramidal cell. Comparable period in **(B1)** shows that AM251 prevents DSI, thus confirming that the L-IPSC activity (e.g., expanded trace in **A2**), despite being partially suppressed by the long-lasting ACh-induced mobilization of eCBs, could be further depressed by a sudden release of eCBs (i.e., DSI). Physostigmine and 4-AP are present. **(A)** and **(B)** modified with permission from Nagode et al. (2011). **(C)** Representative trace from a rat hippocampal slice pretreated and continuously perfused with the gap junction blocker, mefloquine (50 μM). Inset shows an expanded time scale of the indicated region in the top trace. The autocorrelogram of the expanded region demonstrates rhythmic, CCh-induced IPSCs despite the presence of mefloquine. Typical results (n = 5). From D.A. Nagode Ph.D. thesis at http://archive.hshsl.umaryland.edu/handle/10713/2315.

of GABA from CCK+ cells can be suppressed by eCBs (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001), and when eCBs are released from other CCK+ cells the eCBs reduce the strength of chemical inhibition (Iball and Ali, 2011). By decreasing the chemical synaptic inhibition among CCK+ cells this simultaneously strengthens the electrical coupling between them. The release of eCBs depresses the strengths of individual GABAergic synapses onto other CCKs cells *and* increases their tendency to fire together. Importantly, the two kinds of synaptic junctions are independently regulated in the hippocampus; eCBs only suppress the release of GABA, they do not affect the electrical coupling. Thus inhibition can do more than simply veto or permit cell firing, it can directly shift the mode of firing within interneuronal circuits. The functional aspects of this concept has not been explored in the context of mAChR control of oscillations. However, given the ability of mAChRs to mobilize eCBs, mAChR-dependent stimulation of CCK+ cell mediated oscillations could in part reflect dual regulation of chemical and electrical signaling, although the gap junction blocker, mefloquine (Cruikshank et al., 2004), did not alter the ACh-induced θ IPSCs (**Figure 6C**).

PV+ cells are also electrically as well as chemically coupled and their tendency to fire together is facilitated by electrical synapses (Galarreta and Hestrin, 1999). Activation of mAChRs induces the occurrence of γ rhythms driven by PV+ cells in hippocampal CA3, but PV+ cells but do not express CB1Rs and are therefore not directly affected by eCBs. The PV+ cell-mediated inhibitory γ rhythms are suppressed by *exogenous* cannabinoids because activation of the CB1Rs on the glutamate terminals that excite the PV+ cells is suppressed and the resulting loss of excitatory drive

keeps the cells from firing (Holderith et al., 2011). Surprisingly, the eCBs released by activation of M1/M3 mAChRs on PC apparently do not affect the CB1Rs on the glutamate terminals. This conclusion follows from the observation that the CB1R antagonist that prevents the inhibition of γ by exogenous eCBs, when applied by itself does not alter mAChR-driven oscillations (Gulyás et al., 2010). Given that a mAChR agonist very efficiently suppressed GABA release via eCB action in these experiments, it is clear that the eCBs were mobilized. Probably the eCBs simply did not reach the CB1Rs on glutamatergic terminals. The powerful eCB uptake and degradation systems, together with the fact that eCBs cannot travel far from their site of production/release (Kano et al., 2009) could have limited their movements. The restricted actions of the eCB system help sharpen the targeting of ACh actions, even if ACh is released in the volume conduction mode.

Release of GABA from PV+ cells is regulated by opioids, because these cells strongly express μORs on their nerve terminals (Drake and Milner, 2002). Thus μOR agonists, such as enkephalins, may have the analogous effects on the development of PV+ driven inhibitory rhythms as eCBs do on the CCK+ cell rhythms. Mobilization of endogenous opioids in the hippocampus by ACh has not yet been explored in this context to the best of our knowledge.

It is established that the CCK+ cells and the PV+ cells predominate in different kinds of neuronal oscillations. The preferred frequencies of the oscillations, γ for the PV/FS cells and the slower θ rhythms for the CCK/RS, cells will largely be set by intrinsic membrane properties, including the kinetics of their AHPs, that enable the PV/FS cells to fire at higher frequency than the CCK/RS cells, as well as by the kinetics of the chemical transmission that they each mediate—IPSPs mediated by PV/FS cells are faster than those of the CCK/RS cells. Most importantly, the circuitry underlying the rhythms, at least in the hippocampus, is likely to be quite different. The inhibitory θ in CA1 is probably generated by an interconnected inhibitory network of CCK+ cells that express CB1Rs, and perhaps also μORs. This rhythm is independent of fast excitatory glutamatergic synaptic input. Rather, the rhythmic output of this circuit is produced when the cells receive a slow cholinergic input that activates their mAChRs for at least several seconds. Inhibitory synaptic interactions among the interneurons then gives rise to synchronous rhythmic firing within the network, and IPSCs are projected onto groups of PCs. In contrast, the faster inhibitory γ rhythms in CA3 are generated mainly by excitatory synaptic interactions among CA3 PCs, which activate PV+ interneurons that then feed back inhibitory inputs to the PCs. These rhythms are abolished by iGluR blockers, or CB1R agonists, which prevent stimulation of the PV+ cells; they are also abolished by activation of μORs on the PV+ cell terminals. The schematic diagram in **Figure 7** summarizes these conclusions. Note that this schematic is intended only to illustrate the circuitry for the inhibitory rhythms, it does not include other circuitry such as that described by Pietersen et al. (2014) that produces an intrinsic γ rhythm that is entrained by cholinergic inputs and is dependent on excitatory synaptic inputs (hence a PING model) in CA1.

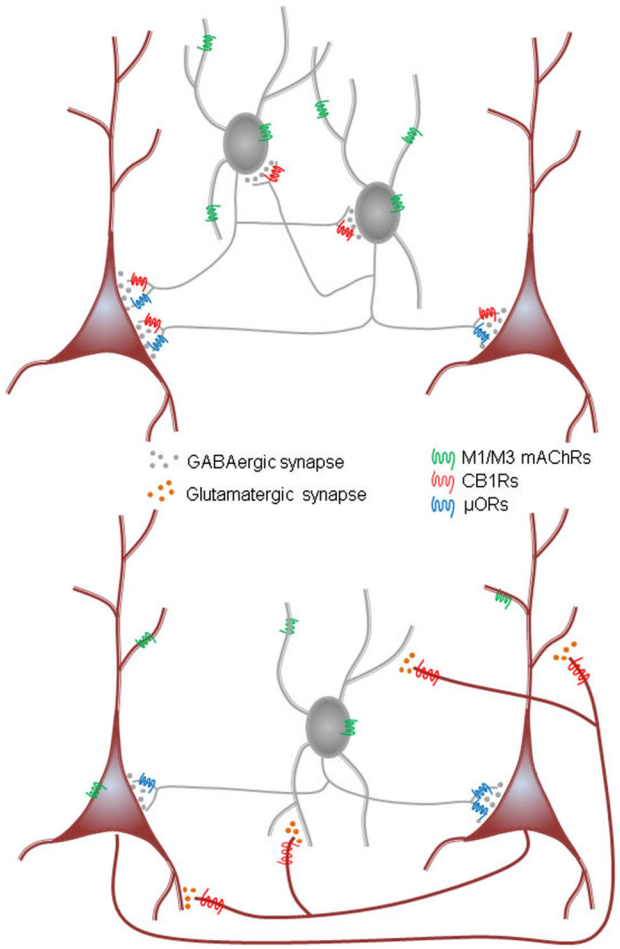

FIGURE 7 | Diagrams of two models for mACh-induced inhibitory rhythmic IPSCs in hippocampus. **Top**, synaptically connected interneuron network is tonically activated by activation of M1/M3 mAChRs on interneurons in CA1. Interneuron firing is induced by mAChR-induced depolarization that, when integrated with intrinsic interneuron firing properties and incoming GABAergic IPSPs from other interneurons of the group, generates rhythmic synchronous interneuron firing. The target PCs receive a rhythmic barrage of IPSPs. This is analogous to the ING ("interneuron gamma") model of gamma rhythms. Cannabinoids interrupt rhythms generated by this network by inhibiting the release of GABA from the CB1R-expressing (mainly CCK+) interneurons; opioids probably inhibit the network by acting on μORs present on a subset of the CB1R+ cells. Note evidence of Pietersen et al. (2014) for an intrinsic γ generator in CA1 that would involve a PING mechanism. **Bottom**, ACh drives action potential firing in an interconnected excitatory network (such as the CA3, but not the CA1, PCs) as well as in the interneurons. The glutamatergic output of the PCs excites interneurons that feed GABAergic IPSPs back onto the PCs. Interactions between the excitatory and inhibitory cells generates the rhythms. This is analogous to the PING ("pyramidal-interneuron gamma") model. Cannabinoids inhibit rhythms generated by this network by inhibiting the release of glutamate from the PCs; opioids inhibit the rhythms by acting on the μORs on the (mainly PV+) interneurons.

FUTURE AREAS FOR EXPLORATION OF mAChR FUNCTION IN THE BRAIN

Despite the enormous amount of investigation into muscarinic cholinergic systems in the brain there are still many areas about

which little is known. We highlight a few opportunities related to their roles of in hippocampal and cortical oscillations or behaviors related to them.

mAChRs, PAMs, and endocannabinoids

Deficiencies in mAChR actions are implicated in various kinds of cognitive dysfunction. Attempts to develop effective therapeutic agents that act directly on specific brain mAChRs have not been successful, largely because of difficulties in restricting the agents to particular mAChR subtypes (Bubser et al., 2012). These agents are generally agonists that bind to the active site of the molecule, or generally enhance the availability of ACh (by preventing its uptake, for example). In either case, side effects occur when unintended receptors are also activated. It has been difficult to devise agonists that only activate one mAChR subtype because the agonist binding site is highly conserved across subtypes. An alternative approach targets sites that are away from this highly conserved region. Ligands at these sites, allosteric modulators, are more specific because they bind to relatively less well-conserved parts of the receptor molecule, i.e., sites that vary widely between subtypes and hence offer more opportunities for specific binding. Allosteric modulators do not directly activate the receptor but enhance the effects of ACh or other ligands that activate it directly. For example, positive allosteric modulators (PAMs) that are specific for M1, M4 or M5 mAChRs have been developed (Bubser et al., 2012). In the presence of a PAM for M1, a low concentration of ACh that produces predominantly M1 mAChR dependent effects would increase the ability of naturally released ACh to activate M1 selectively on its normal postsynaptic target cells. This approach should improve the specificity of action over the usual systemic therapeutic drug application method. Similar strategies have been used in the case of nAChRs; e.g., the weak AChE inhibitor and PAM of nAChRs, galantamine, attenuates nicotine self-administration and seeking rats (Hopkins et al., 2012).

Because activation of M1 (or M3) mAChRs potently stimulates the release of eCBs, an M1-specific PAM should enhance the ability of cholinergic agonists to mobilize eCBs. This hypothesis has not been tested, but could easily be investigated in *in vitro* brain slice preparations. If PAMs do facilitate eCB mobilization by mAChRs, it might have clinical applications, particularly in view of the preliminary results suggesting efficacy of CB1R agonists in alleviating certain consequences of Alzheimer's dementia (agitation, lack of nutritional intact, sleep disturbances; Aso and Ferrer, 2014). Perhaps a mAChR PAM given in conjunction with low concentrations of a CB1R agonist would be beneficial and further reduce the possibility of untoward side effects of either drug alone. Alternatively, an eCB uptake inhibitor, by increasing the concentration of eCBs near their normal site of action, might be beneficial in boosting the eCB mobilizing ability of M1 mAChR activation.

mAChRs, glia, and eCBs

No longer thought to be passive supporting partners of neurons, glia are now understood to have active roles in the regulation of synaptic transmission. Glial cells, mainly astrocytes, express a diversity of mAChRs including M1 and M3 (Pap et al., 2009). In several brain regions activation of mAChRs on glia cause elevations in intracellular glial $[Ca^{2+}]_i$ (Araque et al., 2002; Pap et al., 2009; Takata et al., 2011; Navarrete et al., 2012). Glia participate in the induction of synaptic plasticity in the hippocampus, including a form of LTP at the CA3-CA1 synapses that is dependent on activation of mAChRs (Navarrete et al., 2012). Stimulation of glial mAChRs by application of cholinergic agonists, or stimulation of ACh release from septal cholinergic fibers causes an increase in hippocampal Ca^{2+} in glia (Araque et al., 2002). *In vivo* sensory stimulation or electrical stimulation of the MS increases Ca^{2+} in hippocampal astrocytes and induces LTP of CA3-CA1 synapses (Navarrete et al., 2012). This cholinergic LTP induction depends on activation of mAChRs and mGluRs. Rises in glial cell Ca^{2+} result from activation of IP_3Rs (Takata et al., 2011; Navarrete et al., 2012), and are associated with the release of various factors (Sul et al., 2004) including glutamate (Halassa and Haydon, 2010, for review). Astrocytes are also activated by endocannabinoids (Navarrete and Araque, 2008; Min and Nevian, 2012). The glial induction of LTP in the hippocampus is caused by Ca^{2+}-dependent glutamate release from the astrocytes and subsequent activation of hippocampal PC mGluRs. A similar cholinergically-driven, astrocyte-Ca^{2+} mediated synaptic plasticity in the mouse barrel cortex is dependent on mAChRs and NMDARs (Takata et al., 2011), indicating that mAChR activation stimulates glutamate release there as well. Given that glutamate activation of mGluRs is a potent stimulus for eCB mobilization from PCs (Maejima et al., 2001; Varma et al., 2001) elevation of glial cell $[Ca^{2+}]_i$ should also mobilize eCBs indirectly from the PCs following mGluR stimulation. Given the eCB-mediated influences on cortical and hippocampal rhythms, glia could also participate in regulation of rhythms via eCBs. This hypothesis has evidently not been tested, but if true, would add another potent element to the array of effects mediated by mAChR.

mAChR regulation of rhythms by controlling ectopic axonal activity

It is generally assumed that axons simply transmit signals from neuronal somata to synaptic terminals, and therefore that they automatically follow somatic activity. Two corollaries follow from this assumption: (1) somatic action potential activity is an accurate guide to the activity reaching the terminals; and (2) axons do not act independently of somata.

However, under some circumstances axonal action potentials can be initiated independently of somatic depolarizations. These were initially described in the context of disease or other aberrant conditions, but new challenges to the simple picture have arisen in physiological contexts. In hippocampal slices, Dugladze et al. (2012) report that kainic acid-induced γ oscillations in the field potentials around the PCs of the CA3 region are accompanied by much higher frequency firing in the distal axonal branches of the PCs. Remarkably, this high frequency of axonal firing was not reflected in the somatic action potential firing of the PC somato-dendritic regions. It appeared that the two cellular compartments—axon and soma-dendrite—were in essence operating independently. When GABA-A receptors were pharmacologically blocked, however, the axonal action potentials did invade the somato-dendritic region, implying that normally

they were actively prevented from doing so by a persistent GABAergic inhibition. The investigators discovered that a continual high frequency firing of the axo-axonic interneurons, which specifically target the axon hillock region of the PCs, were responsible. In fact, a single axo-axonic cell was capable of fully controlling the antidromic invasion of the somato-dendritic region of a PC. As noted earlier, the axo-axonic cells in CA3 are strongly activated by mAChR activation, but the IPSPs produced by these cells do not directly contribute to oscillations. Rather, Dugladze et al. (2012) suggest that the main function of the axo-axonic cells is to preserve the independence of axonal and somato-dendritic signaling. It will be important to determine if mAChR-induced oscillations share the ability to modulate PC function in this novel and powerful way.

mAChRs, eCBs, and Fragile X Syndrome

Endocannabinoid modulation of CCK+ cells may underlie some of the deficits in oscillations in Fragile X Syndrome (FXS). In the hippocampus of a mouse model of FXS, *Fmr1*$^{-/-}$ mice, there is enhanced coupling of mGluRs to eCB release at inhibitory synapses in both hippocampus (Zhang and Alger, 2010) and striatum (Maccarrone et al., 2010). Surprisingly, the eCB actions at excitatory synapses are actually decreased, not increased, in this model (Jung et al., 2012). While the molecular basis for this striking difference is not fully understood, the distinctive molecular architecture of excitatory and inhibitory synapses will undoubtedly constitute a major factor. DGL_{α} is normally precisely localized in the spine heads of excitatory synapses (Katona et al., 2006), and it has been found that the disease is associated with an enhanced distance between mGluRs and DGL_{α} (Jung et al., 2012) which could explain the decreased efficiency of eCB production. DGL_{α} has not yet been found at inhibitory synapses (e.g., Lafourcade et al., 2007), and the explanation for enhanced eCB actions at those synapses is unknown, although biochemical targets are being identified (Busquets-Garcia et al., 2013). It should be emphasized that the functional consequences of both decreased eCB action at excitatory synapses and increased eCB action at inhibitory synapses will be the same: an overall increase in network excitability. The same could be true of mAChR-induced eCB release, as overactive signaling through M1 mAChRs has been hypothesized to contribute to the FXS phenotype (D'Antuono et al., 2003). There is enhanced CCh-induced LTD in CA1 hippocampal slices from FRX mice (Volk et al., 2007), and M1 and M4 antagonists reduce the induction of audiogenic seizures (Veeraragavan et al., 2011a,b). The relationship between mAChRs and eCBs deserves further study in the context of FXS, in part because the availability of clinically tested CB1R ligands that could be candidates for inclusion in the therapeutic arsenal for treatment of symptoms of this serious disorder.

CONCLUSION

Studies of the mAChR system in the brain continue to yield exciting new insights and information on a wide variety of neurophysiological problems. Undoubtedly the future holds enormous promise for novel and valuable advances both in the basic understanding of this powerful and ubiquitous regulatory system, and in eventual clinical applications.

CONTRIBUTIONS OF AUTHORS

This review was written jointly by all of the authors. The recent research referred to from the Alger laboratory was supervised by Bradley E. Alger and conducted by Daniel A. Nagode and Ai-Hui Tang, who also had major roles in the experimental design, data analysis, and interpretation of results.

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REFERENCES

Alger, B. E. (2002). Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog. Neurobiol.* 68, 247–286. doi: 10.1016/s0301-0082(02)00080-1

Alger, B. E., and Kim, J. (2011). Supply and demand for endocannabinoids. *Trends Neurosci.* 34, 304–315. doi: 10.1016/j.tins.2011.03.003

Alger, B. E., Pitler, T. A., Wagner, J. J., Martin, L. A., Morishita, W., Kirov, S. A., et al. (1996). Retrograde signaling in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. *J. Physiol.* 496, 197–209.

Ali, A. B. (2007). Presynaptic inhibition of GABAA receptor-mediated unitary IPSPs by cannabinoid receptors at synapses between CCK-positive interneurons in rat hippocampus. *J. Neurophysiol.* 98, 861–869. doi: 10.1152/jn.00156.2007

Allen, T. G., Abogadie, F. C., and Brown, D. A. (2006). Simultaneous release of glutamate and acetylcholine from single magnocellular "cholinergic" basal forebrain neurons. *J. Neurosci.* 26, 1588–1595. doi: 10.1523/jneurosci.3979-05.2006

Araque, A., Martín, E. D., Perea, G., Arellano, J. I., and Buño, W. (2002). Synaptically released acetylcholine evokes Ca2+ elevations in astrocytes in hippocampal slices. *J. Neurosci.* 22, 2443–2450.

Aso, E., and Ferrer, I. (2014). Cannabinoids for treatment of Alzheimer's disease: moving toward the clinic. *Front. Pharmacol.* 5:37. eCollection 2014. doi: 10.3389/fphar.2014.00037

Atasoy, D., Aponte, Y., Su, H. H., and Sternson, S. M. (2008). A FLEX switch targets channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. *J. Neurosci.* 28, 7025–7030. doi: 10.1523/JNEUROSCI.1954-08.2008

Axmacher, N., Mormann, F., Fernandez, G., Elger, C. E., and Fell, J. (2006). Memory formation by neuronal synchronization. *Brain Res. Rev.* 52, 170–182. doi: 10.1016/j.brainresrev.2006.01.007

Bartos, M., Alle, H., and Vida, I. (2011). Role of microcircuit structure and input integration in hippocampal interneuron recruitment and plasticity. *Neuropharmacology* 60, 730–739. doi: 10.1016/j.neuropharm.2010.12.017

Bartos, M., and Elgueta, C. (2012). Functional characteristics of parvalbumin- and cholecystokinin- expressing basket cells. *J. Physiol.* 590, 669–681. doi: 10.1113/jphysiol.2011.226175

Bayraktar, T., Staiger, J. F., Acsady, L., Cozzari, C., Freund, T. F., and Zilles, K. (1997). Co-localization of vasoactive intestinal polypeptide, gamma-aminobutyric acid and choline acetyltransferase in neocortical interneurons of the adult rat. *Brain Res.* 757, 209–217. doi: 10.1016/s0006-8993(97)00218-7

Bell, L. A., Bell, K. A., and McQuiston, A. R. (2013). Synaptic muscarinic response types in hippocampal CA1 interneurons depend on different levels of presynaptic activity and different muscarinic receptor subtypes. *Neuropharmacology* 73, 160–173. doi: 10.1016/j.neuropharm.2013.05.026

Bell, K. A., Shim, H., Chen, C. K., and McQuiston, A. R. (2011). Nicotinic excitatory postsynaptic potentials in hippocampal ca1 interneurons are predominantly mediated by nicotinic receptors that contain $\alpha 4$ and $\beta 2$ subunits. *Neuropharmacology* 61, 1379–1388. doi: 10.1016/j.neuropharm.2011.08.024

Bland, B. H., Colom, L. V., Konopacki, J., and Roth, S. H. (1988). Intracellular records of carbachol-induced theta rhythm in hippocampal slices. *Brain Res.* 447, 364–368. doi: 10.1016/0006-8993(88)91141-9

Bonner, T. I. (1989). New subtypes of muscarinic acetylcholine receptors. *Trends Pharmacol. Sci.* Suppl.15.

Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268. doi: 10.1038/nn1525

Bragin, A., Jando, G., Nadasdy, Z., Hetke, J., Wise, K., and Buzsaki, G. (1995). Gamma (40–100 hz) oscillation in the hippocampus of the behaving rat. *J. Neurosci.* 15, 47–60.

Brazhnik, E. S., and Fox, S. E. (1997). Intracellular recordings from medial septal neurons during hippocampal theta rhythm. *Exp. Brain Res.* 114, 442–453. doi: 10.1007/pl00005653

Broicher, T., Wettschureck, N., Munsch, T., Coulon, P., Meuth, S. G., Kanyshkova, T., et al. (2008). Muscarinic ACh receptor-mediated control of thalamic activity via G(q)/G (11)-family G-proteins. *Pflugers Arch.* 456, 1049–1060. doi: 10.1007/s00424-008-0473-x

Brown, D. A. (2010). Muscarinic acetylcholine receptors (mAChRs) in the nervous system: some functions and mechanisms. *J. Mol. Neurosci.* 41, 340–346. doi: 10.1007/s12031-010-9377-2

Brown, D. A., and Adams, P. R. (1980). Muscarinic suppression of a novel voltage-sensitive K+ current in a vertebrate neurone. *Nature* 283, 673–676. doi: 10.1038/283673a0

Bubser, M., Byun, N., Wood, M. R., and Jones, C. K. (2012). Muscarinic receptor pharmacology and circuitry for the modulation of cognition. *Handb. Exp. Pharmacol.* 208, 121–166. doi: 10.1007/978-3-642-23274-9_7

Busquets-Garcia, A., Gomis-González, M., Guegan, T., Agustín-Pavón, C., Pastor, A., Mato, S., et al. (2013). Targeting the endocannabinoid system in the treatment of fragile X syndrome. *Nat. Med.* 19, 603–607. doi: 10.1038/nm.3127

Buzsáki, G. (2002). Theta oscillations in the hippocampus. *Neuron* 33, 325–340. doi: 10.1016/s0896-6273(02)00586-x

Buzsáki, G. (2005). Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. *Hippocampus* 15, 827–840. doi: 10.1002/hipo.20113

Buzsáki, G., Anastassiou, C. A., and Koch, C. (2012). The origin of extracellular fields and currents—EEG, ECoG, LFP and spikes. *Nat. Rev. Neurosci.* 13, 407–420. doi: 10.1038/nrn3241

Carta, M., Lanore, F., Rebola, N., Szabo, Z., Da Silva, S. V., Lourenço, J., et al. (2014). Membrane lipids tune synaptic transmission by direct modulation of presynaptic potassium channels. *Neuron* 81, 787–799. doi: 10.1016/j.neuron.2013.12.028

Caulfield, M. P., and Birdsall, N. J. (1998). International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50, 279–290.

Cea-del Rio, C. A., Lawrence, J. J., Erdelyi, F., Szabo, G., and McBain, C. J. (2011). Cholinergic modulation amplifies the intrinsic oscillatory properties of CA1 hippocampal cholecystokinin-positive interneurons. *J. Physiol.* 589, 609–627. doi: 10.1113/jphysiol.2010.199422

Cea-del Rio, C. A., Lawrence, J. J., Tricoire, L., Erdelyi, F., Szabo, G., and McBain, C. J. (2010). M3 muscarinic acetylcholine receptor expression confers differential cholinergic modulation to neurochemically distinct hippocampal basket cell subtypes. *J. Neurosci.* 30, 6011–6024. doi: 10.1523/JNEUROSCI.5040-09.2010

Cea-del Rio, C. A., McBain, C. J., and Pelkey, K. A. (2012). An update on cholinergic regulation of cholecystokinin-expressing basket cells. *J. Physiol.* 590, 695–702. doi: 10.1113/jphysiol.2011.225342

Chapman, C. A., and Lacaille, J. C. (1999). Cholinergic induction of theta-frequency oscillations in hippocampal inhibitory interneurons and pacing of pyramidal cell firing. *J. Neurosci.* 19, 8637–8645.

Chédotal, A., Cozzari, C., Faure, M. P., Hartman, B. K., and Hamel, E. (1994). Distinct choline acetyltransferase (ChAT) and vasoactive intestinal polypeptide (VIP) bipolar neurons project to local blood vessels in the rat cerebral cortex. *Brain Res.* 646, 181–193. doi: 10.1016/0006-8993(94)90076-0

Cobb, S. R., Buhl, E. H., Halasy, K., Paulsen, O., and Somogyi, P. (1995). Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378, 75–78. doi: 10.1038/378075a0

Cobb, S. R., and Davies, C. H. (2005). Cholinergic modulation of hippocampal cells and circuits. *J. Physiol.* 562, 81–88. doi: 10.1113/jphysiol.2004.076539

Cole, A. E., and Nicoll, R. A. (1983). Acetylcholine mediates a slow synaptic potential in hippocampal pyramidal cells. *Science* 221, 1299–1301. doi: 10.1126/science.6612345

Colgin, L. L., Kramár, E. A., Gall, C. M., and Lynch, G. (2003). Septal modulation of excitatory transmission in hippocampus. *J. Neurophysiol.* 90, 2358–2366. doi: 10.1152/jn.00262.2003

Cruikshank, S. J., Hopperstad, M., Younger, M., Connors, B. W., Spray, D. C., and Srinivas, M. (2004). Potent block of Cx36 and Cx50 gap junction channels by mefloquine. *Proc. Natl. Acad. Sci. U S A* 101, 12364–12369. doi: 10.1073/pnas.0402044101

Csicsvari, J., Jamieson, B., Wise, K. D., and Buzsaki, G. (2003). Mechanisms of gamma oscillations in the hippocampus of the behaving rat. *Neuron* 37, 311–322. doi: 10.1016/S0896-6273(02)01169-8

Curley, A. A., and Lewis, D. A. (2012). Cortical basket cell dysfunction in schizophrenia. *J. Physiol.* 590, 715–724. doi: 10.1113/jphysiol.2011.224659

D'Antuono, M., Merlo, D., and Avoli, M. (2003). Involvement of cholinergic and gabaergic systems in the fragile x knockout mice. *Neuroscience* 119, 9–13. doi: 10.1016/s0306-4522(03)00103-9

Degroot, A., Kofalvi, A., Wade, M. R., Davis, R. J., Rodrigues, R. J., Rebola, N., et al. (2006). CB1 receptor antagonism increases hippocampal acetylcholine release: site and mechanism of action. *Mol. Pharmacol.* 70, 1236–1245. doi: 10.1124/mol.106.024661

Diana, M. A., and Marty, A. (2003). Characterization of depolarization-induced suppression of inhibition using paired interneuron—Purkinje cell recordings. *J. Neurosci.* 23, 5906–5918.

Dodd, J., Dingledine, R., and Kelly, J. S. (1981). The excitatory action of acetylcholine on hippocampal neurones of the guinea pig and rat maintained in vitro. *Brain Res.* 207, 109–127. doi: 10.1016/0006-8993(81)90682-x

Drake, C. T., and Milner, T. A. (2002). Mu opioid receptors are in discrete hippocampal interneuron subpopulations. *Hippocampus* 12, 119–136. doi: 10.1002/hipo.1107

Dringenberg, H. C., and Vanderwolf, C. H. (1998). Involvement of direct and indirect pathways in electrocorticographic activation. *Neurosci. Biobehav. Rev.* 22, 243–257. doi: 10.1016/s0149-7634(97)00012-2

Dugladze, T., Schmitz, D., Whittington, M. A., Vida, I., and Gloveli, T. (2012). Segregation of axonal and somatic activity during fast network oscillations. *Science* 336, 1458–1461. doi: 10.1126/science.1222017

Edwards, D. A., Kim, J., and Alger, B. E. (2006). Multiple mechanisms of endocannabinoid response initiation in hippocampus. *J. Neurophysiol.* 95, 67–75. doi: 10.1152/jn.00813.2005

Edwards, D. A., Zhang, L., and Alger, B. E. (2008). Metaplastic control of the endocannabinoid system at inhibitory synapses in hippocampus. *Proc. Natl. Acad. Sci. U S A* 105, 8142–8147. doi: 10.1073/pnas.0803558105

Eichenbaum, H. (2013). Memory on time. *Trends Cogn. Sci.* 17, 81–88. doi: 10.1016/j.tics.2012.12.007

Fenno, L., Yizhar, O., and Deisseroth, K. (2011). The development and application of optogenetics. *Annu. Rev. Neurosci.* 34, 389–412. doi: 10.1146/annurev-neuro-061010-113817

Fisahn, A., Pike, F. G., Buhl, E. H., and Paulsen, O. (1998). Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro. *Nature* 394, 186–189. doi: 10.1038/28179

Fisahn, A., Yamada, M., Duttaroy, A., Gan, J. W., Deng, C. X., McBain, C. J., et al. (2002). Muscarinic induction of hippocampal gamma oscillations requires coupling of the M1 receptor to two mixed cation currents. *Neuron* 33, 615–624. doi: 10.1016/s0896-6273(02)00587-1

Fischer, V., Both, M., Draguhn, A., and Egorov, A. V. (2014). Choline-mediated modulation of hippocampal sharp wave-ripple complexes in vitro. *J. Neurochem.* 129, 792–805. doi: 10.1111/jnc.12693

Földy, C., Lee, S. Y., Szabadics, J., Neu, A., and Soltesz, I. (2007). Cell type-specific gating of perisomatic inhibition by cholecystokinin. *Nat. Neurosci.* 10, 1128–1130. doi: 10.1038/nn1952

Fortin, D. A., Trettel, J., and Levine, E. S. (2004). Brief trains of action potentials enhance pyramidal neuron excitability via endocannabinoid-mediated suppression of inhibition. *J. Neurophysiol.* 92, 2105–2112. doi: 10.1152/jn.00351.2004

Freund, T. F., and Katona, I. (2007). Perisomatic inhibition. *Neuron* 56, 33–42. doi: 10.1016/j.neuron.2007.09.012

Fukudome, Y., Ohno-Shosaku, T., Matsui, M., Omori, Y., Fukaya, M., Tsubokawa, H., et al. (2004). Two distinct classes of muscarinic action on hippocampal inhibitory synapses: M2-mediated direct suppression and M1/M3-mediated indirect suppression through endocannabinoid signalling. *Eur. J. Neurosci.* 19, 2682–2692. doi: 10.1111/j.0953-816x.2004.03384.x

Galarreta, M., Erdélyi, F., Szabó, G., and Hestrin, S. (2004). Electrical coupling among irregular-spiking GABAergic interneurons expressing cannabinoid receptors. *J. Neurosci.* 24, 9770–9778. doi: 10.1523/jneurosci.3027-04.2004

Galarreta, M., and Hestrin, S. (1999). A network of fast-spiking cells in the neocortex connected by electrical synapses. *Nature* 402, 72–75. doi: 10.1038/47029

Gao, Y., Vasilyev, D. V., Goncalves, M. B., Howell, F. V., Hobbs, C., Reisenberg, M., et al. (2010). Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *J. Neurosci.* 30, 2017–2024. doi: 10.1523/JNEUROSCI.5693-09.2010

Gaykema, R. P., Luiten, P. G., Nyakas, C., and Traber, J. (1989). Cortical projection patterns of the medial septum-diagonal band complex. *J. Comp. Neurol.* 293, 103–124. doi: 10.1002/cne.902930109

Gibson, J. R., Beierlein, M., and Connors, B. W. (1999). Two networks of electrically coupled inhibitory neurons in neocortex. *Nature* 402, 75–79. doi: 10.1038/47035

Gifford, A. N., Ashby, C. R. Jr. (1996). Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 552122 and is potentiated by the cannabinoid antagonist, SR 141716A. *J. Pharmacol. Exp. Ther.* 277, 1431–1436.

Glickfeld, L. L., Atallah, B. V., and Scanziani, M. (2008). Complementary modulation of somatic inhibition by opioids and cannabinoids. *J. Neurosci.* 28, 1824–1832. doi: 10.1523/JNEUROSCI.4700-07.2008

Gu, Z., and Yakel, J. L. (2011). Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. *Neuron* 14, 155–165. doi: 10.1016/j.neuron.2011.04.026

Gulyás, A. I., Szabó, G. G., Ulbert, I., Holderith, N., Monyer, H., Erdélyi, F., et al. (2010). Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. *J. Neurosci.* 30, 15134–15145. doi: 10.1523/JNEUROSCI.4104-10.2010

Haj-Dahmane, S., and Andrade, R. (1999). Muscarinic receptors regulate two different calcium-dependent non-selective cation currents in rat prefrontal cortex. *Eur. J. Neurosci.* 11, 1973–1980. doi: 10.1046/j.1460-9568.1999.00612.x

Hájos, N., Pálhalmi, J., Mann, E. O., Németh, B., Paulsen, O., and Freund, T. F. (2004). Spike timing of distinct types of GABAergic interneuron during hippocampal gamma oscillations in vitro. *J. Neurosci.* 24, 9127–9137. doi: 10.1523/jneurosci.2113-04.2004

Hájos, N., Papp, E. C., Acsády, L., Levey, A. I., and Freund, T. F. (1998). Distinct interneuron types express m2 muscarinic receptor immunoreactivity on their dendrites or axon terminals in the hippocampus. *Neurosci.* 82, 355–376. doi: 10.1016/s0306-4522(97)00300-x

Hájos, N., and Paulsen, O. (2009). Network mechanisms of gamma oscillations in the CA3 region of the hippocampus. *Neural Netw.* 22, 1113–1119. doi: 10.1016/j.neunet.2009.07.024

Halassa, M. M., and Haydon, P. G. (2010). Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu. Rev. Physiol.* 72, 335–355. doi: 10.1146/annurev-physiol-021909-135843

Halliwell, J. V. (1990). Physiological mechanisms of cholinergic action in the hippocampus. *Prog. Brain Res.* 84, 255–272. doi: 10.1016/s0079-6123(08)60910-3

Hampson, R. E., Zhuang, S. Y., Weiner, J. L., and Deadwyler, S. A. (2003). Functional significance of cannabinoid-mediated, depolarization-induced suppression of inhibition (DSI) in the hippocampus. *J. Neurophysiol.* 90, 55–64. doi: 10.1152/jn.01161.2002

Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., et al. (2012). Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell* 148, 1039–1050. doi: 10.1016/j.cell.2012.01.037

Hashimotodani, Y., Ohno-Shosaku, T., Tsubokawa, H., Ogata, H., Emoto, K., Maejima, T., et al. (2005). Phospholipase Cbeta serves as a coincidence detector through its Ca2+ dependency for triggering retrograde endocannabinoid signal. *Neuron* 45, 257–268. doi: 10.1016/j.neuron.2005.01.004

Hentschke, H., Perkins, M. G., Pearce, R. A., and Banks, M. I. (2007). Muscarinic blockade weakens interaction of gamma with theta rhythms in mouse hippocampus. *Eur. J. Neurosci.* 26, 1642–1656. doi: 10.1111/j.1460-9568.2007.05779.x

Holderith, N., Nemeth, B., Papp, O. I., Veres, J. M., Nagy, G. A., and Hajos, N. (2011). Cannabinoids attenuate hippocampal gamma oscillations by suppressing excitatory synaptic input onto ca3 pyramidal neurons and fast spiking basket cells. *J. Physiol.* 589, 4921–4934. doi: 10.1113/jphysiol.2011.216259

Hopkins, T. J., Rupprecht, L. E., Hayes, M. R., Blendy, J. A., and Schmidt, H. D. (2012). Galantamine, an acetylcholinesterase inhibitor and positive allosteric modulator of nicotinic acetylcholine receptors, attenuates nicotine taking and seeking in rats. *Neuropsychopharmacology* 37, 2310–2321. doi: 10.1038/npp.2012.83

Huang, C. C., and Hsu, K. S. (2010). Activation of muscarinic acetylcholine receptors induces a nitric oxide-dependent long-term depression in rat medial prefrontal cortex. *Cereb. Cortex* 20, 982–996. doi: 10.1093/cercor/bhp161

Hull, C., Adesnik, H., and Scanziani, M. (2009). Neocortical disynatpic inhibition requires somatodendritic integration in interneurons. *J. Neurosci.* 29, 8991–8995. doi: 10.1523/JNEUROSCI.5717-08.2009

Iball, J., and Ali, A. B. (2011). Endocannabinoid release modulates electrical coupling between cck cells connected via chemical and electrical synapses in CA1. *Front. Neural Circuits* 5:17. doi: 10.3389/fncir.2011.00017

Jung, K. M., Sepers, M., Henstridge, C. M., Lassalle, O., Neuhofer, D., Martin, H., et al. (2012). Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. *Nat. Commun.* 3:1080. doi: 10.1038/ncomms2045

Kalmbach, A., Hedrick, T., and Waters, J. (2012). Selective optogenetic stimulation of cholinergic axons in neocortex. *J. Neurophysiol.* 107, 2008–2019. doi: 10.1152/jn.00870.2011

Kalmbach, A., and Waters, J. (2014). Modulation of high- and low-frequency components of the cortical local field potential via nicotinic and muscarinic acetylcholine receptors in anesthetized mice. *J. Neurophysiol.* 111, 258–272. doi: 10.1152/jn.00244.2013

Kano, M., Ohno-Shosaku, T., Hashimotodani, Y., Uchigashima, M., and Watanabe, M. (2009). Endocannabinoid-mediated control of synaptic transmission. *Physiol. Rev.* 89, 309–380. doi: 10.1152/physrev.00019.2008

Karson, M. A., Tang, A.-H., Milner, T. A., and Alger, B. E. (2009). Synaptic cross talk between perisomatic-targeting interneuron classes expressing cholecystokinin and parvalbumin in hippocampus. *J. Neurosci.* 29, 4140–4154. doi: 10.1523/JNEUROSCI.5264-08.2009

Karson, M. A., Whittington, K. C., and Alger, B. E. (2008). Cholecystokinin inhibits endocannabinoid-sensitive hippocampal IPSPs and stimulates others. *Neuropharmacology* 54, 117–128. doi: 10.1016/j.neuropharm.2007.06.023

Kathmann, M., Weber, B., Zimmer, A., and Schlicker, E. (2001). Enhanced acetylcholine release in the hippocampus of cannabinoid CB(1) receptor-deficient mice. *Br. J. Pharmacol.* 132, 1169–1173. doi: 10.1038/sj.bjp.0703987

Katona, I., Urbán, G. M., Wallace, M., Ledent, C., Jung, K. M., Piomelli, D., et al. (2006). Molecular composition of the endocannabinoid system at glutamatergic synapses. *J. Neurosci.* 26, 5628–5637. doi: 10.1523/jneurosci.0309-06.2006

Kawaguchi, Y. (1997). Selective cholinergic modulation of cortical GABAergic cell subtypes. *J. Neurophysiol.* 78, 1743–1747.

Kilb, W., and Luhmann, H. J. (2003). Carbachol-induced network oscillations in the intact cerebral cortex of the newborn rat. *Cereb. Cortex* 13, 409–421. doi: 10.1093/cercor/13.4.409

Kim, J., Isokawa, M., Ledent, C., and Alger, B. E. (2002). Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. *J. Neurosci.* 22, 10182–10191.

Klausberger, T., Magill, P. J., Márton, L. F., Roberts, J. D., Cobden, P. M., Buzsáki, G., et al. (2003). Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. *Nature* 421, 844–848. doi: 10.1038/nature01374

Klausberger, T., and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* 321, 53–57. doi: 10.1126/science.1149381

Knox, D., and Berntson, G. G. (2008). Cortical modulation by nucleus basalis magnocellularis corticopetal cholinergic neurons during anxiety-like states is reflected by decreases in delta. *Brain Res.* 1227, 142–152. doi: 10.1016/j.brainres.2008.06.060

Kolisnyk, B., Guzman, M. S., Raulic, S., Fan, J., Magalhães, A. C., Feng, G., et al. (2013). ChAT-ChR2-EYFP mice have enhanced motor endurance but show deficits in attention and several additional cognitive domains. *J. Neurosci.* 33, 10427–10438. doi: 10.1523/JNEUROSCI.0395-13.2013

Konopacki, J., and Goebiewski, H. (1992). Theta rhythms in the rat medial entorhinal cortex in vitro: evidence for involvement of muscarinic receptors. *Neurosci. Lett.* 141, 93–96. doi: 10.1016/0304-3940(92)90342-5

Korotkova, T., Fuchs, E. C., Ponomarenko, A., von Engelhardt, J., and Monyer, H. (2010). NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations and working memory. *Neuron* 68, 557–569. doi: 10.1016/j.neuron.2010.09.017

Kreitzer, A. C., and Regher, W. G. (2001). Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29, 717–727. doi: 10.1016/s0896-6273(01)00246-x

Krook-Magnuson, E., Luu, L., Lee, S. H., Varga, C., and Soltesz, I. (2011). Ivy and neurogliaform interneurons are a major target of μ-opioid receptor modulation. *J. Neurosci.* 31, 14861–14870. doi: 10.1523/JNEUROSCI.2269-11.2011

Lafourcade, M., Elezgarai, I., Mato, S., Bakiri, Y., Grandes, P., and Manzoni, O. J. (2007). Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex. *PLoS One* 2:e709. doi: 10.1371/journal.pone.0000709

Lamotte d'Incamps, B., and Ascher, P. (2008). Four excitatory postsynaptic ionotropic receptors coactivated at the motoneuron-Renshaw cell synapse. *J. Neurosci.* 28, 14121–14131. doi: 10.1523/JNEUROSCI.3311-08.2008

Larson, J., and Lynch, G. (1986). Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. *Science* 232, 985–988. doi: 10.1126/science.3704635

Lawrence, J. J. (2008). Cholinergic control of GABA release: emerging parallels between neocortex and hippocampus. *Trends Neurosci.* 31, 317–327. doi: 10.1016/j.tins.2008.03.008

Lawrence, J. J., Grinspan, Z. M., Statland, J. M., and McBain, C. J. (2006a). Muscarinic receptor activation tunes mouse stratum oriens interneurones to amplify spike reliability. *J. Physiol.* 571, 555–562. doi: 10.1113/jphysiol.2005.103218

Lawrence, J. J., Statland, J. M., Grinspan, Z. M., and McBain, C. J. (2006b). Cell type-specific dependence of muscarinic signalling in mouse hippocampal stratum oriens interneurones. *J. Physiol.* 570, 595–610. doi: 10.1113/jphysiol.2005.100875

Lee, M. G., Chrobak, J. J., Sik, A., Wiley, R. G., and Buzsaki, G. (1994). Hippocampal theta activity following selective lesion of the septal cholinergic system. *Neuroscience* 62, 1033–1047. doi: 10.1016/0306-4522(94)90341-7

Leung, L. W. (1985). Spectral analysis of hippocampal EEG in the freely moving rat: effects of centrally active drugs and relations to evoked potentials. *Electroencephalogr. Clin. Neurophysiol.* 60, 65–77. doi: 10.1016/0013-4694(85)90952-6

Levey, A. I., Edmunds, S. M., Koliatsos, V., Wiley, R. G., and Heilman, C. J. (1995). Expression of m1–m4 muscarinic acetylcholine receptor proteins in rat hippocampus and regulation by cholinergic innervation. *J. Neurosci.* 15, 4077–4092.

Lewis, P. R., and Shute, C. C. (1967). The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system and the subfornical organ and supra-optic crest. *Brain* 90, 521–540. doi: 10.1093/brain/90.3.521

Losonczy, A., Biró, A. A., and Nusser, Z. (2004). Persistently active cannabinoid receptors mute a subpopulation of hippocampal interneurons. *Proc. Natl. Acad. Sci. U S A* 101, 1362–1367. doi: 10.1073/pnas.0304752101

Lukatch, H. S., and MacIver, M. B. (1997). Physiology, pharmacology and topography of cholinergic neocortical oscillations in vitro. *J. Neurophysiol.* 77, 2427–2445.

Lund, J. S., and Lewis, D. A. (1993). Local circuit neurons of developing and mature macaque prefrontal cortex: golgi and immunocytochemical characteristics. *J. Comp. Neurol.* 328, 282–312. doi: 10.1002/cne.903280209

Maccarrone, M., Rossi, S., Bari, M., De Chiara, V., Rapino, C., Musella, A., et al. (2010). Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. *Neuropsychopharmacology* 35, 1500–1509. doi: 10.1038/npp.2010.19

Maejima, T., Hashimoto, K., Yoshida, T., Aiba, A., and Kano, M. (2001). Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron* 31, 463–475. doi: 10.1016/s0896-6273(01)00375-0

Maejima, T., Oka, S., Hashimotodani, Y., Ohno-Shosaku, T., Aiba, A., Wu, D., et al. (2005). Synaptically driven endocannabinoid release requires Ca2+-assisted metabotropic glutamate receptor subtype 1 to phospholipase Cbeta4 signaling cascade in the cerebellum. *J. Neurosci.* 25, 6826–6835. doi: 10.1523/jneurosci.0945-05.2005

Makara, J. K., Katona, I., Nyíri, G., Németh, B., Ledent, C., Watanabe, M., et al. (2007). Involvement of nitric oxide in depolarization-induced suppression of inhibition in hippocampal pyramidal cells during activation of cholinergic receptors. *J. Neurosci.* 27, 10211–10222. doi: 10.1523/jneurosci.2104-07.2007

Malomouzh, A. I., Mukhtarov, M. R., Nikolsky, E. E., and Vyskocil, F. (2007). Muscarinic M1 acetylcholine receptors regulate the non-quantal release of acetylcholine in the rat neuromuscular junction via NO-dependent mechanism. *J. Neurochem.* 102, 2110–2117. doi: 10.1111/j.1471-4159.2007.04696.x

Mann, E. O., and Paulsen, O. (2007). Role of GABAergic inhibition in hippocampal network oscillations. *Trends Neurosci.* 30, 343–349. doi: 10.1016/j.tins.2007.05.003

Manzanares, J., Corchero, J., Romero, J., Fernandez-Ruiz, J. J., Ramos, J. A., and Fuentes, J. A. (1999). Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends Pharmacol. Sci.* 20, 287–294. doi: 10.1016/s0165-6147(99)01339-5

Martin, L. A., and Alger, B. E. (1999). Muscarinic facilitation of the occurrence of depolarizationinduced suppression of inhibition in rat hippocampus. *Neuroscience* 1999, 61–71. doi: 10.1016/s0306-4522(98)00745-3

Mattis, J., Tye, K. M., Ferenczi, E. A., Ramakrishnan, C., O'Shea, D. J., Prakash, R., et al. (2011). Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. *Nat. Methods* 9, 159–172. doi: 10.1038/nmeth.1808

McCormick, D. A., and Prince, D. A. (1986). Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex in vitro. *J. Physiol.* 375, 169–194.

McQuiston, A. R., and Madison, D. V. (1999a). Muscarinic receptor activity has multiple effects on the resting membrane potentials of CA1 hippocampal interneurons. *J. Neurosci.* 19, 5693–5702.

McQuiston, A. R., and Madison, D. V. (1999b). Muscarinic receptor activity induces an afterdepolarization in a subpopulation of hippocampal CA1 interneurons. *J. Neurosci.* 19, 5703–5710.

Min, R., and Nevian, T. (2012). Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. *Nat. Neurosci.* 15, 746–753. doi: 10.1038/nn.3075

Morishita, W., Kirov, S. A., and Alger, B. E. (1998). Evidence for metabotropic glutamate receptor activation in the induction of depolarization-induced suppression of inhibition in hippocampal CA1. *J. Neurosci.* 18, 4870–4882.

Morishita, W., Kirov, S. A., Pitler, T. A., Martin, L. A., Lenz, R. A., and Alger, B. E. (1997). N-ethylmaleimide blocks depolarization-induced suppression of inhibition and enhances GABA release in the rat hippocampal slice in vitro. *J. Neurosci.* 17, 941–950.

Nagode, D. A., Tang, A.-H., Karson, M. A., Klugmann, M., and Alger, B. E. (2011). Optogenetic release of ACh induces rhythmic bursts of perisomatic ipscs in hippocampus. *PLoS One* 6:e27691. doi: 10.1371/journal.pone.0027691

Nagode, D. A., Tang, A. H., Yang, K., and Alger, B. E. (2014). Optogenetic identification of an intrinsic cholinergically driven inhibitory oscillator sensitive to cannabinoids and opioids in hippocampal CA1. *J. Physiol.* 592, 103–123. doi: 10.1113/jphysiol.2013.257428

Narushima, M., Uchigashima, M., Fukaya, M., Matsui, M., Manabe, T., Hashimoto, K., et al. (2007). Tonic enhancement of endocannabinoid-mediated retrograde suppression of inhibition by cholinergic interneuron activity in the striatum. *J. Neurosci.* 27, 496–506. doi: 10.1523/jneurosci.4644-06.2007

Nathanson, J. L., Yanagawa, Y., Obata, K., and Callaway, E. M. (2009). Preferential labeling of inhibitory and excitatory cortical neurons by endogenous tropism of adeno-associated virus and lentivirus vectors. *Neuroscience* 161, 441–450. doi: 10.1016/j.neuroscience.2009.03.032

Naumann, T., Kermer, P., and Frotscher, M. (1994). Fine structure of rat septohippocampal neurons. III. Recovery of choline acetyltransferase immunoreactivity after fimbria-fornix transection. *J. Comp. Neurol.* 350, 161–170. doi: 10.1002/cne.903500202

Navarrete, M., and Araque, A. (2008). Endocannabinoids mediate neuron-astrocyte communication. *Neuron* 57, 883–893. doi: 10.1016/j.neuron.2008.01.029

Navarrete, M., Perea, G., Fernandez de Sevilla, D., Gómez-Gonzalo, M., Núñez, A., Martín, E. D., et al. (2012). Astrocytes mediate in vivo cholinergic-induced synaptic plasticity. *PLoS Biol.* 10:e1001259. doi: 10.1371/journal.pbio.1001259

Nelson, A. B., Hammack, N., Yang, C. F., Shah, N. M., Seal, R. P., and Kreitzer, A. C. (2014). Striatal cholinergic interneurons drive GABA release from dopamine terminals. *Neuron* 82, 63–70. doi: 10.1016/j.neuron.2014.01.023

Neu, A., Foldy, C., and Soltesz, I. (2007). Postsynaptic origin of CB1-dependent tonic inhibition of GABA release at cholecystokinin-positive basket cell to

pyramidal cell synapses in the ca1 region of the rat hippocampus. *J. Physiol.* 578, 233–247. doi: 10.1113/jphysiol.2006.115691

Newman, E. L., Climer, J. R., and Hasselmo, M. E. (2014). Grid cell spatial tuning reduced following systemic muscarinic receptor blockade. *Hippocampus* 24, 643–655. doi: 10.1002/hipo.22253

Newman, Z., Malik, P., Wu, T. Y., Ochoa, C., Watsa, N., and Lindgren, C. (2007). Endocannabinoids mediate muscarine-induced synaptic depression at the vertebrate neuromuscular junction. *Eur. J. Neurosci.* 25, 1619–1630. doi: 10.1111/j.1460-9568.2007.05422.x

Ohno-Shosaku, T., Maejima, T., and Kano, M. (2001). Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 29, 729–738. doi: 10.1016/s0896-6273(01)00247-1

Ohno-Shosaku, T., Matsui, M., Fukudome, Y., Shosaku, J., Tsubokawa, H., Taketo, M. M., et al. (2003). Postsynaptic M1 and M3 receptors are responsible for the muscarinic enhancement of retrograde endocannabinoid signalling in the hippocampus. *Eur. J. Neurosci.* 18, 109–116. doi: 10.1046/j.1460-9568.2003.02732.x

Oke, O. O., Magony, A., Anver, H., Ward, P. D., Jiruska, P., Jefferys, J. G., et al. (2010). High-frequency gamma oscillations coexist with low-frequency gamma oscillations in the rat visual cortex in vitro. *Eur. J. Neurosci.* 31, 1435–1445. doi: 10.1111/j.1460-9568.2010.07171.x

Oren, I., Hajos, N., and Paulsen, O. (2010). Identification of the current generator underlying cholinergically induced gamma frequency field potential oscillations in the hippocampal CA3 region. *J. Physiol.* 588, 785–797. doi: 10.1113/jphysiol.2009.180851

Oren, I., Mann, E. O., Paulsen, O., and Hajos, N. (2006). Synaptic currents in anatomically identified CA3 neurons during hippocampal gamma oscillations in vitro. *J. Neurosci.* 26, 9923–9934. doi: 10.1523/jneurosci.1580-06.2006

Pap, P., Koszeghy, A., Szucs, G., and Rusznák, Z. (2009). Cytoplasmic Ca(2+) concentration changes evoked by cholinergic stimulation in primary astrocyte cultures prepared from the rat cochlear nucleus. *Hear. Res.* 255, 73–83. doi: 10.1016/j.heares.2009.05.006

Pereda, A. E. (2014). Electrical synapses and their functional interactions with chemical synapses. *Nat. Rev. Neurosci.* 15, 250–263. doi: 10.1038/nrn3708

Petreanu, L., Huber, D., Sobczyk, A., and Svoboda, K. (2007). Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. *Nat. Neurosci.* 10, 663–668. doi: 10.1038/nn1891

Pietersen, A. N., Ward, P. D., Hagger-Vaughan, N., Wiggins, J., Jefferys, J. G., and Vreugdenhil, M. (2014). Transition between fast and slow gamma modes in rat hippocampus area CA1 in vitro is modulated by slow CA3 gamma oscillations. *J. Physiol.* 592, 605–620. doi: 10.1113/jphysiol.2013.263889

Pinto, L., Goard, M. J., Estandian, D., Xu, M., Kwan, A. C., Lee, S. H., et al. (2013). Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat. Neurosci.* 16, 1857–1863. doi: 10.1038/nn.3552

Pitler, T. A., and Alger, B. E. (1990). Activation of the pharmacologically defined M3 muscarinic receptor depolarizes hippocampal pyramidal cells. *Brain Res.* 534, 257–262. doi: 10.1016/0006-8993(90)90137-z

Pitler, T. A., and Alger, B. E. (1992a). Postsynaptic spike firing reduces synaptic GABAA responses in hippocampal pyramidal cells. *J. Neurosci.* 12, 4122–4132.

Pitler, T. A., and Alger, B. E. (1992b). Cholinergic excitation of GABAergic interneurons in the rat hippocampal slice. *J. Physiol.* 450, 127–142.

Povysheva, N. V., Zaitsev, A. V., Gonzalez-Burgos, G., and Lewis, D. A. (2013). Electrophysiological heterogeneity of fast-spiking interneurons: chandelier versus basket cells. *PLoS One* 12:e70553. doi: 10.1371/journal.pone.0070553

Ramírez, B. G., Blazquez, C., Gomez del Pulgar, T., Guzman, M., and de Ceballos, M. L. (2005). Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J. Neurosci.* 25, 1904–1913. doi: 10.1523/jneurosci.4540-04.2005

Reich, C. G., Karson, M. A., Karnup, S. V., Jones, L., and Alger, B. E. (2005). Regulation of IPSP theta rhythms by muscarinic receptors and endocannabinoids in hippocampus. *J. Neurophysiol.* 94, 4290–4299. doi: 10.1152/jn.00480.2005

Ren, J., Qin, C., Hu, F., Tan, J., Qiu, L., Zhao, S., et al. (2011). Habenula "cholinergic" neurons co-release glutamate and acetylcholine and activate postsynaptic neurons via distinct transmission modes. *Neuron* 69, 445–452. doi: 10.1016/j.neuron.2010.12.038

Rodriguez, R., Kallenbach, U., Singer, W., and Munk, M. H. (2004). Short- and long-term effects of cholinergic modulation on gamma oscillations and response synchronization in the visual cortex. *J. Neurosci.* 24, 10369–10378. doi: 10.1523/jneurosci.1839-04.2004

Sanchez-Alavez, M., Robledo, P., Wills, D. N., Havstad, J., and Ehlers, C. L. (2014). Cholinergic modulation of event-related oscillations (ERO). *Brain Res.* 1559, 11–25. doi: 10.1016/j.brainres.2014.02.043

Sarter, M., Parikh, V., and Howe, W. M. (2009). Phasic acetylcholine release and the volume transmission hypothesis: time to move on. *Nat. Rev. Neurosci.* 10, 383–390. doi: 10.1038/nrn2635

Singer, W., and Gray, C. M. (1995). Visual feature integration and the temporal correlation hypothesis. *Annu. Rev. Neurosci.* 18, 555–586. doi: 10.1146/annurev.neuro.18.1.555

Stewart, M., and Fox, S. E. (1989). Detection of an atropine-resistant component of the hippocampal theta rhythm in urethane-anesthetized rats. *Brain Res.* 500, 55–60. doi: 10.1016/0006-8993(89)90299-0

Stewart, M., and Fox, S. E. (1990). Do septal neurons pace the hippocampal theta rhythm? *Trends Neurosci.* 13, 163–168. doi: 10.1016/0166-2236(90)90040-h

Sul, J. Y., Orosz, G., Givens, R. S., and Haydon, P. G. (2004). Astrocytic connectivity in the hippocampus. *Neuron Glia Biol.* 1, 3–11. doi: 10.1017/s1740925x04000031

Takata, N., Mishima, T., Hisatsune, C., Nagai, T., Ebisui, E., Mikoshiba, K., et al. (2011). Astrocyte calcium signaling transforms cholinergic modulation to cortical plasticity in vivo. *J. Neurosci.* 31, 18155–18165. doi: 10.1523/JNEUROSCI.5289-11.2011

Tang, A. H., Karson, M. A., Nagode, D. A., McIntosh, J. M., Uebele, V. N., Renger, J. J., et al. (2011). Nerve terminal nicotinic acetylcholine receptors initiate quantal GABA release from perisomatic interneurons by activating axonal T-type (Cav3) Ca^{2+} channels and Ca^{2+} release from stores. *J. Neurosci.* 31, 13546–13561. doi: 10.1523/JNEUROSCI.2781-11.2011

Tanimura, A., Yamazaki, M., Hashimotodani, Y., Uchigashima, M., Kawata, S., Abe, M., et al. (2010). The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. *Neuron* 65, 320–327. doi: 10.1016/j.neuron.2010.01.021

Tóth, K., Freund, T. F., and Miles, R. (1997). Disinhibition of rat hippocampal pyramidal cells by GABAergic afferents from the septum. *J. Physiol.* 500, 463–474.

Tort, A. B., Komorowski, R. W., Manns, J. R., Kopell, N. J., and Eichenbaum, H. (2009). Theta-gamma coupling increases during the learning of item-context associations. *Proc. Natl. Acad. Sci. U S A* 106, 20942–20947. doi: 10.1073/pnas.0911331106

Traub, R. D., Cunningham, M. O., Gloveli, T., LeBeau, F. E., Bibbig, A., Buhl, E. H., et al. (2003). GABA-enhanced collective behavior in neuronal axons underlies persistent gamma-frequency oscillations. *Proc. Natl. Acad. Sci. U S A* 100, 11047–11052. doi: 10.1073/pnas.1934854100

Trettel, J., Fortin, D. A., and Levine, E. S. (2004). Endocannabinoid signalling selectively targets perisomatic inhibitory inputs to pyramidal neurones in juvenile mouse neocortex. *J. Physiol.* 556, 95–107. doi: 10.1113/jphysiol.2003.058198

Tzavara, E. T., Wade, M., and Nomikos, G. G. (2003). Biphasic effects of cannabinoids on acetylcholine release in the hippocampus: site and mechanism of action. *J. Neurosci.* 23, 9374–9384.

van Aerde, K. I., Mann, E. O., Canto, C. B., Heistek, T. S., Linkenkaer-Hansen, K., Mulder, A. B., et al. (2009). Flexible spike timing of layer 5 neurons during dynamic beta oscillation shifts in rat prefrontal cortex. *J. Physiol.* 587, 5177–5196. doi: 10.1113/jphysiol.2009.178384

van der Zee, E. A., and Luiten, P. G. M. (1999). Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: a review of immunocytochemical localization in relation to learning and memory. *Prog. Neurobiol.* 58, 409–471. doi: 10.1016/s0301-0082(98)00092-6

Varma, N., Brager, D., Morishita, W., Lenz, R. A., London, B., and Alger, B. E. (2002). Presynaptic factors in the regulation of DSI expression in hippocampus. *Neuropharmacology* 43, 550–562. doi: 10.1016/s0028-3908(02)00168-5

Varma, N., Carlson, G. C., Ledent, C., and Alger, B. E. (2001). Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J. Neurosci.* 21, RC188.

Veeraragavan, S., Bui, N., Perkins, J. R., Yuva-Paylor, L. A., and Paylor, R. (2011a). The modulation of fragile X behaviors by the muscarinic M4 antagonist, tropicamide. *Behav. Neurosci.* 125, 783–790. doi: 10.1037/a0025202

Veeraragavan, S., Bui, N., Perkins, J. R., Yuva-Paylor, L. A., Carpenter, R. L., and Paylor, R. (2011b). Modulation of behavioral phenotypes by a muscarinic M1 antagonist in a mouse model of fragile X syndrome. *Psychopharmacology (Berl)* 217, 143–151. doi: 10.1007/s00213-011-2276-6

Vizi, E. S., and Kiss, J. P. (1998). Neurochemistry and pharmacology of the major hippocampal transmitter systems: synaptic and nonsynaptic interactions. *Hippocampus* 8, 566–607. doi: 10.1002/(sici)1098-1063(1998)8:6<566::aid-hipo2>3.0.co;2-w

Volk, L. J., Pfeiffer, B. E., Gibson, J. R., and Huber, K. M. (2007). Multiple Gq-coupled receptors converge on a common protein synthesis-dependent long-term depression that is affected in fragile x syndrome mental retardation. *J. Neurosci.* 27, 11624–11634. doi: 10.1523/jneurosci.2266-07.2007

Wang, X. J. (2010). Neurophysiological and computational principles of cortical rhythms in cognition. *Physiol. Rev.* 90, 1195–1268. doi: 10.1152/physrev.00035.2008

Whittington, M. A., Cunningham, M. O., LeBeau, F. E., Racca, C., and Traub, R. D. (2011). Multiple origins of the cortical γ rhythm. *Dev. Neurobiol.* 71, 92–106. doi: 10.1002/dneu.20814

Whittington, M. A., and Traub, R. D. (2003). Interneuron diversity series: inhibitory interneurons and network oscillations in vitro. *Trends Neurosci.* 26, 676–682. doi: 10.1016/j.tins.2003.09.016

Whittington, M. A., Traub, R. D., Faulkner, H. J., Jefferys, J. G., and Chettiar, K. (1998). Morphine disrupts long-range synchrony of gamma oscillations in hippocampal slices. *Proc. Natl. Acad. Sci. U S A* 95, 5807–5811. doi: 10.1073/pnas.95.10.5807

Widmer, H., Ferrigan, L., Davies, C. H., and Cobb, S. R. (2006). Evoked slow muscarinic acetylcholinergic synaptic potentials in rat hippocampal interneurons. *Hippocampus* 16, 617–628. doi: 10.1002/hipo.20191

Williams, J. H., and Kauer, J. A. (1997). Properties of carbachol-induced oscillatory activity in rat hippocampus. *J. Neurophysiol.* 78, 2631–2640.

Wilson, R. I., and Nicoll, R. A. (2001). Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. *Nature* 410, 588–592. doi: 10.1038/35069076

Winson, J. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science* 201, 160–163. doi: 10.1126/science.663646

Woolf, N. J. (1991). Cholinergic systems in mammalian brain and spinal cord. *Prog. Neurobiol.* 37, 475–524. doi: 10.1016/0301-0082(91)90006-m

Yamada-Hanff, J., and Bean, B. P. (2013). Persistent sodium current drives conditional pacemaking in CA1 pyramidal neurons under muscarinic stimulation. *J. Neurosci.* 33, 15011–15021. doi: 10.1523/JNEUROSCI.0577-13.2013

Yamasaki, M., Matsui, M., and Watanabe, M. (2010). Preferential localization of muscarinic M1 receptor on dendritic shaft and spine of cortical pyramidal cells and its anatomical evidence for volume transmission. *J. Neurosci.* 30, 4408–4418. doi: 10.1523/JNEUROSCI.5719-09.2010

Yamawaki, N., Stanford, I. M., Hall, S. D., and Woodhall, G. L. (2008). Pharmacologically induced and stimulus evoked rhythmic neuronal oscillatory activity in the primary motor cortex in vitro. *Neuroscience* 151, 386–395. doi: 10.1016/j.neuroscience.2007.10.021

Yoshino, H., Miyamae, T., Hansen, G., Zambrowicz, B., Flynn, M., Pedicord, D., et al. (2011). Postsynaptic diacylglycerol lipase mediates retrograde endocannabinoid suppression of inhibition in mouse prefrontal cortex. *J. Physiol.* 589, 4857–4884. doi: 10.1113/jphysiol.2011.212225

Zhang, L., and Alger, B. E. (2010). Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. *J. Neurosci.* 30, 5724–5729. doi: 10.1523/JNEUROSCI.0795-10.2010

Zhang, H., Lin, S. C., and Nicolelis, M. A. (2010). Spatiotemporal coupling between hippocampal acetylcholine release and theta oscillations in vivo. *J. Neurosci.* 30, 13431–13440. doi: 10.1523/JNEUROSCI.1144-10.2010

Zoli, M., Jansson, A., Syková, E., Agnati, L. F., and Fuxe, K. (1999). Volume transmission in the CNS and its relevance for neuropsychopharmacology. *Trends Pharmacol. Sci.* 20, 142–150. doi: 10.1016/s0165-6147(99)01343-7

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Acetylcholine release and inhibitory interneuron activity in hippocampal CA1

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Acetylcholine release in the central nervous system (CNS) has an important role in attention, recall, and memory formation. One region influenced by acetylcholine is the hippocampus, which receives inputs from the medial septum and diagonal band of Broca complex (MS/DBB). Release of acetylcholine from the MS/DBB can directly affect several elements of the hippocampus including glutamatergic and GABAergic neurons, presynaptic terminals, postsynaptic receptors, and astrocytes. A significant portion of acetylcholine's effect likely results from the modulation of GABAergic inhibitory interneurons, which have crucial roles in controlling excitatory inputs, synaptic integration, rhythmic coordination of principal neurons, and outputs in the hippocampus. Acetylcholine affects interneuron function in large part by altering their membrane potential via muscarinic and nicotinic receptor activation. This minireview describes recent data from mouse hippocampus that investigated changes in CA1 interneuron membrane potentials following acetylcholine release. The interneuron subtypes affected, the receptor subtypes activated, and the potential outcome on hippocampal CA1 network function is discussed.

Keywords: hippocampus, acetylcholine, muscarinic, nicotinic, inhibitory interneuron

INTRODUCTION

Acetylcholine is released throughout the mammalian central nervous system (CNS) where it impacts global brain function by affecting sleep-wake cycles, attention, and memory formation. One region of the brain heavily innervated by cholinergic afferents from the medial septum and diagonal band of Broca complex (MS/DBB) is the hippocampus (Dutar et al., 1995). Functionally, acetylcholine release in the hippocampus has been proposed to aid in the formation or retrieval of memories depending on the extracellular concentration of acetylcholine (Power et al., 2003; Hasselmo and Giocomo, 2006; Kenney and Gould, 2008; Deiana et al., 2011; Hasselmo and Sarter, 2011; Easton et al., 2012; Blake et al., 2014). The mechanism by which MS/DBB cholinergic terminals affect hippocampal network function is through the activation of both muscarinic and nicotinic receptors located on dendrites, cell bodies, and axon terminals of pyramidal neurons and inhibitory interneurons, as well as on astrocytes (Cobb and Davies, 2005; Teles-Grilo Ruivo and Mellor, 2013). Although acetylcholine affects multiple sites on several different cell types, a portion of its influence likely arises from its effects on interneuron function.

Inhibitory interneurons play a crucial role in information processing in the hippocampus. Interneurons are very diverse in anatomical structure and presumed function (Freund and Buzsaki, 1996; Klausberger and Somogyi, 2008). Depending on the interneuron subtype and where it innervates the pyramidal cell, an individual interneuron can completely block activity in a dendrite, change action potential firing phase at the soma, or completely prevent action potential firing at the pyramidal cell body (Miles et al., 1996; Larkum et al., 1999). At the network level, interneurons contribute to the generation of synchronous activity among populations of principal neurons at a variety of behaviorally relevant frequencies (Buzsaki, 2002; Buzsaki and Wang, 2012). Given the significant impact individual interneurons have on neuronal network function, it is probable that a considerable proportion of acetylcholine's influence on hippocampal activity arises through interneuron modulation. Although cholinergic receptors have been shown to affect inhibitory presynaptic terminals (Behrends and Ten Bruggencate, 1993; Tang et al., 2011) and interneuron excitability (McQuiston and Madison, 1999b; Griguoli et al., 2009; Cea-Del Rio et al., 2010, 2011), this minireview will limit its focus to recent studies that have investigated the effect of acetylcholine release on changes in interneuron membrane potential, specifically in hippocampal CA1.

MS/DBB CHOLINERGIC NEURON ACTIVITY AND ACETYLCHOLINE RELEASE IN HIPPOCAMPAL CA1

The impact that acetylcholine release has in hippocampal CA1 and the extent to which different interneuron subtypes are affected will depend on the specific location and density of cholinergic axon terminals as well as its inactivating enzyme, acetylcholinesterase. Notably, both cholinergic fibers and acetylcholinesterase have been shown to be differentially distributed across layers in hippocampal CA1. In mouse, cholinergic fibers were shown to be evenly distributed except for two bands of higher density in the stratum pyamidale (SP) and at the border between the stratum radiatum (SR) and stratum lacunosum-moleculare (SLM) (Aznavour et al., 2002). In rat, similar higher density bands were observed in the SP and at the border of SR and SLM. However, compared to the stratum oriens (SO),

lower densities were seen in the SR and even lower densities in SLM (SO > SR > SLM) (Aznavour et al., 2002). The distribution of acetylcholinesterase in hippocampal CA1 complements that of cholinergic input, with higher densities observed between SP and SO as well as another peak in SLM near the border with SR (Storm-Mathisen, 1970). Consistent with these anatomical data, measurements of increased acetylcholine release during theta rhythms have shown that acetylcholine concentrations were highest near the stratum pyramidale (Zhang et al., 2010). This differential distribution of cholinergic fibers and extracellular acetylcholine levels is particularly important when considering that not all cholinergic terminals in the hippocampus appear to transmit acetylcholine synaptically. In both the hippocampus and neocortex, 85–93% of cholinergic axon terminals were estimated to have no postsynaptic specialization and thus the majority of cholinergic terminals were proposed to transmit acetylcholine by volume or non-synaptic transmission (Umbriaco et al., 1994, 1995). However, other groups have estimated that the majority of cholinergic terminals (66–67%) in the neocortex make classical synaptic connections (Smiley et al., 1997; Turrini et al., 2001). Regardless of this discrepancy, a significant portion of terminals appear to release acetylcholine into the extracellular space in a paracrine-like manner. This requires terminally released acetylcholine to diffuse significant distances past acetylcholinesterase to bind to receptors on postsynaptic elements. Thus, regions or layers with favorable densities of cholinergic terminals (higher) and/or acetylcholinesterase (lower) may result in larger extracellular concentrations of acetylcholine that may be more effective at transmitting acetylcholine through volume transmission. Furthermore, it is possible that there is a subset of terminals that are more active, have a higher probability of release, or may release more neurotransmitter. These terminals may be more effective at mediating volume transmission and influencing nearby inhibitory interneurons.

Acetylcholine release from cholinergic terminals will depend on the activity of the cholinergic neurons in the MS/DBB. However, the firing patterns of MS/DBB cholinergic neurons reported in the literature have shown some variability (Barrenechea et al., 1995; Brazhnik and Fox, 1997, 1999; Simon et al., 2006). A small number of anatomically identified MS/DBB cholinergic neurons recorded in awake restrained rodents have been reported to have low irregular firing rates (<2 Hz) (Simon et al., 2006). In contrast, anatomically unidentified neurons with action potential waveforms consistent with MS/DBB cholinergic neurons have been reported to fire at rates up to 30 Hz (Brazhnik and Fox, 1999). Thus, it remains unclear which rates best describe the firing patterns of cholinergic neurons in the MS/DBB or whether they fall along a wide continuum. Nevertheless, potential differences in the firing frequency or the duration of activity of cholinergic neurons could have variable effects on different interneuron subtypes through local differences in acetylcholine concentrations.

EFFECTS OF MUSCARINIC RECEPTOR ACTIVATION ON HIPPOCAMPAL CA1 INHIBITORY INTERNEURONS

Disruption of the MS/DBB cholinergic function by systemic blockade of muscarinic receptors or direct injection of muscarinic receptor antagonists into the hippocampus can impair memory and the encoding of spatial information (Blokland et al., 1992; Atri et al., 2004; Hasselmo, 2006). A potential role for inhibitory interneurons in muscarinic receptor modulation of hippocampal function was initially based on observations that the exogenous application of cholinergic agonists resulted in an increase in spontaneous inhibitory postsynaptic currents (sIPSCs) in CA1 pyramidal neurons (Pitler and Alger, 1992). These data indirectly suggested that a subset of inhibitory interneurons may be depolarized by muscarinic receptor activation and were subsequently confirmed by direct recordings (Parra et al., 1998; McQuiston and Madison, 1999a). However, not all interneurons responded to muscarinic receptor activation by depolarizing. Some interneurons were hyperpolarized or exhibited biphasic responses, and some failed to respond to the exogenous application of muscarinic agonist (Parra et al., 1998; McQuiston and Madison, 1999a). Moreover, each muscarinic response type could not be correlated with a morphological subtype of interneuron. These findings were further complicated by the observation that muscarinic receptors can inhibit the release of GABA from a subset of perisomatic inhibitory interneurons (Behrends and Ten Bruggencate, 1993; Fukudome et al., 2004; Szabo et al., 2010) and muscarinic receptor activation can increase interneuron excitability through the generation of after depolarizations (McQuiston and Madison, 1999b; Lawrence et al., 2006). Thus, the impact that acetylcholine release has on the interneuron population is complex and results in the recruitment of some interneurons while inhibiting others.

ACTIVATION OF MUSCARINIC RECEPTORS IN HIPPOCAMPAL CA1 INTERNEURONS FOLLOWING ACETYLCHOLINE RELEASE

Although cholinergic muscarinic synaptic responses were first measured in CA1 pyramidal neurons in 1983 (Cole and Nicoll, 1983), it was not until 2006 that muscarinic responses to electrically evoked acetylcholine release were measured in hippocampal CA1 inhibitory interneurons (Widmer et al., 2006). This study showed that terminally released acetylcholine had divergent effects on different interneuron subtypes. Interneurons could respond by depolarizing, hyperpolarizing, or with biphasic responses. Overall, the majority of responding interneurons produced depolarizations (64%) whereas hyperpolarizations were infrequently observed (13%) (Widmer et al., 2006). Moreover, like previous studies using exogenous application of muscarinic agonists (Parra et al., 1998; McQuiston and Madison, 1999a), the different electrically evoked muscarinic response types could not be correlated with specific interneuron anatomical subtypes (Widmer et al., 2006). These findings have been recently confirmed by optogenetic studies using evoked release in response to light-activation (Nagode et al., 2011; Bell et al., 2013). However, in one of these optogenetic studies, interneurons responding with biphasic (25%), hyperpolarizing (35%), and depolarizing (40%) muscarinic responses were more equally distributed among the different response types (Bell et al., 2013). Importantly, optogenetically released acetylcholine predominantly produced muscarinic responses (80%) vs. nicotinic responses (17%). The remaining 3% of responding interneurons had both muscarinic

and nicotinic responses. Furthermore, the muscarinic hyperpolarizations were mediated by the activation of M_4 receptors whereas the depolarizations were likely produced by M_3 receptor activation (Bell et al., 2013). Similar to the electrical stimulation studies, muscarinic response type could not be correlated with anatomical interneuron subtypes. Importantly, both studies showed that perisomatically projecting interneurons (likely parvalbumin-expressing basket cells) could respond to acetylcholine release with any one of the three muscarinic response types (Widmer et al., 2006; Bell et al., 2013). In different optogenetic studies, CA1 interneuron membrane potential was indirectly assessed by measuring sIPSC frequency in CA1 pyramidal neurons (Nagode et al., 2011, 2014). Optogenetically released acetylcholine resulted in an increase in large amplitude sIPSCs with frequencies that fell within the theta bandwidth (4–12 Hz) (Nagode et al., 2011). Importantly, this increase in sIPSCs could be inhibited by endocannabinoids suggesting that they resulted from the activation of cholecystokinin positive interneurons (Nagode et al., 2011). Furthermore, the sIPSCs were not affected by optogenetic suppression of parvalbumin positive cells, suggesting they did not arise from the activation of parvalbumin basket cells, axo-axonic, bistratified or oriens-lacunosum-moleculare interneurons (Nagode et al., 2014). These findings are consistent with synaptic stimulation studies, which recorded from an interneuron with cholecystokinin basket cell morphology that produced a biphasic response to acetylcholine release (Widmer et al., 2006). Therefore, based on effects on the membrane potential alone, endogenously activated muscarinic receptors on hippocampal CA1 interneurons will have complex effects on network function (see **Table 1**).

Although different muscarinic response types were almost uniformly observed in CA1 interneurons, not all response types were as easily evoked by optogenetic stimulation (Bell et al., 2013). Consistent with some *in vivo* recordings (Brazhnik and Fox, 1999), acetylcholine released from MS/DBB cholinergic terminals by blue light flashes delivered at 20 Hz was capable of producing

Table 1 | Cholinergic responses vary in similar and different anatomical interneuron subtypes.

Interneuron axonal arborization	Muscarinic depol.	Muscarinic hyperpol.	Muscarinic biphasic	Nicotinic α7	Nicotinic α4β2	Nicotinic α2
Perisomatic SP	Agonist: Parra et al., 1998; McQuiston and Madison, 1999a	Agonist: McQuiston and Madison, 1999a	Agonist: McQuiston and Madison, 1999a	Agonist: McQuiston and Madison, 1999c; Buhler and Dunwiddie, 2001	Agonist: Not identified	Agonist: Not identified
	Synaptic: Widmer et al., 2006; Nagode et al., 2011, 2014; Bell et al., 2013	Synaptic: Widmer et al., 2006; Bell et al., 2013	Synaptic: Widmer et al., 2006; Bell et al., 2013	Synaptic: Not identified	Synaptic: Not identified	Synaptic: Not observed
Proximal dendritic SR or SO	Agonist: Parra et al., 1998; McQuiston and Madison, 1999a	Agonist: McQuiston and Madison, 1999a	Agonist: McQuiston and Madison, 1999a	Agonist: McQuiston and Madison, 1999c; Buhler and Dunwiddie, 2001	Agonist: Not identified	Agonist: Not identified
	Synaptic: Widmer et al., 2006; Bell et al., 2013	Synaptic: Widmer et al., 2006; Bell et al., 2013	Synaptic: Widmer et al., 2006; Bell et al., 2013	Synaptic: Not identified	Synaptic: Bell et al., 2011	Synaptic: Not observed
Distal dendritic SLM	Agonist: Parra et al., 1998; McQuiston and Madison, 1999a	Agonist: Parra et al., 1998; McQuiston and Madison, 1999a	Agonist: McQuiston and Madison, 1999a	Agonist: McQuiston and Madison, 1999c; Buhler and Dunwiddie, 2001; Griguoli et al., 2009	Agonist: Griguoli et al., 2009	Agonist: McQuiston and Madison, 1999c; Griguoli et al., 2009
	Synaptic: Widmer et al., 2006; Bell et al., 2013	Synaptic: Widmer et al., 2006; Bell et al., 2013	Synaptic: Bell et al., 2013	Synaptic: Not identified	Synaptic: Bell et al., 2011	Synaptic: Not observed

Cholinergic responsive interneurons are categorized based on the anatomical location of their axons (left column). References are reported for cholinergic response types observed in each class of interneuron. Agonist refers to responses elicited by exogenous agonist application. Stimulation refers to endogenous acetylcholine responses elicited electrically or optogenetically. Not identified—indicates that such a response type has not been observed in that class of interneuron. Not observed—indicates that no such response type has been observed in any interneuron class.

each response type in hippocampal CA1 interneurons (Bell et al., 2013). However, the number of flashes affected the probability of observing a particular response type. In hyperpolarizing interneurons, 10 flashes were sufficient (91% of hyperpolarizing interneurons) to observe a response. In contrast, 10 flashes were not sufficient to produce a response in the majority of depolarizing interneurons (58%). Similarly, the depolarizing phase could not be observed in the majority of biphasic interneurons (55%) when only 10 stimuli were delivered. Therefore, muscarinic hyperpolarizations may require less presynaptic MS/DBB cholinergic activity compared to depolarizing responses in hippocampal CA1 interneurons. It may be that suppression of interneuron excitability will be the predominant effect in response to low levels of MS/DBB cholinergic activity.

EFFECTS OF NICOTINIC RECEPTOR ACTIVATION ON HIPPOCAMPAL CA1 INTERNEURONS

Activation of nicotinic receptors in the hippocampus has a significant impact on physiological and pathophysiological memory formation (Levin, 2002; Levin et al., 2002, 2009; Buccafusco et al., 2005; Davis and Gould, 2006, 2009; Nott and Levin, 2006; Davis et al., 2007). Of the 11 different nicotinic receptor subunits found in the mammalian CNS, 9 have been reported to be expressed in hippocampal CA1 neurons (Sudweeks and Yakel, 2000). Using exogenous application of nicotinic agonists, functional nicotinic receptors that contain α7 (Alkondon et al., 1997; Jones and Yakel, 1997; Frazier et al., 1998b; McQuiston and Madison, 1999c), α4β2 (McQuiston and Madison, 1999c; Sudweeks and Yakel, 2000), or α2 subunits (McQuiston and Madison, 1999c; Sudweeks and Yakel, 2000; Jia et al., 2009) have been observed in hippocampal CA1 interneurons. Although hippocampal interneurons appeared to express a diverse collection nicotinic receptor subtypes, α7 containing receptors were more frequently observed and produced larger responses (McQuiston and Madison, 1999c; Sudweeks and Yakel, 2000). Indeed, α7 nicotinic receptors in the hippocampus have been associated with memory formation (Levin, 2002; Levin et al., 2002; Nott and Levin, 2006) and their dysfunction may play a role in some forms of schizophrenia (Freedman et al., 1994; Leonard et al., 1996; Adler et al., 1998). However, despite their lower expression levels, the α4β2 containing nicotinic receptors have been reported to play a significant role in memory formation (Davis and Gould, 2006; Davis et al., 2007) and in hippocampal-dependent nicotine addiction (Perry et al., 1999; Davis and Gould, 2009). α4β2 containing receptors have also been correlated with cognitive deficits associated with aging and Alzheimer's disease (Kellar et al., 1987; Wu et al., 2004; Gahring et al., 2005). To fully understand the role that different nicotinic subunits play in the hippocampus, the effect of endogenously released acetylcholine on individual hippocampal cells and the hippocampal network has begun to be investigated.

ACTIVATION OF NICOTINIC RECEPTORS IN HIPPOCAMPAL CA1 INTERNEURONS FOLLOWING ACETYLCHOLINE RELEASE

Acetylcholine release from MS/DBB cholinergic terminals in hippocampal CA1 has been demonstrated to activate nicotinic receptors on interneurons (Alkondon et al., 1998; Frazier et al., 1998a; Stone, 2007). Nicotinic excitatory postsynaptic currents (EPSCs) were first observed using electrical stimulation and whole cell patch clamping in acute rat brain slices. These nicotinic EPSCs had fast kinetics and were blocked by α7 nicotinic receptor antagonists (Alkondon et al., 1998; Frazier et al., 1998a), consistent with studies that applied nicotinic receptor agonists directly onto interneuron cell bodies (Alkondon et al., 1997; Jones and Yakel, 1997; Frazier et al., 1998b; McQuiston and Madison, 1999c). However, more recent optogenetic studies in mouse brain slices were not able to reproduce these earlier observations (Bell et al., 2011). Instead, optogenetically released acetylcholine primarily activated nicotinic receptors that contained α4β2 subunits. Furthermore, the α4β2 responses were mostly subthreshold and had very slow kinetics. These data were suggestive of acetylcholine diffusing a significant distance before binding to the α4β2 containing nicotinic receptors (McQuiston and Madison, 1999c; Bennett et al., 2012), consistent with volume or non-synaptic transmission (Vizi et al., 2010). Although these small nicotinic responses could temporally summate, their ability to excite interneurons was limited through muscarinic presynaptic inhibition. Because the nicotinic responses were mostly subthreshold, nicotinic transmission onto CA1 interneurons may be primarily modulatory in nature. The optogenetic studies also examined the nicotinic responses using voltage-sensitive dye (VSD) imaging. The nicotinic VSD signals were completely blocked by the α4β2 receptor antagonist DHβE and were found to be significantly larger in the distal dendritic region of CA1 pyramidal neurons, which overlaps with inputs from the entorhinal cortex and nucleus reuniens of the thalamus (Bell et al., 2011). Importantly, because the VSD stains all elements of the tissue, the VSD data suggest that α4β2 containing nicotinic receptors are the most prevalent receptor that mediates depolarizing nicotinic responses in mouse hippocampal CA1. Notably, nicotinic responses could be produced by a single flash of light (Bell et al., 2011) suggesting that acetylcholine release from MS/DBB cholinergic terminals may help recruit interneurons via nicotinic receptor activation before they are affected by muscarinic receptor activation.

EFFECTS OF ACETYLCHOLINE RELEASE ON HIPPOCAMPAL CA1 NETWORK FUNCTION FROM THE PERSPECTIVE OF THE INTERNEURON MEMBRANE POTENTIAL

Because CA1 inhibitory interneuron membrane potentials can be differentially modulated by both muscarinic and nicotinic receptor activation following acetylcholine release, the consequential effect on network function is undoubtedly complex. Muscarinic receptor activation can result in varying and opposing effects, even within the same interneuron (see **Table 1**). Unfortunately, our understanding of how each subtype of interneuron can be affected by muscarinic or nicotinic receptor activation remains incomplete. Nevertheless, the number of stimuli required to produce each type of response varied in a consistent manner. Nicotinic responses were most easily evoked requiring the fewest number of stimuli (Bell et al., 2011) whereas depolarizing muscarinic responses were the most difficult to produce requiring the largest number stimuli (Bell et al., 2013). Therefore, it can be hypothesized that low levels of MS/DBB cholinergic

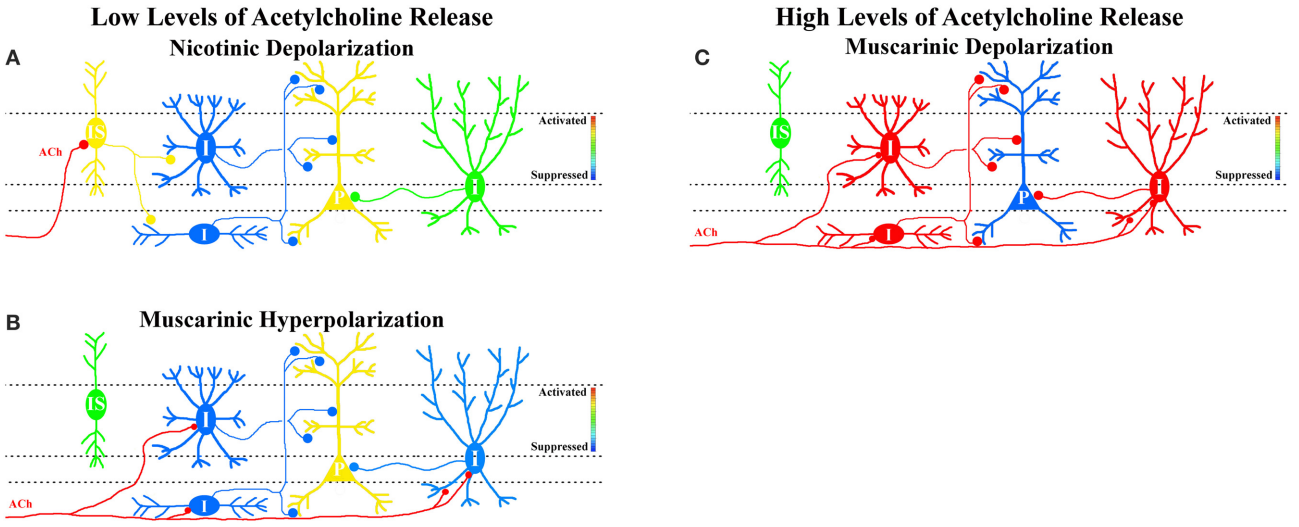

FIGURE 1 | Hypothesis that MS/DBB cholinergic inputs either suppress or activate interneuron networks in hippocampal CA1 depending on cholinergic neuron activity. (A) Low levels of MS/DBB cholinergic activity preferentially activate subsets of interneurons through the activation of nicotinic receptors. We postulate that nicotinic-driven interneurons are interneurons-selective interneurons (IS, yellow—activation) that specifically inhibit other interneurons (blue). Increasing their activity results in disinhibition of pyramidal neurons (P, yellow—activation, and increased output). **(B)** Low levels of MS/DBB cholinergic activity also hyperpolarize subsets of interneurons through the activation of muscarinic receptors (I, blue—suppression) resulting in disinhibition of pyramidal neurons (P, yellow—activation, and increased output). **(C)** Increasing cholinergic neuron activity causes subsets of interneurons to be depolarized by muscarinic receptor activation (I, red—activation, and increased synaptic inhibition) resulting in suppression of pyramidal neurons (P, blue—suppressed output).

neuron activity and lower concentrations of extracellular acetylcholine favor the activation nicotinic receptors or a muscarinic hyperpolarization in specific subsets of CA1 interneurons.

Because muscarinic hyperpolarization of CA1 interneurons requires less presynaptic cholinergic activity, disinhibition (indirect activation) of hippocampal CA1 pyramidal cells may be favored during low levels of MS/DBB cholinergic activity (**Figure 1B**). Furthermore, postulating that nicotinic responses preferentially affect interneurons that selectively inhibit other interneurons (interneuron-selective or IS), nicotinic receptor activation may also result in disinhibition of CA1 pyramidal neurons (**Figure 1A**). Together, low levels of MS/DBB cholinergic activity would favor a net disinhibition of hippocampal CA1 permitting higher probability of output from CA1 pyramidal neurons. Increased output from CA1 may result in the facilitation of recall and memory consolidation in other areas of the CNS as is thought to occur during slow wave sleep (Gais and Born, 2004; Hasselmo and McGaughy, 2004). In contrast, higher levels of MS/DBB cholinergic neuron activity coupled to higher extracellular concentrations of acetylcholine will subsequently recruit different subsets of interneurons that respond via muscarinic depolarizations. Some of these depolarizing interneurons may impose rhythmic inhibition of CA1 pyramidal neurons at theta frequencies (Nagode et al., 2011, 2014), a network rhythm observed during higher levels of acetylcholine release (Zhang et al., 2010). This would result in inhibition of hippocampal CA1 pyramidal neuron output (partly rhythmic) while facilitating synaptic integration within hippocampal CA1 pyramidal cell dendrites through cholinergic effects on glutamatergic receptors and dendritic function (**Figure 1C**) (Tsubokawa and Ross, 1997; Tsubokawa, 2000; Fernandez De Sevilla and Buno, 2010; Giessel and Sabatini, 2010). Indeed, such a dynamic role for acetylcholine concentrations in learning and memory formation has been previously proposed (Hasselmo, 2006; Hasselmo and Giocomo, 2006; Giocomo and Hasselmo, 2007; Hasselmo and Sarter, 2011). In this scheme, lower acetylcholine concentrations permit intrahippocampal (Schaffer collaterals) synaptic interactions to dominate thus increasing hippocampal CA1 output and memory retrieval, whereas higher acetylcholine concentrations favor processing of inputs from outside the hippocampus permitting the transient formation of memories in hippocampal CA1. Therefore, the combined effect of acetylcholine release on glutamatergic inputs and interneuron function may play important roles in tuning the hippocampal CA1 network for recall or to form new memories.

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REFERENCES

Adler, L. E., Olincy, A., Waldo, M., Harris, J. G., Griffith, J., Stevens, K., et al. (1998). Schizophrenia, sensory gating, and nicotinic receptors. *Schizophr. Bull.* 24, 189–202. doi: 10.1093/oxfordjournals.schbul.a033320

Alkondon, M., Pereira, E. F., and Albuquerque, E. X. (1998). alpha-bungarotoxin- and methyllycaconitine-sensitive nicotinic receptors mediate fast synaptic transmission in interneurons of rat hippocampal slices. *Brain Res.* 810, 257–263. doi: 10.1016/S0006-8993(98)00880-4

Alkondon, M., Pereira, E. F., Barbosa, C. T., and Albuquerque, E. X. (1997). Neuronal nicotinic acetylcholine receptor activation modulates gamma-aminobutyric acid release from CA1 neurons of rat hippocampal slices. *J. Pharmacol. Exp. Ther.* 283, 1396–1411.

Atri, A., Sherman, S., Norman, K. A., Kirchhoff, B. A., Nicolas, M. M., Greicius, M. D., et al. (2004). Blockade of central cholinergic receptors impairs new learning and increases proactive interference in a word paired-associate memory task. *Behav. Neurosci.* 118, 223–236. doi: 10.1037/0735-7044.118.1.223

Aznavour, N., Mechawar, N., and Descarries, L. (2002). Comparative analysis of cholinergic innervation in the dorsal hippocampus of adult mouse and rat: a quantitative immunocytochemical study. *Hippocampus* 12, 206–217. doi: 10.1002/hipo.1108

Barrenechea, C., Pedemonte, M., Nunez, A., and Garcia-Austt, E. (1995). *In vivo* intracellular recordings of medial septal and diagonal band of Broca neurons: relationships with theta rhythm. *Exp. Brain Res.* 103, 31–40. doi: 10.1007/BF00241962

Behrends, J. C., and Ten Bruggencate, G. (1993). Cholinergic modulation of synaptic inhibition in the guinea pig hippocampus *in vitro*: excitation of GABAergic interneurons and inhibition of GABA-release. *J. Neurophysiol.* 69, 626–629.

Bell, K. A., Shim, H., Chen, C. K., and McQuiston, A. R. (2011). Nicotinic excitatory postsynaptic potentials in hippocampal CA1 interneurons are predominantly mediated by nicotinic receptors that contain alpha4 and beta2 subunits. *Neuropharmacology* 61, 1379–1388. doi: 10.1016/j.neuropharm.2011.08.024

Bell, L. A., Bell, K. A., and McQuiston, A. R. (2013). Synaptic muscarinic response types in hippocampal CA1 interneurons depend on different levels of presynaptic activity and different muscarinic receptor subtypes. *Neuropharmacology* 73, 160–173. doi: 10.1016/j.neuropharm.2013.05.026

Bennett, C., Arroyo, S., Berns, D., and Hestrin, S. (2012). Mechanisms generating dual-component nicotinic EPSCs in cortical interneurons. *J. Neurosci.* 32, 17287–17296. doi: 10.1523/JNEUROSCI.3565-12.2012

Blake, M. G., Krawczyk, M. C., Baratti, C. M., and Boccia, M. M. (2014). Neuropharmacology of memory consolidation and reconsolidation: insights on central cholinergic mechanisms. *J. Physiol. Paris*. doi: 10.1016/j.jphysparis.2014.04.005. [Epub ahead of print]

Blokland, A., Honig, W., and Raaijmakers, W. G. (1992). Effects of intra-hippocampal scopolamine injections in a repeated spatial acquisition task in the rat. *Psychopharmacology (Berl).* 109, 373–376. doi: 10.1007/BF02245886

Brazhnik, E. S., and Fox, S. E. (1997). Intracellular recordings from medial septal neurons during hippocampal theta rhythm. *Exp. Brain Res.* 114, 442–453. doi: 10.1007/PL00005653

Brazhnik, E. S., and Fox, S. E. (1999). Action potentials and relations to the theta rhythm of medial septal neurons *in vivo*. *Exp. Brain Res.* 127, 244–258. doi: 10.1007/s002210050794

Buccafusco, J. J., Letchworth, S. R., Bencherif, M., and Lippiello, P. M. (2005). Long-lasting cognitive improvement with nicotinic receptor agonists: mechanisms of pharmacokinetic-pharmacodynamic discordance. *Trends Pharmacol. Sci.* 26, 352–360. doi: 10.1016/j.tips.2005.05.007

Buhler, A. V., and Dunwiddie, T. V. (2001). Regulation of the activity of hippocampal stratum oriens interneurons by alpha7 nicotinic acetylcholine receptors. *Neuroscience* 106, 55–67. doi: 10.1016/S0306-4522(01)00257-3

Buzsaki, G. (2002). Theta oscillations in the hippocampus. *Neuron* 33, 325–340. doi: 10.1016/S0896-6273(02)00586-X

Buzsaki, G., and Wang, X. J. (2012). Mechanisms of gamma oscillations. *Annu. Rev. Neurosci.* 35, 203–225. doi: 10.1146/annurev-neuro-062111-150444

Cea-Del Rio, C. A., Lawrence, J. J., Erdelyi, F., Szabo, G., and McBain, C. J. (2011). Cholinergic modulation amplifies the intrinsic oscillatory properties of CA1 hippocampal cholecystokinin-positive interneurons. *J. Physiol.* 589, 609–627. doi: 10.1113/jphysiol.2010.199422

Cea-Del Rio, C. A., Lawrence, J. J., Tricoire, L., Erdelyi, F., Szabo, G., and McBain, C. J. (2010). M3 muscarinic acetylcholine receptor expression confers differential cholinergic modulation to neurochemically distinct hippocampal basket cell subtypes. *J. Neurosci.* 30, 6011–6024. doi: 10.1523/JNEUROSCI.5040-09.2010

Cobb, S. R., and Davies, C. H. (2005). Cholinergic modulation of hippocampal cells and circuits. *J. Physiol.* 562, 81–88. doi: 10.1113/jphysiol.2004.076539

Cole, A. E., and Nicoll, R. A. (1983). Acetylcholine mediates a slow synaptic potential in hippocampal pyramidal cells. *Science* 221, 1299–1301. doi: 10.1126/science.6612345

Davis, J. A., and Gould, T. J. (2006). The effects of DHBE and MLA on nicotine-induced enhancement of contextual fear conditioning in C57BL/6 mice. *Psychopharmacology (Berl).* 184, 345–352. doi: 10.1007/s00213-005-0047-y

Davis, J. A., and Gould, T. J. (2009). Hippocampal nAChRs mediate nicotine withdrawal-related learning deficits. *Eur. Neuropsychopharmacol.* 19, 551–561. doi: 10.1016/j.euroneuro.2009.02.003

Davis, J. A., Kenney, J. W., and Gould, T. J. (2007). Hippocampal alpha4beta2 nicotinic acetylcholine receptor involvement in the enhancing effect of acute nicotine on contextual fear conditioning. *J. Neurosci.* 27, 10870–10877. doi: 10.1523/JNEUROSCI.3242-07.2007

Deiana, S., Platt, B., and Riedel, G. (2011). The cholinergic system and spatial learning. *Behav. Brain Res.* 221, 389–411. doi: 10.1016/j.bbr.2010.11.036

Dutar, P., Bassant, M. H., Senut, M. C., and Lamour, Y. (1995). The septohippocampal pathway: structure and function of a central cholinergic system. *Physiol. Rev.* 75, 393–427.

Easton, A., Douchamps, V., Eacott, M., and Lever, C. (2012). A specific role for septohippocampal acetylcholine in memory? *Neuropsychologia* 50, 3156–3168. doi: 10.1016/j.neuropsychologia.2012.07.022

Fernandez De Sevilla, D., and Buno, W. (2010). The muscarinic long-term enhancement of NMDA and AMPA receptor-mediated transmission at Schaffer collateral synapses develop through different intracellular mechanisms. *J. Neurosci.* 30, 11032–11042. doi: 10.1523/JNEUROSCI.1848-10.2010

Frazier, C. J., Buhler, A. V., Weiner, J. L., and Dunwiddie, T. V. (1998a). Synaptic potentials mediated via alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons. *J. Neurosci.* 18, 8228–8235.

Frazier, C. J., Rollins, Y. D., Breese, C. R., Leonard, S., Freedman, R., and Dunwiddie, T. V. (1998b). Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. *J. Neurosci.* 18, 1187–1195.

Freedman, R., Adler, L. E., Bickford, P., Byerley, W., Coon, H., Cullum, C. M., et al. (1994). Schizophrenia and nicotinic receptors. *Harv. Rev. Psychiatry* 2, 179–192. doi: 10.3109/10673229409017136

Freund, T. F., and Buzsaki, G. (1996). Interneurons of the hippocampus. *Hippocampus* 6, 347–470.

Fukudome, Y., Ohno-Shosaku, T., Matsui, M., Omori, Y., Fukaya, M., Tsubokawa, H., et al. (2004). Two distinct classes of muscarinic action on hippocampal inhibitory synapses: M2-mediated direct suppression and M1/M3-mediated indirect suppression through endocannabinoid signalling. *Eur. J. Neurosci.* 19, 2682–2692. doi: 10.1111/j.0953-816X.2004.03384.x

Gahring, L. C., Persiyanov, K., and Rogers, S. W. (2005). Mouse strain-specific changes in nicotinic receptor expression with age. *Neurobiol. Aging* 26, 973–980. doi: 10.1016/j.neurobiolaging.2004.07.005

Gais, S., and Born, J. (2004). Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2140–2144. doi: 10.1073/pnas.0305404101

Giessel, A. J., and Sabatini, B. L. (2010). M1 muscarinic receptors boost synaptic potentials and calcium influx in dendritic spines by inhibiting postsynaptic SK channels. *Neuron* 68, 936–947. doi: 10.1016/j.neuron.2010.09.004

Giocomo, L. M., and Hasselmo, M. E. (2007). Neuromodulation by glutamate and acetylcholine can change circuit dynamics by regulating the relative influence of afferent input and excitatory feedback. *Mol. Neurobiol.* 36, 184–200. doi: 10.1007/s12035-007-0032-z

Griguoli, M., Scuri, R., Ragozzino, D., and Cherubini, E. (2009). Activation of nicotinic acetylcholine receptors enhances a slow calcium-dependent potassium conductance and reduces the firing of stratum oriens interneurons. *Eur. J. Neurosci.* 30, 1011–1022. doi: 10.1111/j.1460-9568.2009.06914.x

Hasselmo, M. E. (2006). The role of acetylcholine in learning and memory. *Curr. Opin. Neurobiol.* 16, 710–715. doi: 10.1016/j.conb.2006.09.002

Hasselmo, M. E., and Giocomo, L. M. (2006). Cholinergic modulation of cortical function. *J. Mol. Neurosci.* 30, 133–135. doi: 10.1385/JMN:30:1:133

Hasselmo, M. E., and McGaughy, J. (2004). High acetylcholine levels set circuit dynamics for attention and encoding and low acetylcholine levels set dynamics for consolidation. *Prog. Brain Res.* 145, 207–231. doi: 10.1016/S0079-6123(03)45015-2

Hasselmo, M. E., and Sarter, M. (2011). Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology* 36, 52–73. doi: 10.1038/npp.2010.104

Jia, Y., Yamazaki, Y., Nakauchi, S., and Sumikawa, K. (2009). Alpha2 nicotine receptors function as a molecular switch to continuously excite a subset of interneurons in rat hippocampal circuits. *Eur. J. Neurosci.* 29, 1588–1603. doi: 10.1111/j.1460-9568.2009.06706.x

Jones, S., and Yakel, J. L. (1997). Functional nicotinic ACh receptors on interneurones in the rat hippocampus. *J. Physiol.* 504(Pt 3), 603–610. doi: 10.1111/j.1469-7793.1997.603bd.x

Kellar, K. J., Whitehouse, P. J., Martino-Barrows, A. M., Marcus, K., and Price, D. L. (1987). Muscarinic and nicotinic cholinergic binding sites in Alzheimer's disease cerebral cortex. *Brain Res.* 436, 62–68. doi: 10.1016/0006-8993(87)91556-3

Kenney, J. W., and Gould, T. J. (2008). Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. *Mol. Neurobiol.* 38, 101–121. doi: 10.1007/s12035-008-8037-9

Klausberger, T., and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* 321, 53–57. doi: 10.1126/science.1149381

Larkum, M. E., Zhu, J. J., and Sakmann, B. (1999). A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* 398, 338–341. doi: 10.1038/18686

Lawrence, J. J., Statland, J. M., Grinspan, Z. M., and McBain, C. J. (2006). Cell type-specific dependence of muscarinic signalling in mouse hippocampal stratum oriens interneurones. *J. Physiol.* 570, 595–610. doi: 10.1113/jphysiol.2005.100875

Leonard, S., Adams, C., Breese, C. R., Adler, L. E., Bickford, P., Byerley, W., et al. (1996). Nicotinic receptor function in schizophrenia. *Schizophr. Bull.* 22, 431–445. doi: 10.1093/schbul/22.3.431

Levin, E. D. (2002). Nicotinic receptor subtypes and cognitive function. *J. Neurobiol.* 53, 633–640. doi: 10.1002/neu.10151

Levin, E. D., Bradley, A., Addy, N., and Sigurani, N. (2002). Hippocampal alpha 7 and alpha 4 beta 2 nicotinic receptors and working memory. *Neuroscience* 109, 757–765. doi: 10.1016/S0306-4522(01)00538-3

Levin, E. D., Petro, A., Rezvani, A. H., Pollard, N., Christopher, N. C., Strauss, M., et al. (2009). Nicotinic alpha7- or beta2-containing receptor knockout: effects on radial-arm maze learning and long-term nicotine consumption in mice. *Behav. Brain Res.* 196, 207–213. doi: 10.1016/j.bbr.2008.08.048

McQuiston, A. R., and Madison, D. V. (1999a). Muscarinic receptor activity has multiple effects on the resting membrane potentials of CA1 hippocampal interneurons. *J. Neurosci.* 19, 5693–5702.

McQuiston, A. R., and Madison, D. V. (1999b). Muscarinic receptor activity induces an afterdepolarization in a subpopulation of hippocampal CA1 interneurons. *J. Neurosci.* 19, 5703–5710.

McQuiston, A. R., and Madison, D. V. (1999c). Nicotinic receptor activation excites distinct subtypes of interneurons in the rat hippocampus. *J. Neurosci.* 19, 2887–2896.

Miles, R., Toth, K., Gulyas, A. I., Hajos, N., and Freund, T. F. (1996). Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* 16, 815–823. doi: 10.1016/S0896-6273(00)80101-4

Nagode, D. A., Tang, A. H., Karson, M. A., Klugmann, M., and Alger, B. E. (2011). Optogenetic release of ACh induces rhythmic bursts of perisomatic IPSCs in hippocampus. *PLoS ONE* 6:e27691. doi: 10.1371/journal.pone.0027691

Nagode, D. A., Tang, A. H., Yang, K., and Alger, B. E. (2014). Optogenetic identification of an intrinsic cholinergically driven inhibitory oscillator sensitive to cannabinoids and opioids in hippocampal CA1. *J. Physiol.* 592, 103–123. doi: 10.1113/jphysiol.2013.257428

Nott, A., and Levin, E. D. (2006). Dorsal hippocampal alpha7 and alpha4beta2 nicotinic receptors and memory. *Brain Res.* 1081, 72–78. doi: 10.1016/j.brainres.2006.01.052

Parra, P., Gulyas, A. I., and Miles, R. (1998). How many subtypes of inhibitory cells in the hippocampus? *Neuron* 20, 983–993. doi: 10.1016/S0896-6273(00)80479-1

Perry, D. C., Davila-Garcia, M. I., Stockmeier, C. A., and Kellar, K. J. (1999). Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. *J. Pharmacol. Exp. Ther.* 289, 1545–1552.

Pitler, T. A., and Alger, B. E. (1992). Cholinergic excitation of GABAergic interneurons in the rat hippocampal slice. *J. Physiol.* 450, 127–142.

Power, A. E., Vazdarjanova, A., and McGaugh, J. L. (2003). Muscarinic cholinergic influences in memory consolidation. *Neurobiol. Learn. Mem.* 80, 178–193. doi: 10.1016/S1074-7427(03)00086-8

Simon, A. P., Poindessous-Jazat, F., Dutar, P., Epelbaum, J., and Bassant, M. H. (2006). Firing properties of anatomically identified neurons in the medial septum of anesthetized and unanesthetized restrained rats. *J. Neurosci.* 26, 9038–9046. doi: 10.1523/JNEUROSCI.1401-06.2006

Smiley, J. F., Morrell, F., and Mesulam, M. M. (1997). Cholinergic synapses in human cerebral cortex: an ultrastructural study in serial sections. *Exp. Neurol.* 144, 361–368. doi: 10.1006/exnr.1997.6413

Stone, T. W. (2007). Kynurenic acid blocks nicotinic synaptic transmission to hippocampal interneurons in young rats. *Eur. J. Neurosci.* 25, 2656–2665. doi: 10.1111/j.1460-9568.2007.05540.x

Storm-Mathisen, J. (1970). Quantitative histochemistry of acetylcholinesterase in rat hippocampal region correlated to histochemical staining. *J. Neurochem.* 17, 739–750. doi: 10.1111/j.1471-4159.1970.tb03344.x

Sudweeks, S. N., and Yakel, J. L. (2000). Functional and molecular characterization of neuronal nicotinic ACh receptors in rat CA1 hippocampal neurons. *J. Physiol.* 527(Pt 3), 515–528. doi: 10.1111/j.1469-7793.2000.00515.x

Szabo, G. G., Holderith, N., Gulyas, A. I., Freund, T. F., and Hajos, N. (2010). Distinct synaptic properties of perisomatic inhibitory cell types and their different modulation by cholinergic receptor activation in the CA3 region of the mouse hippocampus. *Eur. J. Neurosci.* 31, 2234–2246. doi: 10.1111/j.1460-9568.2010.07292.x

Tang, A. H., Karson, M. A., Nagode, D. A., McIntosh, J. M., Uebele, V. N., Renger, J. J., et al. (2011). Nerve terminal nicotinic acetylcholine receptors initiate quantal GABA release from perisomatic interneurons by activating axonal T-type (Cav3) Ca(2)(+) channels and Ca(2)(+) release from stores. *J. Neurosci.* 31, 13546–13561. doi: 10.1523/JNEUROSCI.2781-11.2011

Teles-Grilo Ruivo, L. M., and Mellor, J. R. (2013). Cholinergic modulation of hippocampal network function. *Front. Synaptic Neurosci.* 5:2. doi: 10.3389/fnsyn.2013.00002

Tsubokawa, H. (2000). Control of Na+ spike backpropagation by intracellular signaling in the pyramidal neuron dendrites. *Mol. Neurobiol.* 22, 129–141. doi: 10.1385/MN:22:1-3:129

Tsubokawa, H., and Ross, W. N. (1997). Muscarinic modulation of spike backpropagation in the apical dendrites of hippocampal CA1 pyramidal neurons. *J. Neurosci.* 17, 5782–5791.

Turrini, P., Casu, M. A., Wong, T. P., De, K. Y., Ribeiro-Da-Silva, A., and Cuello, A. C. (2001). Cholinergic nerve terminals establish classical synapses in the rat cerebral cortex: synaptic pattern and age-related atrophy. *Neuroscience* 105, 277–285. doi: 10.1016/S0306-4522(01)00172-5

Umbriaco, D., Garcia, S., Beaulieu, C., and Descarries, L. (1995). Relational features of acetylcholine, noradrenaline, serotonin and GABA axon terminals in the stratum radiatum of adult rat hippocampus (CA1). *Hippocampus* 5, 605–620. doi: 10.1002/hipo.450050611

Umbriaco, D., Watkins, K. C., Descarries, L., Cozzari, C., and Hartman, B. K. (1994). Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections. *J. Comp. Neurol.* 348, 351–373. doi: 10.1002/cne.903480304

Vizi, E. S., Fekete, A., Karoly, R., and Mike, A. (2010). Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment. *Br. J. Pharmacol.* 160, 785–809. doi: 10.1111/j.1476-5381.2009.00624.x

Widmer, H., Ferrigan, L., Davies, C. H., and Cobb, S. R. (2006). Evoked slow muscarinic acetylcholinergic synaptic potentials in rat hippocampal interneurons. *Hippocampus* 16, 617–628. doi: 10.1002/hipo.20191

Wu, J., Kuo, Y. P., George, A. A., Xu, L., Hu, J., and Lukas, R. J. (2004). beta-Amyloid directly inhibits human alpha4beta2-nicotinic acetylcholine receptors heterologously expressed in human SH-EP1 cells. *J. Biol. Chem.* 279, 37842–37851. doi: 10.1074/jbc.M400335200

Zhang, H., Lin, S. C., and Nicolelis, M. A. (2010). Spatiotemporal coupling between hippocampal acetylcholine release and theta oscillations *in vivo*. *J. Neurosci.* 30, 13431–13440. doi: 10.1523/JNEUROSCI.1144-10.2010

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Striatal cholinergic interneuron regulation and circuit effects

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The striatum plays a central role in motor control and motor learning. Appropriate responses to environmental stimuli, including pursuit of reward or avoidance of aversive experience all require functional striatal circuits. These pathways integrate synaptic inputs from limbic and cortical regions including sensory, motor and motivational information to ultimately connect intention to action. Although many neurotransmitters participate in striatal circuitry, one critically important player is acetylcholine (ACh). Relative to other brain areas, the striatum contains exceptionally high levels of ACh, the enzymes that catalyze its synthesis and breakdown, as well as both nicotinic and muscarinic receptor types that mediate its postsynaptic effects. The principal source of striatal ACh is the cholinergic interneuron (ChI), which comprises only about 1–2% of all striatal cells yet sends dense arbors of projections throughout the striatum. This review summarizes recent advances in our understanding of the factors affecting the excitability of these neurons through acute effects and long term changes in their synaptic inputs. In addition, we discuss the physiological effects of ACh in the striatum, and how changes in ACh levels may contribute to disease states during striatal dysfunction.

Keywords: acetylcholine, cholinergic interneuron, Parkinson's disease, plasticity, striatum

INTRODUCTION

The striatum is a subcortical brain region crucial for integrating motivation and action (Da Cunha et al., 2012). Convergence of inputs from motor cortex, thalamus and limbic areas create associations between actions and outcomes that ultimately contribute to survival. The essential nature of the striatum is evidenced by the presence of homologs structures throughout vertebrate evolution over hundreds of millions of years (Stephenson-Jones et al., 2011). Pathological changes in the striatum and associated basal ganglia structures are implicated in a wide variety of neurological and psychiatric disorders that involve the combination of motivation and action, including drug addiction (Koob, 1992), binge-eating (Norgren et al., 2006), obsessive-compulsive disorder (Aouizerate et al., 2004), attention deficit and hyperactivity disorder (Chudasama and Robbins, 2006), Huntington's disease (Cepeda et al., 2007), Parkinson's disease (Albin et al., 1989; Ellens and Leventhal, 2013), and L-DOPA induced dyskinesia (Barroso-Chinea and Bezard, 2010). Because so many human pathologies involve striatal dysfunction, a better understanding of neurotransmission in this brain structure will provide insight into the etiology of these conditions, potentially leading to the development of new pharmacotherapies. One neurotransmitter that is highly enriched in the striatum and vitally important for normal function is acetylcholine (ACh). This review will focus on the regulation of striatal ACh release as well as the functional consequences of cholinergic neurotransmission.

Unlike brain structures that show ordered laminar organization such as the hippocampus or cortex, the striatum is a heterogeneous mix of different cell types. The vast majority of striatal neurons (∼95%) are the GABA-ergic medium spiny neurons (MSNs), also referred to as spiny projection neurons, which are the principal output cell type. The MSNs that express dopamine (DA) D1 receptors project to and inhibit cells in the internal capsule of the globus pallidus as well as the substantia nigra pars reticulata. These projections are referred to as the direct pathway, or the GO pathway, and activation of this class of cells leads to enhanced locomotion. Another MSN population expresses dopamine D2 receptors, and these projections inhibit cells in the external capsule of the globus pallidus. This is the indirect, or the NO-GO pathway, and activation of this pathway decreases locomotion. Both pathways eventually influence thalamic control of motor cortex to affect motor function. Approximately 6% of MSNs in the dorsal striatum express both D1 and D2 receptors. These cells produce both GABA and glutamate, allowing them to potentially modulate the basal ganglia network bidirectionally (Perreault et al., 2012). The MSN network of basal ganglia connectivity has provided a model for understanding striatal involvement in motor control (Albin et al., 1989; DeLong, 1990; Kravitz et al., 2010). In addition to the MSNs, approximately 4% of striatal neurons are GABA-ergic interneurons. These locally projecting inhibitory cells consist of three types: parvalbumin-expressing fast spiking interneurons (FSIs), NPY/SOM/NOS-expressing persistent depolarization low-threshold spiking interneurons, and

the less understood calretinin-expressing low-threshold calcium spike interneurons (Kawaguchi, 1993). Each of these GABA-interneuron cell types possesses a unique gene expression profile and distinct electrophysiological properties (Tepper et al., 2010).

The remaining cells are the large, aspiny cholinergic interneurons (ChIs) originally described by the anatomist Kölliker in the late 1800's. Although recent tract tracing and electron microscopy studies report cholinergic projections from the rostral pedunculopontine nucleus into the striatum (Dautan et al., 2014), it is generally accepted that ChIs are the main source of striatal ACh (Woolf and Butcher, 1981). ChIs comprise ∼1% of striatal cells, yet they ramify extensively and send projections widely throughout the striatum: each ChI is estimated to produce on average 500,000 axonal varicosities (Bolam et al., 1984; Contant et al., 1996). Anatomically, ChIs are easily distinguished from the other striatal cell types due to their large diameter somata (>15 microns). In addition, ChIs display unique electrophysiological characteristics, which include tonic action potential firing at a rate of 3–10 Hz (Wilson et al., 1990), depolarized resting membrane potential (∼ −60 mV) (Lee et al., 1998), high input resistance (∼ 200 MΩ) (Calabresi et al., 1997), prominent hyperpolarization-activated cation current (I_h) (Deng et al., 2007a), and broad action potential duration (Threlfell et al., 2012). The striatum has the highest levels of cholinergic markers in the brain, including ACh, choline acetyltransferase (ChAT), and acetylcholinesterase (AChE) (Macintosh, 1941; Hebb and Silver, 1961; Woolf et al., 1984). Such a high density of cholinergic markers underscores the importance of ACh neurotransmission in the striatum. Therefore, understanding striatal physiology requires careful consideration of the activity of ChIs and the consequences of changes in cholinergic signaling.

Classically, the striatum is subdivided according to synaptic connectivity. For example, the dorsal striatum receives DA-ergic input primarily from substantia nigra pars compacta (SNc) (Hattori et al., 1991) and sends projections to ventrolateral substantia nigra pars reticulata (SNr) and the globus pallidus. The ventral striatum receives the majority of DA from ventral tegmental area (VTA) projections, and in turn, sends inhibitory projections into the dorsomedial SNr (Maurin et al., 1999) and globus pallidus. In rodents, the dorsal and ventral striatum subserve different functions: The dorsal striatum is implicated in sensorimotor functions such as serial order learning (Yin, 2010), stimulus-response habit formation (Devan et al., 2011), and performance of learned instrumental tasks (Shiflett et al., 2010), whereas the ventral striatum is important for the reinforcement of appetitive behaviors including drugs of abuse (Robinson and Berridge, 2000) and healthy rewards, such as food intake (Kelley, 2004). Different functional roles are attributed to lateral vs. medial regions of the dorsal striatum. The dorsolateral region, or the "sensorimotor striatum," receives strong motor and premotor cortical inputs, and is therefore particularly important for habit formation (Künzle, 1975; Haber et al., 2000). The dorsomedial striatum, referred to as the "associative striatum," receives inputs from limbic regions as well as prefrontal cortex, and is involved in behavioral flexibility, reward-associated motor learning, and reaction time (Hauber and Schmidt, 1994; Ragozzino, 2003). Gradients of afferent connectivity most likely influence overall striatal output (Voorn et al., 2004), suggesting that these subdivisions are an oversimplification of striatal connectivity and function. Accepting that caveat, the distinctions in function of different striatal regions in the rodent brain provide a framework for ongoing investigations into the neural substrates of relevant behaviors, with an ultimate goal of understanding the functional role of analogous structures in the human brain.

ChIs are believed to be the analogs of tonically active neurons (TANs) identified by *in vivo* recordings in the putamen of primates. This correlation is based on similarities in ChAT immunoreactivity, electrophysiological, and morphological characteristics (Inokawa et al., 2010). Changes in TAN activity have been linked to motor and reinforcement learning. In classical sensorimotor Pavlovian conditioning, TANs pause activity within a second after presentation of the conditioned stimulus (CS), followed by a transient increase in activity before recovery to baseline firing. This stereotyped neural behavior was described as the "conditioned pause response" (Kimura et al., 1984; Aosaki et al., 1994). This CS-induced change in firing is not dependent on motor activity, as a similar firing profile was observed when the animal was trained to withhold movement after CS presentation in a NO-GO task (Apicella et al., 1991). TANs also pause in response to aversive-CS, but not to neutral stimuli (Ravel et al., 1999). The conditioned pause response is therefore believed to encode salience value to external stimuli. Thus, changes in TAN activity may contribute to associative learning, particularly the relationship between environmental cues and outcomes. The circuitry responsible for the pause response is debated. Some evidence implicates a dependence on SNc DA-ergic tone (Watanabe and Kimura, 1998; Reynolds et al., 2004; Straub et al., 2014), however others have observed a change in TAN firing even in response to aversive stimuli that do not increase DA-ergic firing (Mirenowicz and Schultz, 1996; Ravel et al., 1999). We know that ChIs respond to many neurotransmitters, and this stereotypical pause in activity could be mediated by a variety of inputs. Synchronous changes in afferent activity likely mediate the pause response among multiple ChIs, resulting in a coordinated change in striatal cholinergic tone. Understanding the connectivity and neurotransmission that influences these cells may thus provide insight into learning phenomena.

STRIATAL CHOLINERGIC DYSFUNCTION IN PARKINSON'S DISEASE AND TREATMENT

The necessity of proper striatal neurotransmission for normal motor function is dramatically and tragically evidenced by the deficits observed in Parkinson's disease (PD). The first medical characterization of PD was published in 1817 (Parkinson, 1817):

> The first symptoms perceived are, a slight sense of weakness, with a proneness to trembling in some particular part; sometimes in the head, but most commonly in one of the hands and arms… After a few more months the patient is found to be less strict than usual in preserving an upright posture: this being most observable whilst walking.

Although this is the first formal description of the disease in Western literature, descriptions of the disease appeared in Eastern

texts as old as 600 BC (Manyam, 1990; Zhang et al., 2006; Raudino, 2012; Ovallath and Deepa, 2013). Some ancient cultures used treatments derived from herbal preparations that contain anticholinergic compounds with similar pharmacology to some therapies prescribed today (Manyam and Sánchez-Ramos, 1999).

Today, we know that the bradykinesia, resting tremor, rigidity, and difficulty in initiating movement observed in PD arise from deficits in basal ganglia DA transmission. However, it is important to consider the balance between the actions of striatal ACh and DA in the etiology of PD. Previous beliefs held that the two neurotransmitters had opposing actions, which was supported by the partial relief of PD symptoms with administration of anticholinergic compounds. These therapies may restore the balance between the two neurotransmitter systems. Drugs with similar pharmacological properties are still in use, particularly for younger PD patients whose primary symptom is tremor (Hristova and Koller, 2000), but cognitive and autonomic side effects preclude their widespread use. While anticholinergic drugs can improve some symptoms of PD (Whyte et al., 1971; Cantello et al., 1986; Baba et al., 2012), it has also been reported that elevation of ACh by treating patients with acetylcholinesterase inhibitors improves motor symptoms of PD (Chung et al., 2010). Although somewhat contradictory to the anticholinergic drug effects, inhibiting ACh degradation might enhance DA transmission through nicotinic acetylcholine receptors (nAChRs) on DA terminals. Alternatively, this treatment could promote cholinergic receptor desensitization to mimic anticholinergic drug effects. It is important to note that similar cholinesterase treatments have seen no effect on motor symptoms of PD (Poewe et al., 2006). Together, these observations highlight the importance of cholinergic transmission in striatal function under healthy and Parkinsonian conditions.

The physiology of ChIs is dramatically altered in PD. In humans, mutations in leucine-rich repeat kinase 2 (LRRK2) or the gene that encodes α-synuclein are both associated with a higher likelihood to develop PD (Simón-Sánchez et al., 2009). These genes are expressed in many basal ganglia cell types and the mechanisms that link these mutations to PD are the subject of ongoing studies (Gasser, 2009). ChIs in both rodents and humans express high levels of LRRK2 (Higashi et al., 2007; West et al., 2014), and abnormal kinase activity may contribute to pathological changes in ChI physiology. α-synuclein inclusions in the somata of ChIs are observed only in late PD but not early PD (Mori et al., 2008), suggesting that Lewy body-related interference of ChI physiology may be observed late in the disease.

In the 6-OHDA lesion model of PD, microdialysis studies have observed that striatal ACh levels are elevated in the DA depleted rat striatum (DeBoer et al., 1993), indicating a dysregulation of ChI excitability. Additionally, functional downregulation of M4—Cav2 coupling results in decreased sensitivity to autoinhibitory cholinergic transmission (Ding et al., 2006). Given the therapeutic effects of anticholinergic compounds in PD mentioned above, and the physiological changes in ChIs seen in animal models of the disease, it is evident that ACh is important in PD.

The development of DA replacement therapy to relieve the symptoms of PD in the 1960's revolutionized our understanding of neurotransmission in the dorsal striatum (Goetz, 2011). Currently, the biochemical precursor to DA, levodopa (L-DOPA) is the most effective clinical treatment for the motor symptoms of PD. L-DOPA crosses the blood brain barrier where it is then converted into DA by aromatic amino acid decarboxylase, thus increasing striatal levels of DA. Although L-DOPA effectively reverses PD locomotor disability, long-term treatment has its shortcomings, including shortening of the therapeutic window and psychiatric or mood disturbances such as impulse control disorders (Lesser et al., 1979; Voon et al., 2009; Santangelo et al., 2013a,b). Another debilitating side effect is the onset of levodopa-induced dyskinesia (LID), which is characterized by dystonia or choreic movements of the limbs, hands, or face. These side effects are potentially more debilitating than PD itself. This condition is highly prevalent, with LID development seen in approximately 40% of patients after 5 years of treatment, rising to nearly 90% after 10 years (Ahlskog and Muenter, 2001; Fabbrini et al., 2007). Interestingly, the age of onset of PD is a strong determinant for the development of LID, with earlier onset patients experiencing a more rapid expression of LID symptoms (Kostic et al., 1991; Kumar et al., 2005).

Pathological changes in striatal ACh signaling are related to the expression of LID. The anticholinergic benzatropine decreased dyskinesia in L-DOPA treated human PD patients (Pourcher et al., 1989), however, there are also reports of increased dyskinesia with anticholinergic treatment (Birket-Smith, 1974; Hauser and Olanow, 1993; Linazasoro, 1994). In a study of dyskinetic monkeys, the mAChR antagonist atropine changed the nature of dyskinesia from dystonia to chorea (Gomez-Mancilla and Bédard, 1993). Although these reports show mixed effects, they do suggest that cholinergic signaling influences the expression of LID. While examining a mouse model of LID, Ding et al. (2011b) observed enhanced levels of phosphorylated extracellular signal-regulated kinase (pERK) specifically in striatal ChIs. Electrophysiological recordings of ChIs revealed higher baseline and dopamine-induced firing rates in LID animals relative to vehicle-treated littermates. The increased ChI excitability and the expression of LID associated behaviors were both inhibited by blockers of MEK/ERK signaling (Ding et al., 2011b). Extending those studies, selective ablation of striatal ChIs decreases LID expression in a unilateral lesion model of PD (Won et al., 2014). Both nicotinic and muscarinic receptors are believed to contribute to LID. Treatment with either nAChR antagonists or nicotine improves abnormal involuntary movements (AIMs) in rodents and primates, which suggests that both drugs are decreasing nAChR function either through receptor blockade or desensitization (Quik et al., 2007; Bordia et al., 2010; Zhang et al., 2013). Although antimuscarinic drugs have mixed effects in human LID patients as mentioned above, there are reports of decreased LID expression (Pourcher et al., 1989), and a recent study of the Pitx3 mouse model of LID, a muscarinic receptor antagonist decreased behavioral expression of dyskinesia (Ding et al., 2011b). These findings support the conclusion that cholinergic transmission is important for mediating some aspects of LID and that pharmacological modulation of this system may help treat this debilitating condition.

Changes in striatal cholinergic signaling have been observed in patients with other movement disorders and psychiatric illnesses.

A partial list of these disorders and the nature of the changes in cholinergic activity can be found in **Table 1**. Considering the importance of striatal ChIs in both normal physiology and in disease, it is clear that this minority of cells plays a major role in the striatum. As such, understanding the nature of efferent and afferent synaptic connectivity of ChIs can provide important insights into striatal physiology.

AFFERENT CONNECTIONS TO ChIs

ChI excitability is affected by a remarkably large number of afferent input types and post-synaptic receptors. A simplified summary of this complex story is presented in **Table 2** and **Figures 1**, **2**. This section outlines the current state of our understanding of afferent control of ChIs.

GABA

ChIs receive a variety of GABA-ergic inputs, both local and extrastriatal in origin. GABA can inhibit cells by activating ionotropic $GABA_A$ receptors, which increases Cl- conductance. Of the $GABA_A$ receptor subunits, the α2, α4, β2/3 subunits are most highly expressed in the striatum (Persohn et al., 1992), and of potential interest, the α3 subunit is expressed only in choline acetyltransferase positive (ChAT+) cells (Rodríguez-Pallares et al., 2000). Local stimulation produces IPSCs in ChIs, and these events are blocked by bicuculline, indicating that ChIs express functional $GABA_A$ receptors (Sato et al., 2014). GABA also activates metabotropic $GABA_B$ receptors, G-protein coupled receptors (GPCRs) which decrease cell excitation by coupling to the $G_{i/o}$ protein and negatively regulating adenylyl cyclase (AC) (Bettler et al., 2004). Neither immunohistochemical examination of $GABA_B$ expression in ChIs nor the electrophysiological effects of selective $GABA_B$ activation on ChI excitability have been reported. *In vivo*, microdialysis experiments suggest that tonic activity at $GABA_A$ receptors regulates ChI excitability, whereas the $GABA_B$ receptors do not tonically inhibit ChI activity (DeBoer and Westerink, 1994).

Locally, GABA-ergic MSNs form synaptic connections with ChIs. Substance P-containing inputs are more prevalent than enkephalin-containing terminals, perhaps indicating that D1 MSNs have a more prominent influence over ChI activity compared to D2 MSNs (Martone et al., 1992). MSNs in the intact brain exist in one of two states of excitability, either an up or down state. In the up state, cells rest at a depolarized membrane potential, and are more likely to fire spontaneous action potentials compared to the relatively hyperpolarized down state (Wilson and Groves, 1981). The excitability state of MSNs will thus influence the inhibitory tone on ChIs. In the slice preparation MSNs are silent, and GABA released from these neurons will have minimal impact on ChIs. However, optogenetic activation of MSNs evoked a small amplitude IPSC in ∼75% of ChIs, suggesting that the MSN-ChI connection is highly prevalent (Chuhma et al., 2011). Ultrastructural analysis of TAN connectivity in primates shows that approximately 24% of all synaptic contacts onto ChAT+ cells originate from MSN axon collaterals (Gonzales et al., 2013). In sum, GABA release from MSN onto ChIs is likely a major determinant of excitability of these cells *in vivo*.

ChIs also receive synaptic inputs from GABA-ergic interneurons. nAChR activation, presumably located on GABA-ergic interneurons, inhibits tonic firing of ChIs (De Rover et al., 2002; Sullivan et al., 2008). Not all GABA-ergic interneurons project to ChIs, however: PV positive FSIs project to MSNs and other interneurons, but do not inhibit ChIs (Szydlowski et al., 2013). Currently, NPY-expressing PLTS GABA-ergic interneurons are the best candidate for inhibition of ChIs, as ultrastructural analysis suggests synaptic connections with choline acetyltransferase-positive striatal neurons (Vuillet et al., 1992). However, there is no direct electrophysiological or functional evidence for this connection. It is unknown if calretinin positive interneurons form connections with ChIs.

Extrastriatal sources of GABA may also inhibit ChIs. Co-release of DA and GABA from nigrostriatal neurons onto MSNs occurs via a VMAT2-dependent vesicular mechanism (Tritsch et al., 2012). Cholinergic activation of nAChRs on those terminals was recently shown to enhance GABAergic inputs to MSNs (Nelson et al., 2014b). We know that DA projections form prominent synaptic contacts onto ChIs (Dimova et al., 1993; Li et al., 2002), and if some of these DA teminals co-release GABA, these inputs could profoundly affect tonic activity of ChIs *in vivo*. In addition, these projections may also have important implications in PD, as the loss of SNc projections could decrease this source of GABA-ergic tone onto ChIs, potentially contributing to increases in striatal ACh levels. Other non-dopaminergic projection neurons may contribute to GABA inhibition of ChIs. In addition to DA neurons, the VTA possesses GABA projection neurons that inhibit ventral striatum ChIs (Brown et al., 2012), but whether dorsal striatum ChIs receive GABA input from non-dopaminergic projection neurons in SNc or VTA is not known. Nigrostriatal non-dopaminergic projections have been observed (Gerfen et al., 1987; Rodríguez and González-Hernández, 1999), but the physiological effects of these presumed GABA projections on ChI activity have not been studied. These extrastriatal GABA projections may provide a means to inhibit ChIs that is independent of intrastriatal GABA sources.

GLUTAMATE – IONOTROPIC RECEPTORS

Glutamatergic innervation of ChIs is predominantly extrastriatal (Künzle, 1975). Glutamate induces rapid depolarization through activation of postsynaptically expressed AMPA, NMDA, or kainate receptors. About half of all ChIs are immunopositive for GluR1, GluR2, and GluR4 subunits (Bernard et al., 1997; Deng et al., 2007b), despite the presence of mRNA of all 4 GluR subunits (Richardson et al., 2000). AMPA receptors expressed on ChIs show rapid deactivation, desensitization, and a relatively high permeability to Ca^{2+}, and these properties differ from the AMPA receptors expressed by MSNs (Götz et al., 1997). mRNA for NR1 and NR2D are present at high levels in ChIs, while expression of NR2A mRNA is contested (Landwehrmeyer et al., 1995; Standaert et al., 1999; Richardson et al., 2000). 90% of ChIs are immunopositive for the kainate receptors GluR5/6/7 (Chen et al., 1996). In a slice preparation, bath application of an NMDAR positive allosteric modulator increases ChI firing rate (Feng et al., 2014), implying that glutamate tone in the slice preparation contributes to baseline ChI excitability. Considering the expression of these

Table 1 | Diseases associated with striatal cholinergic dysfunction.

Disorder	Nature of change	Species	Citations
Parkinson's disease (PD)	Smokers are less likely to develop PD	Human	Morens et al., 1995; Allam et al., 2004; Quik et al., 2012
	↓ symptoms with anticholinergic drugs	Human (drug trial)	Katzenschlager et al., 2003; Lanska, 2010; Fox et al., 2011; Fernandez, 2012
	↓ AChE activity	Human (PET Scan)	Gilman et al., 2010
	↓ nAChR binding	Human (postmortem)	Rinne et al., 1991; Aubert et al., 1992; Court et al., 2000; Hellström-Lindahl and Court, 2000; Bohr et al., 2005; Gotti et al., 2006a
		Monkey (MPTP lesion)	Kulak et al., 2002; Quik and McIntosh, 2006
	↓ M1 binding	Human (PM)	Sirviö et al., 1989; Lange et al., 1993; Piggott et al., 2003
	Changes in CHRNB3 gene	Human (genotyping)	Bar-Shira et al., 2014
Huntington's disease	↓ in symptoms with AChE inhibitor	Rat (3-NP lesion)	Kumar and Kumar, 2009
	↓ ChAT activity	Human	Bird and Iversen, 1974; Enna et al., 1976a; Suzuki et al., 2001a
	↓ ChAT mRNA	Mouse (R6/1 model)	Smith et al., 2006
	↓ mAChR binding	Human (postmortem)	Hiley and Bird, 1974; Enna et al., 1976a,b
Alzheimer's disease	↓ cognitive deficits with AChE	Rat (ketamine induced behavior)	Zugno et al., 2013
	↓ AChE levels (in NAc)	Human (PM)	Hammond and Brimijoin, 1988
	↓ nAChR binding sites in putamen, but not in caudate	Human (postmortem)	Shimohama et al., 1985
	No change in nAChR binding	Human (postmortem)	Aubert et al., 1992; Gotti et al., 2006a
	↓ cognitive deficits with α7 or α4β2 agonists	Human (drug trial)	Haydar and Dunlop, 2010
	↑ M1 binding	Human (postmortem)	Aubert et al., 1992
Schizophrenia	↑ likelihood to smoke	Human	Dalack et al., 1998; McEvoy and Allen, 2002
	↑ ChAT activity	Human (postmortem)	McGeer and McGeer, 1977
	↓ ChAT activity	Human (postmortem)	Bird et al., 1977
	↓ ChAT+ cells	Human (postmortem)	Holt et al., 1999
	↓ cognitive deficits with nicotine, α4β2 agonist	Human (drug trial)	Radek et al., 2010
	↑ nAChR binding	Human (postmortem)	Court et al., 2000
	↓ nAChR binding	Human (postmortem)	Durany et al., 2000
	↓ mAChR binding	Human (SPECT scan)	Raedler et al., 2003
	↓ M1 levels	Human (postmortem)	Dean et al., 1996
	Changes in CHNRA7 gene	Human (postmortem, genotyping)	Leonard et al., 2002
Bipolar disorder	↓ β2* nAChR binding	Human (PET scan)	Hannestad et al., 2013
	Changes in CHRNA7 gene	Human (genotyping)	Hong et al., 2004; Ancín et al., 2010
Tourette syndrome	↓ ChAT+ cells	Human (Postmortem)	Kataoka et al., 2010
	↓ tics with cholinesterase inhibitor	Mouse (DOI induced head tics)	Hayslett and Tizabi, 2003
		Human (drug trial)	Cubo et al., 2008
	↓ tics with nicotine	Mouse (DOI induced head tics)	Hayslett and Tizabi, 2003
		Human (drug trial)	Shytle et al., 1996; McEvoy and Allen, 2002
	↓ tics with nAChR antagonist	Mouse (DOI induced head tics)	Hayslett and Tizabi, 2003
		Human (drug trial)	Sanberg et al., 1998; Silver et al., 2000

(Continued)

Table 1 | Continued

Disorder	Nature of change	Species	Citations
	Alternative splicing in ACh related genes	Human (genotyping)	Tian et al., 2011
Attention Deficit Hyperactivity Disorder	No change in performance on attention tasks with nAChR agonist	Human (drug trial)	Jucaite et al., 2014
	↑ performance on attention tasks with nAChR agonist	Rat (MK801 induced attentional impairment)	Rezvani et al., 2012
		Human (drug trial)	Wilens and Decker, 2007; Bain et al., 2013; Potter et al., 2014
	Changes in choline transporter gene	Human (genotyping)	English et al., 2009
	Changes in CHRNA4 gene	Human (genotyping)	Todd et al., 2003; Lee et al., 2008; Guan et al., 2009; Wallis et al., 2009

glutamate receptors on ChIs, it is not surprising that application of AMPA, NMDA, or kainate excites ChIs (Calabresi et al., 1998b; Vorobjev et al., 2000; Cepeda et al., 2001). Collectively, the expression of the three functional classes of glutamate receptors support the idea that glutamate is an important determinant of ChI excitability.

Electron microscopy has revealed that glutamate synapses comprise 13% of total synaptic connections onto ChIs (Gonzales et al., 2013). In the dorsolateral striatum, glutamate is released from cells located in the sensorimotor cortex and the centromedian/parafascicular nucleus of the thalamus, with the vast majority of excitatory projections being thalamic in origin (Berendse and Groenewegen, 1990; Lapper and Bolam, 1992; Thomas et al., 2000; Ding et al., 2010). Glutamatergic inputs, predominantly thalamostriatal projections are likely responsible for synchronous activation of ChIs, which has been suggested to coordinate DA release through activation of nAChRs on DA terminals (Ding et al., 2010; Threlfell et al., 2012). Another source of glutamate, corelease from SNc dopamine terminals, is a topic of debate—Optogenetic activation of SNc dopaminergic axons can produce a small amplitude EPSC in dorsal striatum MSNs (Tritsch et al., 2012). However, using a similar optogenetic approach, others report that glutamate and DA are only coreleased in the ventral, but not dorsal striatum (Stuber et al., 2010). Both of these studies were performed while recording from MSNs, and the electrophysiological significance of SNc-derived glutamate on ChI excitability has not yet been reported. Due to the close proximity between MSNs and ChIs, one would also expect an influence of this source of glutamate on ChIs (Dimova et al., 1993; Li et al., 2002). In this case, the loss of SNc projections in PD may impact the degree to which glutamate modifies ChI activity.

Serotonergic (5-HT) projection neurons from the dorsal raphe nucleus also express glutamate-like immunoreactivity. In culture, 5-HT cells form glutamatergic autapses, indicating that 5-HT projections may functionally co-release glutamate (Nicholas et al., 1992; Johnson and Yee, 1995). The extent to which changes in 5-HT transmission are associated with altered striatal glutamatergic signaling is unknown, but this may contribute to striatal dysfunction in mood disorders. SSRI treatment of depression will prolong the action of synaptically released 5-HT, and may lead to presynaptic inhibition through autoreceptors. This may alter local excitation via co-released glutamate to decrease striatal excitation.

Striatal ChIs also co-release glutamate with ACh (Higley et al., 2011). One isoform of the vesicular glutamate transporter, VGLUT3, is highly expressed in ChIs, and evidence suggests co-expression of this transporter with the vesicular ACh transporter on the same synaptic vesicles. These transporters act synergistically to optimize vesicular loading of ACh and glutamate (Nelson et al., 2014a). Vglut3 knock-out mice have a hypocholinergic striatum, presumably due to a decrease in loading of both glutamate and ACh into vesicles, and also due to less excitatory drive onto synaptically connected ChIs (Gras et al., 2008). The functional consequences of these non-thalamic/non-cortical sources of glutamatergic drive onto ChIs have not been studied in depth.

GLUTAMATE – METABOTROPIC RECEPTORS

Glutamate also mediates long-term modulation of ChIs via metabotropic glutamate receptors (mGluRs). Excitatory group I mGluRs, which include mGluR1 and mGluR5, are highly expressed on ChIs (Tallaksen-Greene et al., 1998; Bell et al., 2002; Conn et al., 2005), and application of group I agonists induce excitation (Calabresi et al., 1999; Pisani et al., 2001; Berg et al., 2007). This excitation is mediated by a combination of cation currents through TrpC channels downstream of $G_{q\alpha}$, as well as inhibition of the chloride activated K^+ channel Slo2.1 (Berg et al., 2007). The group II mGluRs, which consist of mGluR2 and mGluR3, decrease excitability by inhibiting AC through activation of $G_{i/o\alpha}$ (Diraddo et al., 2014). mGluR2, mRNA expression on ChIs (Testa et al., 1994; Bell et al., 2002) indicate that agonists of these receptors would theoretically decrease cell excitability. However, group II mGluRs are more involved in the modulation of synaptic inputs onto ChIs, as activation of these receptors results in no change in membrane potential, but decreases the amplitude of both excitatory and inhibitory synaptic inputs onto ChIs (Pisani et al., 2002; Martella et al., 2009). Of the group III mGluRs, only mGluR7 is expressed on ChIs, at a prevalence of 38% with no expression of mGluR4, 6, or 8. As with group II mGluRs, group III mGluRs decrease presynaptic release probability by inhibition of the AC pathway (Bell et al., 2002). Although rapid ChI excitation and inhibition are mediated by ionotropic

Table 2 | Neurotransmitter systems and their effects on ChI activity.

Neurotransmitter	Source	Postsynaptic receptor targets	Effect on ChI
GABA	MSN PLTS interneurons SNc (?)	$GABA_A$	$GABA_A$: Inhibition DeBoer and Westerink, 1994
Glutamate	Intralaminar thalamic nuclei Sensorimotor cortex SNc Raphe nucleus ChI	GluR1, 2, 4 GluN1, 2D Kainate	AMPA, NMDA, Kainate: Excitation Calabresi et al., 1998b; Vorobjev et al., 2000; Cepeda et al., 2001 mGluR1,5: Excitation Calabresi et al., 1999; Pisani et al., 2001; Berg et al., 2007 mGluR2: Inhibition Martella et al., 2009 mGluR7: No direct effect Bell et al., 2002
Dopamine	SNc DA-ergic interneurons	D1 (low levels) D2 D5	Increased excitation Aosaki et al., 1998; Centonze et al., 2003; Ding et al., 2011b or Decreased excitation Deng et al., 2007a; Chuhma et al., 2014
5-HT	Raphe nucleus	5-HT2 5-HT6 5-HT7	Increased excitation Blomeley and Bracci, 2005 Excitation Bonsi et al., 2007 Excitation Bonsi et al., 2007 or No effect Blomeley and Bracci, 2005
Histamine	TMN Mast cells	H1 H2 H3	Depolarization and action potential firing Bell et al., 2000
Substance P	D1 MSNs	NK1	Depolarization, inward shift in holding current Aosaki and Kawaguchi, 1996 Increased ACh release Arenas et al., 1991; Preston et al., 2000
Enkephalin	D2 MSNs	DOR	Decreased excitation Mulder et al., 1984
		KOR	Decreased excitation Schoffelmeer et al., 1997 No effect on K+ induced ACh release Arenas et al., 1990; Jackisch et al., 1993
		MOR	Decreased excitation Ponterio et al., 2013
Dynorphin	D1 MSNs	KOR	Excitation at low concentrations of agonist Crain and Shen, 1996 Inhibition at higher concentrations of agonist Gross et al., 1990
Noradrenaline	Locus coeruleus	β1	Depolarization, increased action potential firing Pisani et al., 2003
Adenosine	Degradation of ATP	A_1 A_{2A}	Inhibition of ACh release Brown et al., 1990 Increased ACh release Kurokawa et al., 1994 No change in ACh release Jin and Fredholm, 1997
ATP	Synaptic release	P_2X P_2Y	No change in holding current Scheibler et al., 2004
Nitric oxide	NOS+ PLTS interneurons		Depolarization Centonze et al., 2001

Summary of the neurotransmitter systems and other neuromodulators involved in the regulation of ChI activity. "?" denotes potential yet untested source of neurotransmitter release.

receptors, it is important to consider that glutamate can have long term modulatory effects on ChI excitability via mGluR activation.

DOPAMINE

In addition to GABA and glutamate, there are a number of other neurotransmitter systems that affect ChI activity. Striatal dopamine levels are the highest of any region in the brain and it is a principal determinant of striatal function. The predominant source of dopaminergic innervation of the dorsal striatum is A9 neurons—neurons which have cell bodies in the SNc and project broadly into the striatum, forming hundreds of thousands of synaptic connections per neuron (Kubota et al., 1987; Chang, 1988; Arbuthnott and Wickens, 2007; Moss and Bolam, 2008; Matsuda et al., 2009; Threlfell and Cragg, 2011). Although synaptic connections to MSNs are well documented, some reports demonstrate dopamine cells synapse onto ChAT positive cells (Hattori et al., 1976) while others report that dopamine modulates ChIs through volume transmission (Lehmann and Langer,

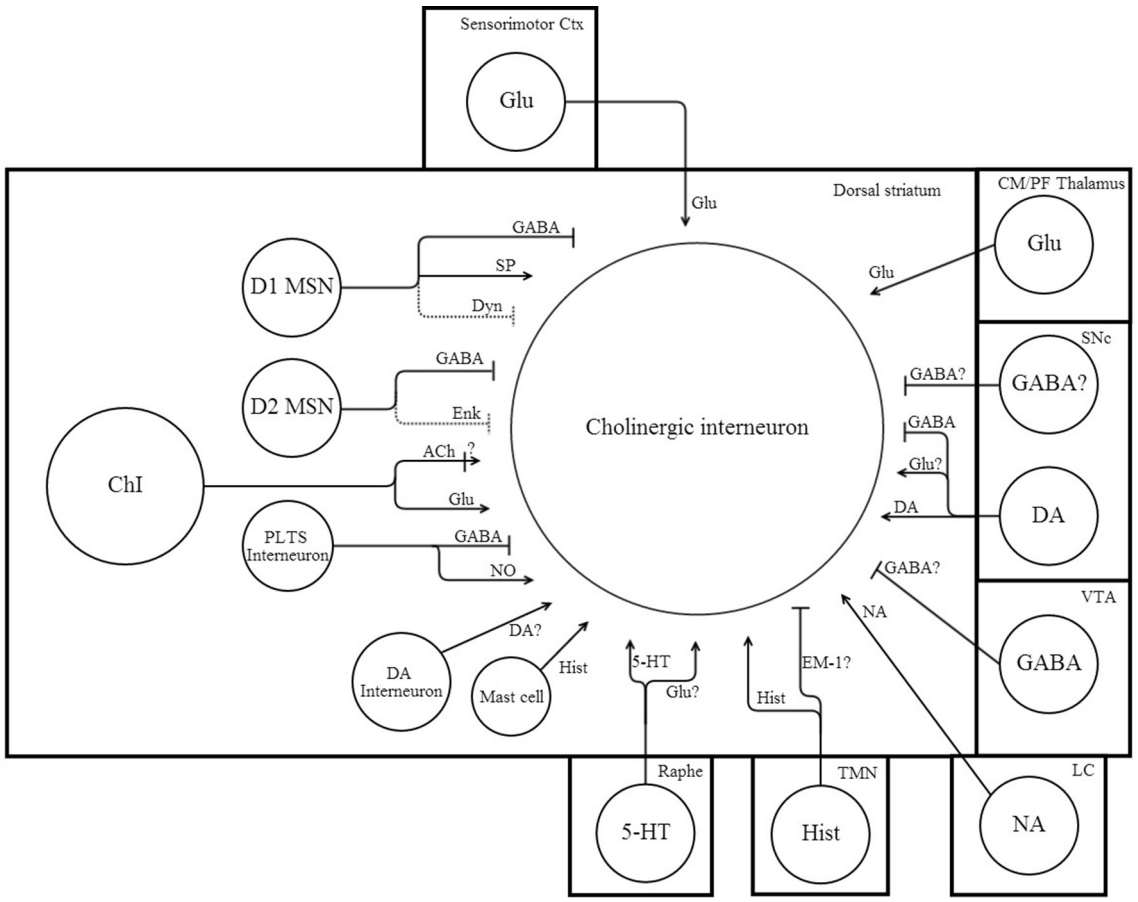

FIGURE 1 | Network connectivity of dorsal striatal cholinergic interneurons. Simplified schematic illustrating some of the afferent synaptic inputs onto ChIs. Arrow heads indicate excitation, while perpendicular lines indicate inhibition. Dotted lines indicate a weak synaptic input. "?" denotes uncertain or untested inputs that are expected by either anatomical or physiological results. References that support afferent connections to ChIs can be found in the text and **Table 2**.

1983). These nigral dopaminergic neurons may exist in 4 different activity states. The tonically active state is independent of excitatory drive, as the neurons will fire at a rate of around 3 Hz *in vivo* or *ex vivo* in a slice preparation (Grace and Bunney, 1984; Hyland et al., 2002; Zhou et al., 2006; Ding et al., 2011a; Henny et al., 2012; Guatteo et al., 2013). They also can transition to burst activity with excitatory inputs increasing activity to around 20 Hz (Grace and Bunney, 1984; Hyland et al., 2002). In addition to these two active states, the cells may exist in one of two silent states, either hyperpolarization below action potential threshold, or depolarization block. Activity of these neurons is crucially important to normal striatal function (Gasser, 2009).

Postsynaptically, the majority of ChIs express D2 and D5 receptors with only about 20% of the neurons expressing low levels of D1 receptors (Dawson et al., 1988; Bergson et al., 1995; Yan et al., 1997). D2 receptors generally decrease neuronal exitability through activation of $G_{i/o\alpha}$, which also inhibits AC activity to decrease cAMP levels. D5 receptors are members of the D1 family that activate AC through G_s and generally increase excitability (Beaulieu and Gainetdinov, 2011). In the slice preparation, bath application of DA can strongly excite ChIs (Aosaki et al., 1998; Centonze et al., 2003; Ding et al., 2011b), however others have reported that DA inhibits ChIs by prolonging slow afterhyperpolarization duration (Deng et al., 2007a), and that optogenetic activation of DA terminals induces a pause in ChI firing (Chuhma et al., 2014). It was also reported that amphetamine-induced increases in striatal DA rhas no effect on ACh efflux *in vivo* (Abercrombie and DeBoer, 1997), implying that, under those conditions, elevated DA does not significantly affect cholinergic tone. Clearly, DA can affect ChI excitability, and the conditions under which DA either excites or inhibits these neurons will require further study.

In addition to this important extrastriatal source of DA, there also exists a small population of striatal DA interneurons in both primates and rodents (Dubach et al., 1987; Cossette et al., 2005; Ibáñez-Sandoval et al., 2010). Interestingly, the number of striatal TH+ cells increases following acute experimental dopamine depletion in both rodents and primates (Tashiro et al., 1989; Betarbet et al., 1997; Smith and Kieval, 2000; Jollivet et al., 2004). This is potentially a compensatory mechanism designed to counteract the loss of DA-ergic innervation from SNc. Strangely, the number of striatal TH+ cells is decreased in humans with PD

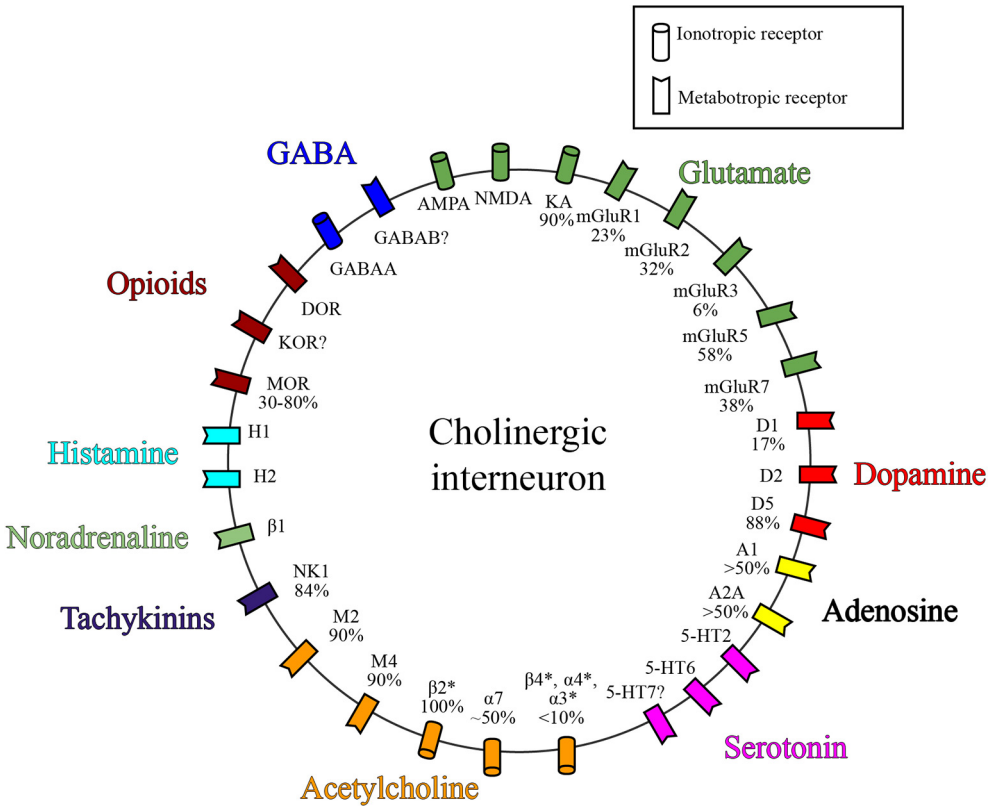

FIGURE 2 | Receptor expression on ChIs. Simplified diagram depicting a subset of known receptor classes expressed on ChIs grouped by neurotransmitter. Values represent percent of ChIs that express the given receptor. References for receptor expression by ChIs can be found in the text and **Table 2**.

(Huot et al., 2007), highlighting one difference between experimentally induced PD and the actual pathogenesis of the disease in humans. These DA interneurons form inhibitory GABA-ergic synapses with MSNs (Ibáñez-Sandoval et al., 2010). Whether or not these TH+ interneurons make DA-ergic and/or GABA-ergic synaptic contacts with ChIs is yet to be determined. Another interesting question to address would be whether or not these TH+ interneurons undergo changes in physiology that serve in a homeostatic role in the Parkinsonian striatum. Independent of changes in cell numbers outlined above, increased excitability following DA depletion may counteract low levels of striatal DA to help maintain striatal function. This small minority of striatal cells remains an interesting focus for future investigations.

ACETYLCHOLINE

ChIs receive synaptic inputs from other ChIs. Both nAChRs and mAChRs are expressed at various levels on ChIs. With respect to nAChR expression, *in situ* hybridization has shown that all ChIs express mRNA for β2 subunits, about half express α7 mRNA, while other subunit mRNAs are expressed at low levels (Azam et al., 2003). In support of the idea that ChIs express nAChRs, nicotine application to a slice preparation induces ACh release (Sandor et al., 1991). Interestingly, that effect was only seen in slices from animals that had undergone dopamine depletion with 6-OHDA treatment or in the presence of the D2 receptor antagonist sulpiride. These data suggest that resolving the nAChR-mediated ACh release requires elimination of D2 receptor mediated inhibition (Sandor et al., 1991).

The mAChR component of ACh modulation is through activation of the $G_{i/o}$ coupled M2 and M4 receptors (Weiner et al., 1990; Smiley et al., 1999; Ding et al., 2006), thus acting as an autoinhibitory clamp to prevent excessive ACh release. No co-expression of M1 and ChAT is observed in the striatum (Dawson et al., 1990; Alcantara et al., 2001).

Synchronized activity in ChIs is observed following presentation of behaviorally salient stimuli (Apicella et al., 1997; Ravel et al., 1999). Although this synchronous firing has been linked to coordinated thalamostriatal inputs (Ding et al., 2010), ChI projections to other ChIs may also contribute to this synchrony through positive feedback control to coordinate strong increases in ACh. Whether or not nAChR mediated transmission contributes to ChI-ChI signaling has yet to be reported.

SEROTONIN

Serotonin is a major determinant of ChI excitability. 5-HT-ergic projections originate from the raphe nucleus in the hind brain. These cells fire tonically at a rate of about 1–2 Hz (Innis and Aghajanian, 1987; Sprouse et al., 1989; Haj-Dahmane et al., 1991), releasing 5-HT into many brain areas including the striatum. 5-HT has an overall direct excitatory effect on ChIs, increasing action potential firing and membrane depolarization (Blomeley and Bracci, 2005; Bonsi et al., 2007). Activation of the G_q-coupled

5-HT2 receptors increases excitability due to a decrease in the amplitude of both the slow and medium afterhyperpolarization (AHP) (Blomeley and Bracci, 2005). It has not been examined if 5-HT receptor classes 1A, 3, or 4 are expressed on ChIs, however selective agonists of these receptor classes do not induce changes in ChI excitability (Blomeley and Bracci, 2005). 5-HT6 activation excites ChIs (Bonsi et al., 2007). The contribution of the 5-HT7 receptor to excitation is debated, as Blomeley and Bracci (2005) reported no effect, while Bonsi et al. (2007) observed depolarization.

HISTAMINE

Histamine (HA) is a neurotransmitter that was first identified as a peripheral vasodilator with an effect on respiratory patterns and muscle tone (Dale and Laidlaw, 1910). Originally described in the brain in 1984, HA immunoreactive fibers were found to project widely throughout the brain including the striatum (Haas et al., 2008). HA-ergic cell bodies reside only in a small region of the posterior hypothalamus, the tuberomamillary nucleus (Panula et al., 1984; Blandina et al., 2012). Histamine is also produced by mast cells (Schwartz et al., 1986).

HA can act on 4 different types of G-protein coupled receptors, H1 through H4 (Parsons and Ganellin, 2006). These receptors are widely expressed, but only H1, H2, and H3 are highly expressed in the striatum, with H1 and H2 expressed on ChIs, and the autoreceptor H3 being expressed presynaptically on HA-ergic terminals. H1 and H2 are excitatory, coupling to G_q and G_s respectively, while the inhibitory H3 receptor is coupled with G_i (Timmerman, 1989; Haas et al., 2008). HA application has the net result of depolarization of ChIs, presumably acting through by H1 receptors (Bell et al., 2000). In the ventral striatum, ACh overflow is increased following H1 activation, while blockade of H2 increases ACh overflow, presumably through activation of H2 on GABA interneurons (Prast et al., 1999b). The same group found an increase in ACh overflow with concurrent H3 activation, an effect mediated by presynaptically expressed GABA interneurons (Prast et al., 1999a).

OPIOIDS

ChIs are also sensitive to opioidergic modulation. δ, κ, and μ opioid receptors (DOR, KOR, and MOR, respectively) are the three major classes of opioid receptors in mammals. These receptors inhibit cell activity through coupling with $G_{i/o}$ proteins (Mansour et al., 1994; Tso and Wong, 2003), and are activated endogenously by a number of tightly regulated peptides. The endogenous opioid enkephalin activates DORs, and is produced by D2 expressing indirect pathway MSNs. mRNA for DORs is expressed in striatal ChIs (Le Moine et al., 1994), and activation of these receptors decreases ACh release (Mulder et al., 1984). The low number of synaptic connections between enkephalinergic cells and ChAT positive cells (Martone et al., 1992) suggests that enkephalin may only minimally inhibit ChI activity endogenously, but this does not preclude the possibility of volume transmission. Systematic investigation of endogenous DOR effects on ChI excitability has not been reported.

KOR is another major class of opioid receptors in the striatum. The endogenous opioid dynorphin is produced by D1 expressing direct pathway MSNs, and activates KORs. Like DORs, KORs are widely expressed in the striatum (Fallon and Leslie, 1986; Mansour et al., 1994). Compared to the DOR or MOR, the KOR receptor can be associated with $G_{i/o}$ as well as G_s. This bipolar effect of KOR activation is concentration dependent. At very low, subnanomolar concentrations of agonist, KOR preferably couples to G_s (Crain and Shen, 1996), but increasing the concentration results in the activation of signaling cascades downstream of $G_{i/o}$ (Gross et al., 1990; Claye et al., 1996). Thus, depending on the level of striatal dynorphin, ChIs may either increase or decrease their excitability. KOR activation decreases ACh release in the striatum (Mulder et al., 1991; Schoffelmeer et al., 1997), however, different studies showed no effect of KOR activation on ACh release (Arenas et al., 1990; Jackisch et al., 1993). These apparently contradictory findings could result from the biphasic dose-dependent intracellular coupling of the KOR. It is also possible that the KOR effects on ACh release occur through indirect modulation, as there has not been a direct demonstration of co-localization of KOR with ChAT expression in striatum, nor is there direct electrophysiological evidence of KOR expression on ChIs.

The MOR is also coexpressed on ChAT positive striatal cells, however with tremendous diurnal variation, fluctuating from 30% coexpression in the daytime to a peak of 80% coexpression in the afternoon (Jabourian et al., 2005). Activation of MOR by exogenous DAMGO decreases ChI firing (Ponterio et al., 2013). MOR-inhibition of ACh release lowers the DA release probability in striatum by limiting activation of presynaptic nAChRs (Britt and McGehee, 2008). Endomorphin-1 (EM-1), an endogenous agonist at the MOR, shows only weak immunoreactivity in the striatum. EM-1 may be co-released by histaminergic neurons, as the EM-1 immunoreactivity signal is very prominent in the posterior hypothalamus (Martin-Schild et al., 1999). In addition, some endogenous agonists have overlapping affinity for different opioid receptor classes, such as Leu-enkephalin, which activates both DORs, and MORs at physiological concentrations (Jabourian et al., 2005).

TACHYKININS

Tachykinins are another class of neuropeptides expressed in the striatum. In addition to producing GABA and dynorphin, D1-expressing MSNs also express the tachykinin Substance P Terminals that contain Substance P. form synaptic connections with ChIs. Substance P. is a potent activator of NK1 receptors, which are expressed by ChIs (Bolam et al., 1986; Richardson et al., 2000). Activation of NK1 results in excitation (Aosaki and Kawaguchi, 1996) and increased ACh release (Arenas et al., 1991; Preston et al., 2000).

EFFERENT CONNECTIONS OF ChIs

Even though the ChIs make up a small fraction of cells in the striatum, they possess a large synaptic arbor and thus send ACh projections broadly throughout the striatum. As such, changes in ChI physiology influence a multitude of postsynaptic targets by activation of nicotinic and muscarinic ACh receptors. This section addresses the effects of ACh neurotransmission.

NICOTINIC ACETYLCHOLINE RECEPTORS

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated, pentameric ion channels that are activated by endogenous ACh, exogenous nicotine, or other ligands. nAChRs can be expressed both pre and postsynaptically, where they induce depolarization and increase excitability. Presynaptic nAChRs enhance release of several different neurotransmitter types (MacDermott et al., 1999). The subunits that are assembled into neuronal nAChRs include α2-α10 and β2-β4 (Patrick et al., 1993; McGehee and Role, 1995; Dani, 2001). They can be composed of homomeric or heteromeric subunit combinations, which determine characteristic pharmacological and biophysical properties of the receptor (Fenster et al., 1997; Gotti et al., 2006b). In the striatum, the most common nAChR subunits are the α4, α6, α7, β2, and β3, although other subunits are present at lower levels (Quik et al., 2007). Generally, nAChR activation induces rapid depolarization, but Ca^{2+} entry, particularly through homomeric α7 nAChRs can lead to rapid changes in neurotransmitter release or long term changes in cellular function through activation of Ca^{2+} dependent intracellular cascades, such as altered transcription through pCREB activation (Mulle et al., 1992; Chang and Berg, 2001; Hu et al., 2002; Wu et al., 2009; Del Barrio et al., 2011).

MUSCARINIC ACETYLCHOLINE RECEPTORS

In comparison to the rapid, excitatory effect of nAChR activation, mAChR activation serves a more long-term modulatory role. Activation of mAChRs can either increase or decrease cell excitability. A total of 5 subtypes of mAChRs have been isolated and cloned, but they are generally divided into 2 classes based on differences in their intracellular signaling cascades. The excitatory mAChRs, consisting of M1, M3, and M5, couple to $G_{q/11}$ and induce activation of the phospholipase C pathway (Lin et al., 2004). The inhibitory receptors, M2, and M4, couple to $G_{i/o}$ proteins and decrease activity of adenylyl cyclase (Wess, 1996). All 5 mAChRs are expressed in the striatum (Yan et al., 2001), however M1 and M4 are more heavily expressed than other isoforms, with a small presence of M2 and very low levels of M3 and M5 expression (Yasuda et al., 1993). Muscarinic receptors are not limited to somatic expression, as terminal expression of mAChRs serves to modulate neurotransmitter release probability. Expression of M2 receptors on ChI terminals serves an autoinhibitory role (Hersch et al., 1994). Additionally, neurotransmitter release at incoming afferents can be sensitive to mAChR modulation, as these mAChRs can receive synaptic inputs from ChIs. Because mAChR activation can either increase or decrease cell excitability, the net effect of ACh release depends on the patterns of postsynaptic mAChR expression. For each major striatal postsynaptic target, both the nicotinic and muscarinic effects on neuronal excitability will be addressed.

MEDIUM SPINY NEURONS

GABA-ergic MSN projection neurons are the sole output of the striatum. Direct activation of AChRs on MSNs therefore represent a direct effect of ACh on striatal output. MSNs are generally believed to lack nAChRs (Matsubayashi et al., 2001; Luo et al., 2013), although Liu et al. (2007) reports that direct activation of nAChRs on MSNs by nicotine induces depolarization. This direct nAChR modulation of MSN activity has not been explored in depth, as the evidence that MSNs express nAChRs is quite limited. Interestingly, lesion studies indicate that only about 20% of α4β2 nAChRs are expressed on DA terminals (Quik and Wonnacott, 2011). Thus, the contribution of these receptors to striatal circuitry likey involves expression on GABAergic interneurons and presynaptic projections from a range of cell types. Resolving the complete physiological role of striatal α4β2 receptors is a topic of ongoing investigations.

The majority of studies of ACh-mediated modulation of MSNs focuses on mAChR activation. Bath application of the mAChR agonist carbachol increases MSN excitation in the absence of synaptic input, both in a slice preparation and in dissociated cell culture (Hsu et al., 1996; Galarraga et al., 1999). Two mechanisms have been proposed to explain this excitation. One involves an M1 mediated decrease in the inhibitory KCNQ potassium (Kv7) current (Shen et al., 2005), while the other an M1 mediated inhibition of Ca^{2+} entry through N and P/Q type channels, which in turn decreases the duration of the AHP (Pérez-Garci et al., 2003; Perez-Rosello et al., 2005). Neither of these studies differentiates between the direct and indirect pathway MSNs, and the mechanisms could differ between these cell types. Inhibitory M4 receptors are expressed on a subpopulation of MSNs (Bernard et al., 1992), and functional electrophysiological evidence suggests that M4 decreases Ca^{2+} influx to decrease excitability (Howe and Surmeier, 1995). Direct pathway MSNs express both M1 and M4, while indirect pathway MSNs express M1. Less than half of indirect pathway MSNs express M4 (Bernard et al., 1992; Yan et al., 2001). Both classes of MSNs would be excited with M1 activation, but the differential expression pattern of the inhibitory M4 could mean that ACh influences the two classes of MSNs in opposing directions.

GABA-ERGIC INTERNEURONS

In addition to directly acting on MSNs, ACh can also modify striatal output through receptors on GABA interneurons that project to MSNs. Optogenetic activation of cholinergic cells produced IPSCs and IPSPs in MSNs that were inhibited by nAChR blockade. This microcircuit is believed to be a disynaptic connection, consisting of nAChR-expressing GABA interneurons that are activated by ACh, which then release GABA onto MSNs (English et al., 2011). The GABA interneurons that contribute to this inhibition of MSNs are likely the parvalbumin-expressing FSIs (Chang and Kita, 1992) and/or the NPY-expressing PLTS interneurons (English et al., 2011). However, English and coworkers did not observe an involvement of FSIs in the ChI-MSN interaction. Consistent with nAChR activation leading to elevated striatal GABA, inhibition of α7 receptors resulted in a decrease in striatal GABA in awake behaving animals (Beggiato et al., 2013), while activation of α7 nAChRs increases GABA levels (Campos et al., 2010).

Muscarinic receptor activation of GABA interneurons that project to MSNs can influence striatal output. Subcellular localization of the M2 receptor has been demonstrated in the NPY+ PLTS interneurons (Bernard et al., 1998). Consistent with this result, ACh decreases striatal GABA release (Marchi et al., 1990). More specifically, this is mediated by an inhibitory mAChR, as

muscarine decreases GABA release onto MSNs (Sugita et al., 1991). Thus far, there are no reports of M1 receptor expression on GABA interneurons, but it is possible that M1-mediated enhancement of GABA output from one of the other interneuron subtypes neurons could contribute to striatal circuitry.

GLUTAMATERGIC TERMINALS

Glutamatergic inputs into the dorsal striatum originate primarily from the intralaminar nuclei of the thalamus and from the sensorimotor cortex, with a small amount of glutamate co-released from other terminals as well (Higley et al., 2011). nAChR expression on glutamatergic terminals provides a mechanism for cholinergic enhancement of excitatory drive onto MSNs. Increased glutamate release through activation of presynaptic nAChRs has been observed in brain regions such as the hippocampus, medial habenula, olfactory bulb and human neocortex (McGehee et al., 1995; Gray et al., 1996; Fisher and Dani, 2000; Girod et al., 2000; Marchi et al., 2002). Glutamate release probability is also modulated by nAChRs in the striatum (Kaiser and Wonnacott, 2000). *In vivo* microdialysis studies demonstrate that α7 nAChR activation in striatum increases glutamate release (Campos et al., 2010). α7 nAChR antagonism decreases glutamate release (Carpenedo et al., 2001), indicating that baseline ACh levels contribute to glutamatergic tone. Because the homomeric α7 subtype is highly Ca^{2+} permeable compared to other nAChRs stoichiometries, Ca^{2+} entry through these receptors may lead directly to enhanced neurotransmitter release (Gray et al., 1996). Activation of the α4β2* subtype also increases glutamate release onto MSNs (Xiao et al., 2009). As glutamatergic inputs originate from various neuronal types and brain regions, differential expression of nAChR stoichiometries may allow ChIs to amplify glutamate inputs differentially.

In contrast, activation of mAChRs negatively modulates striatal glutamate release. In field potential recordings, a mAChR agonist suppressed corticostriatal glutamatergic transmission (Malenka and Kocsis, 1988). Increasing mAChR signaling either by increasing ChI firing rates, or exogenous agonist application decreases excitatory drive onto MSNs (Calabresi et al., 1998a; Pakhotin and Bracci, 2007; Pancani et al., 2014). Muscarinic modulation of glutamatergic terminals occurs through M2 or M4 receptor activation, as mRNA and protein levels for both mAChRs are observed at high levels in striatal somata as well as terminals (Levey et al., 1991; Hersch et al., 1994). M2 or, interestingly enough, M3 activation results in paired pulse facilitation, indicating that mAChR activation decreases glutamate release probability (Hernández-Echeagaray et al., 1998; Ding et al., 2010). The change in release probability is observed in both corticostriatal afferents and thalamostriatal afferents, and when recording from both direct and indirect MSNs, indicating that regardless of the origin of the terminal or the post-synaptic target, release probability at glutamatergic terminals is decreased with mAChR activation (Ding et al., 2010). In agreement with these observations, intrastriatal injections of an M2-selective antagonist increases glutamate overflow (Smolders et al., 1997), providing evidence that tonic levels of ACh contribute to striatal glutamate tone. Additionally, glutamate release is downregulated via mAChR activation (Dodt and Misgeld, 1986). ChIs are thus in a position to regulate excitatory inputs to MSN, both rapidly by acting on nAChRs, and more slowly and persistently via mAChR activation.

DOPAMINERGIC TERMINALS

ACh profoundly modulates DA release in the striatum. In a slice preparation, optogenetic activation of ChIs increases evoked DA release. The quantity of DA released is dependent on frequency of stimulation, and requires synchronous ChI cell activation as well as activation of β2-containing nAChRs expressed on dopaminergic terminals (Cachope et al., 2012; Threlfell et al., 2012). Although the physiological conditions that coordinate synchronous firing of large numbers of ChIs are unknown, cross-talk between ChIs may facilitate simultaneous firing of these neurons to enhance DA release.

Dopaminergic terminals express α4 and β2 subunits at high levels, along with α5, α6, α7, and β3 subunits at variable levels (Le Novère et al., 1996; Sharples et al., 2000; Jones et al., 2001; Klink et al., 2001; Quik et al., 2003; Grady et al., 2007; Keath et al., 2007). Nicotinic agonists increase the efflux of DA in striatal tissue, as measured by microdialysis (Puttfarcken et al., 2000; Campos et al., 2010), and as expected, nAChR antagonists decrease DA efflux by interfering with the effects of local ACh activation of presynaptic nAChRs on DA terminals (Wonnacott et al., 2000; Grady et al., 2007). There is evidence that enhancement of DA release by exogenous activation of nAChRs requires glutamatergic signaling (Garcia-Munoz et al., 1996; Wonnacott et al., 2000), but enhancement of DA release by coordinated ACh release from ChIs is not dependent upon glutamate transmission (Threlfell et al., 2012). Additionally, striatal dopaminergic terminals also corelease GABA. In a recent study, optogenetic activation of ChIs produces a GABAA receptor mediated synaptic response in MSNs. Pharmacological blockade of α4 nAChRs inhibits this GABA current, suggesting that striatal nAChRs regulate GABA levels via modulation of release probability from DA terminals (Nelson et al., 2014b).

The expression of nAChRs on DA terminals not only enhances DA transmission, chronic agonist exposure, such as that achieved in tobacco users can shift DA release probability to suppress release during low frequency activity, but maintain or enhance release during burst firing (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004). These observations were obtained using high resolution fast-scan cylclic voltammetry to assess extracellular DA levels, and they suggest that nicotine may enhance the impact of high frequency DA neuron activity to effectively increase the salience of environmental stimuli. Recent *in vivo* investigations suggest that a chronic nicotine exposure model, which mimics the daily pattern of nicotine exposure by smokers (2 weeks via drinking water), results in a downregulation of electrically stimulated DA release due to persistent desensitization of nAChRs on DA terminals (Koranda et al., 2014). This observation extends the results seen in other preparations including brain slices and non-human primates (Perez et al., 2012, 2013; Exley et al., 2013). Together, these findings have led to the intriguing speculation that the reported protective effects of smoking against PD may result from an adaptation in striatal

circuitry to lower DA levels, thus delaying the onset of symptoms (Koranda et al., 2014).

Exogenous nicotine affects striatal dopamine in interesting and sometime counterintuitive ways, as the interplay between nAChR activation and desensitization can lead to contradictory effects. In contrast, endogenous ACh from ChIs is rapidly degraded by acetylcholinesterase, which is expressed at remarkably high levels in striatum. As alluded to above, synchronous ChI activation can have profound effects on DA release in striatum, through the coordinated activation of presynaptic nAChRs on DA terminals (Threlfell et al., 2012). This study from the Cragg laboratory used optogenetic stimulation to coordinate ChI activity selectively to demonstrate this phenomenon. This is relevant to endogenous activation of ChIs, as synchronous stimulation of these neurons has been reported through coordination of thalamostriatal inputs in response to salient environmental stimuli (Ding et al., 2010; Threlfell et al., 2012).

While mAChRs are also involved in modulation of DA release, the identity of mAChRs expressed by SNc DA cells is unclear, as some observe M2, M4, and M5 (Vilaró et al., 1990; Levey et al., 1991), while others report expression of only M5 receptors (Weiner et al., 1990). Agreement on M5 receptor expression suggests that dopaminergic terminals in the striatum express this mAChR subtype. Electrophysiological evidence supports this, as M5 KO mice show reduced oxotremorine enhancement of potassium-stimulated dopamine release (Zhang et al., 2002). M2 receptors are also involved in tonic DA release, as intrastriatal administration of an M2 antagonist dramatically increases DA levels in freely moving rats (Smolders et al., 1997). Non-selective activation of striatal mAChRs with oxotremorine increases DA release (Lehmann and Langer, 1982; Threlfell et al., 2010), suggesting that M5 activation plays a stronger role in the modulation of DA terminals.

PLASTICITY OF THE EXCITATORY INPUTS TO CHOLINERGIC INTERNEURONS

Activity-dependent modification of synaptic connections is believed to be an important cellular substrate for learning and memory. As the dorsal striatum is believed to be an important site of action for habit formation and motor learning, it is likely that synaptic plasticity contributes to that learning. Considerable effort has focused on understanding the plasticity of the excitatory inputs to MSNs (Calabresi et al., 1996; Mahon et al., 2004; Surmeier et al., 2007), however, LTP/LTD in ChIs has not been explored extensively. Recording in tissue slices from dorsal striatum, Suzuki et al. (2001b) demonstrated LTP in ChIs following a 1 s, 100 Hz train stimulation of the corpus callosum. This LTP was dependent on Ca^{2+} entry, as intrapipette BAPTA blocked LTP induction. The source of Ca^{2+} entry in these studies was not from NMDA receptors, as NMDA blockers had no effect on LTP induction. They also found this LTP to be D5 receptor dependent, as pharmacological blockade of D1/D5 receptors prevents the long term maintenance of enhanced synaptic strength, whereas D2 receptor blockade did not affect LTP induction (Suzuki et al., 2001b). Bonsi and co-workers observed a similar LTP (using three 1 s, 100 Hz trains), however they attribute LTP at these synapses to calcium entry via L-type HVA channels, and not to Ca^{2+} permeable AMPA receptors or NMDA receptors (Bonsi et al., 2004). Using the same HFS stimulation paradigm as Bonsi et al, Picconi and coworkers demonstrate that plasticity of ChIs is not observed in the R6/2 mouse model of Huntington's disease (Picconi et al., 2006). It is not clear why a Huntington's disease model should lack plasticity at these synapses, which highlights the need for better understanding of the underlying mechanisms and functional significance of synaptic plasticity of the inputs to striatal ChIs.

Spike-timing dependent plasticity (STDP) is another experimental paradigm used to induce plasticity. In accordance with Hebbian theory, changing the time between presynaptic activation and postsynaptic depolarization can elicit either a strengthening, weakening, or no change in synaptic strength. First discovered in the cortex, examples of STDP have been observed in other parts of the nervous system including the hippocampus, striatum neuromuscular junction, and cerebellum (Linden et al., 1991; Markram et al., 1997; Wan and Poo, 1999; Nishiyama et al., 2000; Plotkin et al., 2013). To date, only one group has reported STDP in ChIs. Fino and colleagues observed bidirectional plasticity in a majority of ChIs—Post-pre stimulation elicited an LTP in some cells and LTD in others, while pre-post stimulation results in only LTD. Using pharmacology, they found that both forms of plasticity depend upon mGluR activation (Fino et al., 2008). These findings contrast with the Suzuki study where HFS induced-LTP was insensitive to mGluR blockade (Suzuki et al., 2001b). Additionally, post-pre stimulation plasticity was inhibited by NMDA receptor blockade, suggesting that HFS induced-LTP occurs by another mechanism. Further exploration into LTP/LTD of the excitatory inputs to ChIs represents an exciting field of study, as changes in synaptic strength here may contribute to motor skill learning and habit formation.

SUMMARY

Even though ChIs only make up 1–2% of all striatal cells, they send dense projections throughout the striatum. ACh can affect the output of the striatum directly or indirectly, and a wide variety of neurotransmitter systems can influence the activity of these cells. Given that ChIs are in a position to integrate synaptic inputs and modulate the output of the striatum, understanding the physiology of these cells will contribute to our knowledge of striatal function.

ChIs receive afferent inputs from a wide variety of sources which can arise either locally from within the striatum, or from brain regions as distant as the brainstem. Some of these neurotransmitter systems alter cellular excitability rapidly through their actions on ionotropic receptors, producing rapid electrical signals on a millisecond time scale. These changes in membrane properties affect the firing activity of the ChIs, and given the hundreds of thousands of synaptic contacts formed by each ChI, several other cell types are influenced by changes ACh release. The inherent membrane properties of ChIs allows them to be easily modified in either direction during neurotransmitter release: their depolarized resting membrane potential allows excitatory neurotransmitters to easily enhance action potential firing rate, and considering their tonic activity, inhibitory neurotransmitters will inhibit firing, reducing total cholinergic tone. Thus, because

these cells are resting at some intermediate state of activity, they are sensitive to incoming afferents. In addition to the ionotropic receptors on these cells, ChIs express a wide variety of GPCRs. Activation of GPCRs can have a multitude of cellular effects, including the opening of ion channels, changes in plasticity or protein transcription (Altier, 2012; Rojas and Dingledine, 2013). The long time course of the signaling cascades downstream of GPCR activation could indicate that a temporary increase in neurotransmitter activity may lead to long-lasting modifications in ChI physiology that increases or decreases the cellular response to other incoming afferents. Given the sensitivity of these cells to various synaptic inputs, understanding ChI connectivity provides insight into the striatal network.

ACh has a wide variety of effects following release, either by directly activating receptors on postsynaptic cells or indirectly via the modulation of neurotransmitter release at terminals. Changes in striatal cholinergic tone will thus result in a complex series of downstream effects which ultimately may affect the striatal output neurons. Considering the multiple ACh-sensitive neurotransmitter systems that are involved in the striatal network, changes in receptor function or expression on any class of cells may result in a shift in the balance of these systems, potentially resulting in dysfunction. Understanding the nature of synaptic connectivity and the location of receptor expression therefor has a direct connection with human pathology. Using this knowledge in conjunction with an understanding of the changes that occur in disease, we can work toward the development of novel therapies that are aimed at counteracting neurotransmitter dysregulation.

Often times, we oversimplify the nature of the neurotransmitters released at a given terminal, neglecting the co-release of other neurotransmitters whose post-synaptic effects can differ from the primary neurotransmitter. Although there is evidence that co-release occurs, very few have looked in detail at the functional consequences of multiple transmitter release, or whether or not these neurotransmitters are released in sufficient amounts to contribute to cell physiology.

New genetic techniques can improve our understanding of striatal neurotransmission in both the normal and abnormal brain. Optogenetic manipulation of excitability in specific neuronal subtypes is providing important insights into connectivity throughout the nervous system. This robust technology has advantages over other methods of exogenous neural control and certainly provides a means to explore ChI efferent and afferent connections, as well as the nature of the neurotransmitter phenotypes that influence the excitability of these neurons. Extending these methods to *in vivo* analyses can help provide causal links between synaptic information and behavior.

CLARITY is a potentially groundbreaking new technique that allows high resolution visualization of subcellular structures such as individual synapses in the whole brain (Chung and Deisseroth, 2013). Because the preparation for CLARITY removes brain lipids, antibodies can easily permeate the entirety of the brain, permitting the resolution of the striatal connectome in total. This anatomical information will precisely elucidate the synaptic connectivity between striatal cell types.

Ultimately, ongoing efforts to improve our understanding of striatal ChIs will provide valuable insights into the physiology of this important brain area and help identify new pharmacotherapies for striatal disorders.

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REFERENCES

Abercrombie, E. D., and DeBoer, P. (1997). Substantia nigra D1 receptors and stimulation of striatal cholinergic interneurons by dopamine: a proposed circuit mechanism. *J. Neurosci.* 17, 8498–8505.

Ahlskog, J. E., and Muenter, M. D. (2001). Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov. Disord. Off. J. Mov. Disord. Soc.* 16, 448–458. doi: 10.1002/mds.1090

Albin, R. L., Young, A. B., and Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends Neurosci.* 12, 366–375. doi: 10.1016/0166-2236(89)90074-X

Alcantara, A. A., Mrzljak, L., Jakab, R. L., Levey, A. I., Hersch, S. M., and Goldman-Rakic, P. S. (2001). Muscarinic m1 and m2 receptor proteins in local circuit and projection neurons of the primate striatum: anatomical evidence for cholinergic modulation of glutamatergic prefronto-striatal pathways. *J. Comp. Neurol.* 434, 445–460. doi: 10.1002/cne.1186

Allam, M. F., Campbell, M. J., Del Castillo, A. S., and Fernández-Crehuet Navajas, R. (2004). Parkinson's disease protects against smoking? *Behav. Neurol.* 15, 65–71. doi: 10.1155/2004/516302

Altier, C. (2012). GPCR and voltage-gated calcium channels (VGCC) signaling complexes. *Subcell. Biochem.* 63, 241–262. doi: 10.1007/978-94-007-4765-4_13

Ancín, I., Barabash, A., Vázquez-Álvarez, B., Santos, J. L., Sánchez-Morla, E., Martínez, J. L., et al. (2010). Evidence for association of the non-duplicated region of CHRNA7 gene with bipolar disorder but not with Schizophrenia. *Psychiatr. Genet.* 20, 289–297. doi: 10.1097/YPG.0b013e32833a9b7a

Aosaki, T., and Kawaguchi, Y. (1996). Actions of substance P on rat neostriatal neurons *in vitro*. *J. Neurosci.* 16, 5141–5153.

Aosaki, T., Kiuchi, K., and Kawaguchi, Y. (1998). Dopamine D1-like receptor activation excites rat striatal large aspiny neurons *in vitro*. *J. Neurosci.* 18, 5180–5190.

Aosaki, T., Tsubokawa, H., Ishida, A., Watanabe, K., Graybiel, A. M., and Kimura, M. (1994). Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. *J. Neurosci.* 14, 3969–3984.

Aouizerate, B., Guehl, D., Cuny, E., Rougier, A., Bioulac, B., Tignol, J., et al. (2004). Pathophysiology of obsessive-compulsive disorder: a necessary link between phenomenology, neuropsychology, imagery and physiology. *Prog. Neurobiol.* 72, 195–221. doi: 10.1016/j.pneurobio.2004.02.004

Apicella, P., Legallet, E., and Trouche, E. (1997). Responses of tonically discharging neurons in the monkey striatum to primary rewards delivered during different behavioral states. *Exp. Brain Res.* 116, 456–466. doi: 10.1007/PL00005773

Apicella, P., Ljungberg, T., Scarnati, E., and Schultz, W. (1991). Responses to reward in monkey dorsal and ventral striatum. *Exp. Brain Res.* 85, 491–500. doi: 10.1007/BF00231732

Arbuthnott, G. W., and Wickens, J. (2007). Space, time and dopamine. *Trends Neurosci.* 30, 62–69. doi: 10.1016/j.tins.2006.12.003

Arenas, E., Alberch, J., Perez-Navarro, E., Solsona, C., and Marsal, J. (1991). Neurokinin receptors differentially mediate endogenous acetylcholine release evoked by tachykinins in the neostriatum. *J. Neurosci.* 11, 2332–2338.

Arenas, E., Alberch, J., Sanchez Arroyos, R., and Marsal, J. (1990). Effect of opioids on acetylcholine release evoked by K+ or glutamic acid from rat neostriatal slices. *Brain Res.* 523, 51–56. doi: 10.1016/0006-8993(90)91633-R

Aubert, I., Araujo, D. M., Cécyre, D., Robitaille, Y., Gauthier, S., and Quirion, R. (1992). Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. *J. Neurochem.* 58, 529–541. doi: 10.1111/j.1471-4159.1992.tb09752.x

Azam, L., Winzer-Serhan, U., and Leslie, F. M. (2003). Co-expression of alpha7 and beta2 nicotinic acetylcholine receptor subunit mRNAs within rat brain cholinergic neurons. *Neuroscience* 119, 965–977. doi: 10.1016/S0306-4522(03)00220-3

Baba, Y., Higuchi, M.-A., Abe, H., Fukuyama, K., Onozawa, R., Uehara, Y., et al. (2012). Anti-cholinergics for axial symptoms in Parkinson's disease

after subthalamic stimulation. *Clin. Neurol. Neurosurg.* 114, 1308–1311. doi: 10.1016/j.clineuro.2012.03.046

Bain, E. E., Robieson, W., Pritchett, Y., Garimella, T., Abi-Saab, W., Apostol, G., et al. (2013). A randomized, double-blind, placebo-controlled phase 2 study of α4β2 agonist ABT-894 in adults with ADHD. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 38, 405–413. doi: 10.1038/npp.2012.194

Barroso-Chinea, P., and Bezard, E. (2010). Basal Ganglia circuits underlying the pathophysiology of levodopa-induced dyskinesia. *Front. Neuroanat.* 4:131. doi: 10.3389/fnana.2010.00131

Bar-Shira, A., Gana-Weisz, M., Gan-Or, Z., Giladi, E., Giladi, N., and Orr-Urtreger, A. (2014). CHRNB3 c.-57A>G functional promoter change affects Parkinson's disease and smoking. *Neurobiol. Aging* 35, 2179.e1–2179.e6. doi: 10.1016/j.neurobiolaging.2014.03.014

Beaulieu, J.-M., and Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol. Rev.* 63, 182–217. doi: 10.1124/pr.110.002642

Beggiato, S., Antonelli, T., Tomasini, M. C., Tanganelli, S., Fuxe, K., Schwarcz, R., et al. (2013). Kynurenic acid, by targeting α7 nicotinic acetylcholine receptors, modulates extracellular GABA levels in the rat striatum *in vivo*. *Eur. J. Neurosci.* 37, 1470–1477. doi: 10.1111/ejn.12160

Bell, M. I., Richardson, P. J., and Lee, K. (2000). Histamine depolarizes cholinergic interneurones in the rat striatum via a H(1)-receptor mediated action. *Br. J. Pharmacol.* 131, 1135–1142. doi: 10.1038/sj.bjp.0703692

Bell, M. I., Richardson, P. J., and Lee, K. (2002). Functional and molecular characterization of metabotropic glutamate receptors expressed in rat striatal cholinergic interneurones. *J. Neurochem.* 81, 142–149. doi: 10.1046/j.1471-4159.2002.00815.x

Berendse, H. W., and Groenewegen, H. J. (1990). Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. *J. Comp. Neurol.* 299, 187–228. doi: 10.1002/cne.902990206

Berg, A. P., Sen, N., and Bayliss, D. A. (2007). TrpC3/C7 and Slo2.1 are molecular targets for metabotropic glutamate receptor signaling in rat striatal cholinergic interneurons. *J. Neurosci.* 27, 8845–8856. doi: 10.1523/JNEUROSCI.0551-07.2007

Bergson, C., Mrzljak, L., Smiley, J. F., Pappy, M., Levenson, R., and Goldman-Rakic, P. S. (1995). Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J. Neurosci.* 15, 7821–7836.

Bernard, V., Laribi, O., Levey, A. I., and Bloch, B. (1998). Subcellular redistribution of m2 muscarinic acetylcholine receptors in striatal interneurons *in vivo* after acute cholinergic stimulation. *J. Neurosci.* 18, 10207–10218.

Bernard, V., Normand, E., and Bloch, B. (1992). Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. *J. Neurosci.* 12, 3591–3600.

Bernard, V., Somogyi, P., and Bolam, J. P. (1997). Cellular, subcellular, and subsynaptic distribution of AMPA-type glutamate receptor subunits in the neostriatum of the rat. *J. Neurosci.* 17, 819–833.

Betarbet, R., Turner, R., Chockkan, V., DeLong, M. R., Allers, K. A., Walters, J., et al. (1997). Dopaminergic neurons intrinsic to the primate striatum. *J. Neurosci.* 17, 6761–6768.

Bettler, B., Kaupmann, K., Mosbacher, J., and Gassmann, M. (2004). Molecular structure and physiological functions of GABA(B) receptors. *Physiol. Rev.* 84, 835–867. doi: 10.1152/physrev.00036.2003

Bird, E. D., and Iversen, L. L. (1974). Huntington's chorea. Post-mortem measurement of glutamic acid decarboxylase, choline acetyltransferase and dopamine in basal ganglia. *Brain J. Neurol.* 97, 457–472. doi: 10.1093/brain/97.1.457

Bird, E. D., Spokes, E. G., Barnes, J., MacKay, A. V., Iversen, L. L., and Shepherd, M. (1977). Increased brain dopamine and reduced glutamic acid decarboxylase and choline acetyl transferase activity in schizophrenia and related psychoses. *Lancet* 2, 1157–1158. doi: 10.1016/S0140-6736(77)91542-2

Birket-Smith, E. (1974). Abnormal involuntary movements induced by anticholinergic therapy. *Acta Neurol. Scand.* 50, 801–811. doi: 10.1111/j.1600-0404.1974.tb02820.x

Blandina, P., Munari, L., Provensi, G., and Passani, M. B. (2012). Histamine neurons in the tuberomamillary nucleus: a whole center or distinct subpopulations? *Front. Syst. Neurosci.* 6:33. doi: 10.3389/fnsys.2012.00033

Blomeley, C., and Bracci, E. (2005). Excitatory effects of serotonin on rat striatal cholinergic interneurones. *J. Physiol.* 569, 715–721. doi: 10.1113/jphysiol.2005.098269

Bohr, I. J., Ray, M. A., McIntosh, J. M., Chalon, S., Guilloteau, D., McKeith, I. G., et al. (2005). Cholinergic nicotinic receptor involvement in movement disorders associated with Lewy body diseases. An autoradiography study using [(125)I]alpha-conotoxinMII in the striatum and thalamus. *Exp. Neurol.* 191, 292–300. doi: 10.1016/j.expneurol.2004.10.004

Bolam, J. P., Ingham, C. A., Izzo, P. N., Levey, A. I., Rye, D. B., Smith, A. D., et al. (1986). Substance P-containing terminals in synaptic contact with cholinergic neurons in the neostriatum and basal forebrain: a double immunocytochemical study in the rat. *Brain Res.* 397, 279–289. doi: 10.1016/0006-8993(86)90629-3

Bolam, J. P., Wainer, B. H., and Smith, A. D. (1984). Characterization of cholinergic neurons in the rat neostriatum. A combination of choline acetyltransferase immunocytochemistry, Golgi-impregnation and electron microscopy. *Neuroscience* 12, 711–718. doi: 10.1016/0306-4522(84)90165-9

Bonsi, P., Cuomo, D., Ding, J., Sciamanna, G., Ulrich, S., Tscherter, A., et al. (2007). Endogenous serotonin excites striatal cholinergic interneurons via the activation of 5-HT 2C, 5-HT6, and 5-HT7 serotonin receptors: implications for extrapyramidal side effects of serotonin reuptake inhibitors. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 32, 1840–1854. doi: 10.1038/sj.npp.1301294

Bonsi, P., De Persis, C., Calabresi, P., Bernardi, G., and Pisani, A. (2004). Coordinate high-frequency pattern of stimulation and calcium levels control the induction of LTP in striatal cholinergic interneurons. *Learn. Mem.* 11, 755–760. doi: 10.1101/lm.82104

Bordia, T., Campos, C., McIntosh, J. M., and Quik, M. (2010). Nicotinic receptor-mediated reduction in L-DOPA-induced dyskinesias may occur via desensitization. *J. Pharmacol. Exp. Ther.* 333, 929–938. doi: 10.1124/jpet.109.162396

Britt, J. P., and McGehee, D. S. (2008). Presynaptic opioid and nicotinic receptor modulation of dopamine overflow in the nucleus accumbens. *J. Neurosci.* 28, 1672–1681. doi: 10.1523/JNEUROSCI.4275-07.2008

Brown, M. T. C., Tan, K. R., O'Connor, E. C., Nikonenko, I., Muller, D., and Lüscher, C. (2012). Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature* 492, 452–456. doi: 10.1038/nature11657

Brown, S. J., James, S., Reddington, M., and Richardson, P. J. (1990). Both A1 and A2a purine receptors regulate striatal acetylcholine release. *J. Neurochem.* 55, 31–38. doi: 10.1111/j.1471-4159.1990.tb08817.x

Cachope, R., Mateo, Y., Mathur, B. N., Irving, J., Wang, H.-L., Morales, M., et al. (2012). Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: setting the tone for reward processing. *Cell Rep.* 2, 33–41. doi: 10.1016/j.celrep.2012.05.011

Calabresi, P., Ascone, C. M., Centonze, D., Pisani, A., Sancesario, G., D'Angelo, V., et al. (1997). Opposite membrane potential changes induced by glucose deprivation in striatal spiny neurons and in large aspiny interneurons. *J. Neurosci.* 17, 1940–1949.

Calabresi, P., Centonze, D., Gubellini, P., Pisani, A., and Bernardi, G. (1998a). Blockade of M2-like muscarinic receptors enhances long-term potentiation at corticostriatal synapses. *Eur. J. Neurosci.* 10, 3020–3023. doi: 10.1111/j.1460-9568.1998.00348.x

Calabresi, P., Centonze, D., Pisani, A., and Bernardi, G. (1999). Metabotropic glutamate receptors and cell-type-specific vulnerability in the striatum: implication for ischemia and Huntington's disease. *Exp. Neurol.* 158, 97–108. doi: 10.1006/exnr.1999.7092

Calabresi, P., Centonze, D., Pisani, A., Sancesario, G., Gubellini, P., Marfia, G. A., et al. (1998b). Striatal spiny neurons and cholinergic interneurons express differential ionotropic glutamatergic responses and vulnerability: implications for ischemia and Huntington's disease. *Ann. Neurol.* 43, 586–597. doi: 10.1002/ana.410430506

Calabresi, P., Siniscalchi, A., Pisani, A., Stefani, A., Mercuri, N. B., and Bernardi, G. (1996). A field potential analysis on the effects of lamotrigine, GP 47779, and felbamate in neocortical slices. *Neurology* 47, 557–562. doi: 10.1212/WNL.47.2.557

Campos, F., Alfonso, M., and Durán, R. (2010). *In vivo* modulation of alpha7 nicotinic receptors on striatal glutamate release induced by anatoxin-A. *Neurochem. Int.* 56, 850–855. doi: 10.1016/j.neuint.2010.03.010

Cantello, R., Riccio, A., Gilli, M., Delsedime, M., Scarzella, L., Aguggia, M., et al. (1986). Bornaprine vs placebo in Parkinson disease: double-blind controlled cross-over trial in 30 patients. *Ital. J. Neurol. Sci.* 7, 139–143. doi: 10.1007/BF02230432

Carpenedo, R., Pittaluga, A., Cozzi, A., Attucci, S., Galli, A., Raiteri, M., et al. (2001). Presynaptic kynurenate-sensitive receptors inhibit glutamate release. *Eur. J. Neurosci.* 13, 2141–2147. doi: 10.1046/j.0953-816x.2001.01592.x

Centonze, D., Grande, C., Usiello, A., Gubellini, P., Erbs, E., Martin, A. B., et al. (2003). Receptor subtypes involved in the presynaptic and postsynaptic actions of dopamine on striatal interneurons. *J. Neurosci.* 23, 6245–6254.

Centonze, D., Pisani, A., Bonsi, P., Giacomini, P., Bernardi, G., and Calabresi, P. (2001). Stimulation of nitric oxide-cGMP pathway excites striatal cholinergic interneurons via protein kinase G activation. *J. Neurosci.* 21, 1393–1400.

Cepeda, C., Itri, J. N., Flores-Hernández, J., Hurst, R. S., Calvert, C. R., and Levine, M. S. (2001). Differential sensitivity of medium- and large-sized striatal neurons to NMDA but not kainate receptor activation in the rat. *Eur. J. Neurosci.* 14, 1577–1589. doi: 10.1046/j.0953-816x.2001.01783.x

Cepeda, C., Wu, N., André, V. M., Cummings, D. M., and Levine, M. S. (2007). The corticostriatal pathway in Huntington's disease. *Prog. Neurobiol.* 81, 253–271. doi: 10.1016/j.pneurobio.2006.11.001

Chang, H. T. (1988). Dopamine-acetylcholine interaction in the rat striatum: a dual-labeling immunocytochemical study. *Brain Res. Bull.* 21, 295–304. doi: 10.1016/0361-9230(88)90244-4

Chang, H. T., and Kita, H. (1992). Interneurons in the rat striatum: relationships between parvalbumin neurons and cholinergic neurons. *Brain Res.* 574, 307–311. doi: 10.1016/0006-8993(92)90830-3

Chang, K. T., and Berg, D. K. (2001). Voltage-gated channels block nicotinic regulation of CREB phosphorylation and gene expression in neurons. *Neuron* 32, 855–865. doi: 10.1016/S0896-6273(01)00516-5

Chen, Q., Veenman, C. L., and Reiner, A. (1996). Cellular expression of ionotropic glutamate receptor subunits on specific striatal neuron types and its implication for striatal vulnerability in glutamate receptor-mediated excitotoxicity. *Neuroscience* 73, 715–731. doi: 10.1016/0306-4522(96)00011-5

Chudasama, Y., and Robbins, T. W. (2006). Functions of frontostriatal systems in cognition: comparative neuropsychopharmacological studies in rats, monkeys and humans. *Biol. Psychol.* 73, 19–38. doi: 10.1016/j.biopsycho.2006.01.005

Chuhma, N., Mingote, S., Moore, H., and Rayport, S. (2014). Dopamine neurons control striatal cholinergic neurons via regionally heterogeneous dopamine and glutamate signaling. *Neuron* 81, 901–912. doi: 10.1016/j.neuron.2013.12.027

Chuhma, N., Tanaka, K. F., Hen, R., and Rayport, S. (2011). Functional connectome of the striatal medium spiny neuron. *J. Neurosci.* 31, 1183–1192. doi: 10.1523/JNEUROSCI.3833-10.2011

Chung, K. A., Lobb, B. M., Nutt, J. G., and Horak, F. B. (2010). Effects of a central cholinesterase inhibitor on reducing falls in Parkinson disease. *Neurology* 75, 1263–1269. doi: 10.1212/WNL.0b013e3181f6128c

Chung, K., and Deisseroth, K. (2013). CLARITY for mapping the nervous system. *Nat. Methods* 10, 508–513. doi: 10.1038/nmeth.2481

Claye, L. H., Unterwald, E. M., Ho, A., and Kreek, M. J. (1996). Both dynorphin A(1-17) and [Des-Tyr1]dynorphin A(2-17) inhibit adenylyl cyclase activity in rat caudate putamen. *J. Pharmacol. Exp. Ther.* 277, 359–365.

Conn, P. J., Battaglia, G., Marino, M. J., and Nicoletti, F. (2005). Metabotropic glutamate receptors in the basal ganglia motor circuit. *Nat. Rev. Neurosci.* 6, 787–798. doi: 10.1038/nrn1763

Contant, C., Umbriaco, D., Garcia, S., Watkins, K. C., and Descarries, L. (1996). Ultrastructural characterization of the acetylcholine innervation in adult rat neostriatum. *Neuroscience* 71, 937–947. doi: 10.1016/0306-4522(95)00507-2

Cossette, M., Lecomte, F., and Parent, A. (2005). Morphology and distribution of dopaminergic neurons intrinsic to the human striatum. *J. Chem. Neuroanat.* 29, 1–11. doi: 10.1016/j.jchemneu.2004.08.007

Court, J. A., Piggott, M. A., Lloyd, S., Cookson, N., Ballard, C. G., McKeith, I. G., et al. (2000). Nicotine binding in human striatum: elevation in schizophrenia and reductions in dementia with Lewy bodies, Parkinson's disease and Alzheimer's disease and in relation to neuroleptic medication. *Neuroscience* 98, 79–87. doi: 10.1016/S0306-4522(00)00071-3

Crain, S. M., and Shen, K. F. (1996). Modulatory effects of Gs-coupled excitatory opioid receptor functions on opioid analgesia, tolerance, and dependence. *Neurochem. Res.* 21, 1347–1351. doi: 10.1007/BF02532375

Cubo, E., Fernández Jaén, A., Moreno, C., Anaya, B., González, M., and Kompoliti, K. (2008). Donepezil use in children and adolescents with tics and attention-deficit/hyperactivity disorder: an 18-week, single-center, dose-escalating, prospective, open-label study. *Clin. Ther.* 30, 182–189. doi: 10.1016/j.clinthera.2008.01.010

Da Cunha, C., Gomez, A.-A., and Blaha, C. D. (2012). The role of the basal ganglia in motivated behavior. *Rev. Neurosci.* 23, 747–767. doi: 10.1515/revneuro-2012-0063

Dalack, G. W., Healy, D. J., and Meador-Woodruff, J. H. (1998). Nicotine dependence in schizophrenia: clinical phenomena and laboratory findings. *Am. J. Psychiatry* 155, 1490–1501.

Dale, H. H., and Laidlaw, P. P. (1910). The physiological action of beta-iminazolylethylamine. *J. Physiol.* 41, 318–344.

Dani, J. A. (2001). Overview of nicotinic receptors and their roles in the central nervous system. *Biol. Psychiatry* 49, 166–174. doi: 10.1016/S0006-3223(00)01011-8

Dautan, D., Huerta-Ocampo, I., Witten, I. B., Deisseroth, K., Bolam, J. P., Gerdjikov, T., et al. (2014). A major external source of cholinergic innervation of the striatum and nucleus accumbens originates in the brainstem. *J. Neurosci.* 34, 4509–4518. doi: 10.1523/JNEUROSCI.5071-13.2014

Dawson, V. L., Dawson, T. M., Filloux, F. M., and Wamsley, J. K. (1988). Evidence for dopamine D-2 receptors on cholinergic interneurons in the rat caudate-putamen. *Life Sci.* 42, 1933–1939. doi: 10.1016/0024-3205(88)90492-4

Dawson, V. L., Dawson, T. M., and Wamsley, J. K. (1990). Muscarinic and dopaminergic receptor subtypes on striatal cholinergic interneurons. *Brain Res. Bull.* 25, 903–912. doi: 10.1016/0361-9230(90)90186-4

Dean, B., Crook, J. M., Opeskin, K., Hill, C., Keks, N., and Copolov, D. L. (1996). The density of muscarinic M1 receptors is decreased in the caudate-putamen of subjects with schizophrenia. *Mol. Psychiatry* 1, 54–58.

DeBoer, P., Abercrombie, E. D., Heeringa, M., and Westerink, B. H. (1993). Differential effect of systemic administration of bromocriptine and L-dopa on the release of acetylcholine from striatum of intact and 6-OHDA-treated rats. *Brain Res.* 608, 198–203. doi: 10.1016/0006-8993(93)91459-6

DeBoer, P., and Westerink, B. H. (1994). GABAergic modulation of striatal cholinergic interneurons: an *in vivo* microdialysis study. *J. Neurochem.* 62, 70–75. doi: 10.1046/j.1471-4159.1994.62010070.x

Del Barrio, L., Egea, J., León, R., Romero, A., Ruiz, A., Montero, M., et al. (2011). Calcium signalling mediated through α7 and non-α7 nAChR stimulation is differentially regulated in bovine chromaffin cells to induce catecholamine release. *Br. J. Pharmacol.* 162, 94–110. doi: 10.1111/j.1476-5381.2010.01034.x

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends Neurosci.* 13, 281–285. doi: 10.1016/0166-2236(90)90110-V

Deng, P., Zhang, Y., and Xu, Z. C. (2007a). Involvement of I(h) in dopamine modulation of tonic firing in striatal cholinergic interneurons. *J. Neurosci.* 27, 3148–3156. doi: 10.1523/JNEUROSCI.5535-06.2007

Deng, Y. P., Xie, J. P., Wang, H. B., Lei, W. L., Chen, Q., and Reiner, A. (2007b). Differential localization of the GluR1 and GluR2 subunits of the AMPA-type glutamate receptor among striatal neuron types in rats. *J. Chem. Neuroanat.* 33, 167–192. doi: 10.1016/j.jchemneu.2007.02.008

De Rover, M., Lodder, J. C., Kits, K. S., Schoffelmeer, A. N. M., and Brussaard, A. B. (2002). Cholinergic modulation of nucleus accumbens medium spiny neurons. *Eur. J. Neurosci.* 16, 2279–2290. doi: 10.1046/j.1460-9568.2002.02289.x

Devan, B. D., Hong, N. S., and McDonald, R. J. (2011). Parallel associative processing in the dorsal striatum: segregation of stimulus-response and cognitive control subregions. *Neurobiol. Learn. Mem.* 96, 95–120. doi: 10.1016/j.nlm.2011.06.002

Dimova, R., Vuillet, J., Nieoullon, A., and Kerkerian-Le Goff, L. (1993). Ultrastructural features of the choline acetyltransferase-containing neurons and relationships with nigral dopaminergic and cortical afferent pathways in the rat striatum. *Neuroscience* 53, 1059–1071. doi: 10.1016/0306-4522(93)90489-3

Ding, J. B., Guzman, J. N., Peterson, J. D., Goldberg, J. A., and Surmeier, D. J. (2010). Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron* 67, 294–307. doi: 10.1016/j.neuron.2010.06.017

Ding, J., Guzman, J. N., Tkatch, T., Chen, S., Goldberg, J. A., Ebert, P. J., et al. (2006). RGS4-dependent attenuation of M4 autoreceptor function in striatal cholinergic interneurons following dopamine depletion. *Nat. Neurosci.* 9, 832–842. doi: 10.1038/nn1700

Ding, S., Wei, W., and Zhou, F.-M. (2011a). Molecular and functional differences in voltage-activated sodium currents between GABA projection neurons and dopamine neurons in the substantia nigra. *J. Neurophysiol.* 106, 3019–3034. doi: 10.1152/jn.00305.2011

Ding, Y., Won, L., Britt, J. P., Lim, S. A. O., McGehee, D. S., and Kang, U. J. (2011b). Enhanced striatal cholinergic neuronal activity mediates L-DOPA-induced dyskinesia in parkinsonian mice. *Proc. Natl. Acad. Sci. U.S.A.* 108, 840–845. doi: 10.1073/pnas.1006511108

Diraddo, J. O., Miller, E. J., Hathaway, H. A., Grajkowska, E., Wroblewska, B., Wolfe, B. B., et al. (2014). A real-time method for measuring cAMP production modulated by Gαi/o-coupled metabotropic glutamate receptors. *J. Pharmacol. Exp. Ther.* 349, 373–382. doi: 10.1124/jpet.113.211532

Dodt, H. U., and Misgeld, U. (1986). Muscarinic slow excitation and muscarinic inhibition of synaptic transmission in the rat neostriatum. *J. Physiol.* 380, 593–608.

Dubach, M., Schmidt, R., Kunkel, D., Bowden, D. M., Martin, R., and German, D. C. (1987). Primate neostriatal neurons containing tyrosine hydroxylase: immunohistochemical evidence. *Neurosci. Lett.* 75, 205–210. doi: 10.1016/0304-3940(87)90298-9

Durany, N., Zöchling, R., Boissl, K. W., Paulus, W., Ransmayr, G., Tatschner, T., et al. (2000). Human post-mortem striatal alpha4beta2 nicotinic acetylcholine receptor density in schizophrenia and Parkinson's syndrome. *Neurosci. Lett.* 287, 109–112. doi: 10.1016/S0304-3940(00)01144-7

Ellens, D. J., and Leventhal, D. K. (2013). Review: electrophysiology of basal ganglia and cortex in models of Parkinson disease. *J. Park. Dis.* 3, 241–254. doi: 10.3233/JPD-130204

English, B. A., Hahn, M. K., Gizer, I. R., Mazei-Robison, M., Steele, A., Kurnik, D. M., et al. (2009). Choline transporter gene variation is associated with attention-deficit hyperactivity disorder. *J. Neurodev. Disord.* 1, 252–263. doi: 10.1007/s11689-009-9033-8

English, D. F., Ibanez-Sandoval, O., Stark, E., Tecuapetla, F., Buzsaki, G., Deisseroth, K., et al. (2011). Novel GABAergic circuits mediate the reinforcement-related signals of striatal cholinergic interneurons. *Nat. Neurosci.* 15, 123–130. doi: 10.1038/nn.2984

Enna, S. J., Bennett, J. P. Jr., Bylund, D. B., Snyder, S. H., Bird, E. D., and Iversen, L. L. (1976a). Alterations of brain neurotransmitter receptor binding in Huntington's chorea. *Brain Res.* 116, 531–537. doi: 10.1016/0006-8993(76)90502-3

Enna, S. J., Bird, E. D., Bennett, J. P. Jr., Bylund, D. B., Yamamura, H. I., Iversen, L. L., et al. (1976b). Huntington's chorea. Changes in neurotransmitter receptors in the brain. *N. Engl. J. Med.* 294, 1305–1309. doi: 10.1056/NEJM197606102942402

Exley, R., Clements, M. A., Hartung, H., McIntosh, J. M., Franklin, M., Bermudez, I., et al. (2013). Striatal dopamine transmission is reduced after chronic nicotine with a decrease in α6-nicotinic receptor control in nucleus accumbens. *Eur. J. Neurosci.* 22, 1379–1389. doi: 10.1111/ejn.12298

Fabbrini, G., Brotchie, J. M., Grandas, F., Nomoto, M., and Goetz, C. G. (2007). Levodopa-induced dyskinesias. *Mov. Disord. Off. J. Mov. Disord. Soc.* 22, 1379–1389. doi: 10.1002/mds.21475

Fallon, J. H., and Leslie, F. M. (1986). Distribution of dynorphin and enkephalin peptides in the rat brain. *J. Comp. Neurol.* 249, 293–336. doi: 10.1002/cne.902490302

Feng, Z.-J., Zhang, X., and Chergui, K. (2014). Allosteric modulation of NMDA receptors alters neurotransmission in the striatum of a mouse model of Parkinson's disease. *Exp. Neurol.* 255, 154–160. doi: 10.1016/j.expneurol.2014.03.001

Fenster, C. P., Rains, M. F., Noerager, B., Quick, M. W., and Lester, R. A. (1997). Influence of subunit composition on desensitization of neuronal acetylcholine receptors at low concentrations of nicotine. *J. Neurosci.* 17, 5747–5759.

Fernandez, H. H. (2012). Updates in the medical management of Parkinson disease. *Cleve. Clin. J. Med.* 79, 28–35. doi: 10.3949/ccjm.78gr.11005

Fino, E., Deniau, J.-M., and Venance, L. (2008). Cell-specific spike-timing-dependent plasticity in GABAergic and cholinergic interneurons in corticostriatal rat brain slices. *J. Physiol.* 586, 265–282. doi: 10.1113/jphysiol.2007.144501

Fisher, J. L., and Dani, J. A. (2000). Nicotinic receptors on hippocampal cultures can increase synaptic glutamate currents while decreasing the NMDA-receptor component. *Neuropharmacology* 39, 2756–2769. doi: 10.1016/S0028-3908(00)00102-7

Fox, S. H., Katzenschlager, R., Lim, S.-Y., Ravina, B., Seppi, K., Coelho, M., et al. (2011). The movement disorder society evidence-based medicine review update: treatments for the motor symptoms of Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc.* 26(Suppl. 3), S2–S41. doi: 10.1002/mds.23829

Galarraga, E., Hernández-López, S., Reyes, A., Miranda, I., Bermudez-Rattoni, F., Vilchis, C., et al. (1999). Cholinergic modulation of neostriatal output: a functional antagonism between different types of muscarinic receptors. *J. Neurosci.* 19, 3629–3638.

Garcia-Munoz, M., Patino, P., Young, S. J., and Groves, P. M. (1996). Effects of nicotine on dopaminergic nigrostriatal axons requires stimulation of presynaptic glutamatergic receptors. *J. Pharmacol. Exp. Ther.* 277, 1685–1693.

Gasser, T. (2009). Molecular pathogenesis of Parkinson disease: insights from genetic studies. *Expert Rev. Mol. Med.* 11, e22. doi: 10.1017/S1462399409001148

Gerfen, C. R., Herkenham, M., and Thibault, J. (1987). The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. *J. Neurosci.* 7, 3915–3934.

Gilman, S., Koeppe, R. A., Nan, B., Wang, C.-N., Wang, X., Junck, L., et al. (2010). Cerebral cortical and subcortical cholinergic deficits in parkinsonian syndromes. *Neurology* 74, 1416–1423. doi: 10.1212/WNL.0b013e3181dc1a55

Girod, R., Barazangi, N., McGehee, D., and Role, L. W. (2000). Facilitation of glutamatergic neurotransmission by presynaptic nicotinic acetylcholine receptors. *Neuropharmacology* 39, 2715–2725. doi: 10.1016/S0028-3908(00)00145-3

Goetz, C. G. (2011). The history of Parkinson's disease: early clinical descriptions and neurological therapies. *Cold Spring Harb. Perspect. Med.* 1:a008862. doi: 10.1101/cshperspect.a008862

Gomez-Mancilla, B., and Bédard, P. J. (1993). Effect of nondopaminergic drugs on L-dopa-induced dyskinesias in MPTP-treated monkeys. *Clin. Neuropharmacol.* 16, 418–427. doi: 10.1097/00002826-199310000-00004

Gonzales, K. K., Pare, J.-F., Wichmann, T., and Smith, Y. (2013). GABAergic inputs from direct and indirect striatal projection neurons onto cholinergic interneurons in the primate putamen. *J. Comp. Neurol.* 521, 2502–2522. doi: 10.1002/cne.23295

Gotti, C., Moretti, M., Bohr, I., Ziabreva, I., Vailati, S., Longhi, R., et al. (2006a). Selective nicotinic acetylcholine receptor subunit deficits identified in Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies by immunoprecipitation. *Neurobiol. Dis.* 23, 481–489. doi: 10.1016/j.nbd.2006.04.005

Gotti, C., Zoli, M., and Clementi, F. (2006b). Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol. Sci.* 27, 482–491. doi: 10.1016/j.tips.2006.07.004

Götz, T., Kraushaar, U., Geiger, J., Lübke, J., Berger, T., and Jonas, P. (1997). Functional properties of AMPA and NMDA receptors expressed in identified types of basal ganglia neurons. *J. Neurosci.* 17, 204–215.

Grace, A. A., and Bunney, B. S. (1984). The control of firing pattern in nigral dopamine neurons: single spike firing. *J. Neurosci.* 4, 2866–2876.

Grady, S. R., Salminen, O., Laverty, D. C., Whiteaker, P., McIntosh, J. M., Collins, A. C., et al. (2007). The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum. *Biochem. Pharmacol.* 74, 1235–1246. doi: 10.1016/j.bcp.2007.07.032

Gras, C., Amilhon, B., Lepicard, E. M., Poirel, O., Vinatier, J., Herbin, M., et al. (2008). The vesicular glutamate transporter VGLUT3 synergizes striatal acetylcholine tone. *Nat. Neurosci.* 11, 292–300. doi: 10.1038/nn2052

Gray, R., Rajan, A. S., Radcliffe, K. A., Yakehiro, M., and Dani, J. A. (1996). Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature* 383, 713–716. doi: 10.1038/383713a0

Gross, R. A., Moises, H. C., Uhler, M. D., and Macdonald, R. L. (1990). Dynorphin A and cAMP-dependent protein kinase independently regulate neuronal calcium currents. *Proc. Natl. Acad. Sci. U.S.A.* 87, 7025–7029. doi: 10.1073/pnas.87.18.7025

Guan, L., Wang, B., Chen, Y., Yang, L., Li, J., Qian, Q., et al. (2009). A high-density single-nucleotide polymorphism screen of 23 candidate genes in attention deficit hyperactivity disorder: suggesting multiple susceptibility genes among Chinese Han population. *Mol. Psychiatry* 14, 546–554. doi: 10.1038/sj.mp.4002139

Guatteo, E., Yee, A., McKearney, J., Cucchiaroni, M. L., Armogida, M., Berretta, N., et al. (2013). Dual effects of L-DOPA on nigral dopaminergic neurons. *Exp. Neurol.* 247, 582–594. doi: 10.1016/j.expneurol.2013.02.009

Haas, H. L., Sergeeva, O. A., and Selbach, O. (2008). Histamine in the nervous system. *Physiol. Rev.* 88, 1183–1241. doi: 10.1152/physrev.00043.2007

Haber, S. N., Fudge, J. L., and McFarland, N. R. (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *J. Neurosci.* 20, 2369–2382. doi: 10.0270-6474/00/202369-14

Haj-Dahmane, S., Hamon, M., and Lanfumey, L. (1991). K+ channel and 5-hydroxytryptamine1A autoreceptor interactions in the rat dorsal raphe nucleus: an *in vitro* electrophysiological study. *Neuroscience* 41, 495–505. doi: 10.1016/0306-4522(91)90344-N

Hammond, P., and Brimijoin, S. (1988). Acetylcholinesterase in Huntington's and Alzheimer's diseases: simultaneous enzyme assay and immunoassay of multiple brain regions. *J. Neurochem.* 50, 1111–1116. doi: 10.1111/j.1471-4159.1988.tb10580.x

Hannestad, J. O., Cosgrove, K. P., DellaGioia, N. F., Perkins, E., Bois, F., Bhagwagar, Z., et al. (2013). Changes in the cholinergic system between bipolar depression and euthymia as measured with [123I]5IA single photon emission computed tomography. *Biol. Psychiatry* 74, 768–776. doi: 10.1016/j.biopsych.2013.04.004

Hattori, T., Singh, V. K., McGeer, E. G., and McGeer, P. L. (1976). Immunohistochemical localization of choline acetyltransferase containing neostriatal neurons and their relationship with dopaminergic synapses. *Brain Res.* 102, 164–173. doi: 10.1016/0006-8993(76)90583-7

Hattori, T., Takada, M., Moriizumi, T., and Van der Kooy, D. (1991). Single dopaminergic nigrostriatal neurons form two chemically distinct synaptic types: possible transmitter segregation within neurons. *J. Comp. Neurol.* 309, 391–401. doi: 10.1002/cne.903090308

Hauber, W., and Schmidt, W. J. (1994). Differential effects of lesions of the dorsomedial and dorsolateral caudate-putamen on reaction time performance in rats. *Behav. Brain Res.* 60, 211–215. doi: 10.1016/0166-4328(94)90149-X

Hauser, R. A., and Olanow, C. W. (1993). Orobuccal dyskinesia associated with trihexyphenidyl therapy in a patient with Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc.* 8, 512–514. doi: 10.1002/mds.870080417

Haydar, S. N., and Dunlop, J. (2010). Neuronal nicotinic acetylcholine receptors - targets for the development of drugs to treat cognitive impairment associated with schizophrenia and Alzheimer's disease. *Curr. Top. Med. Chem.* 10, 144–152. doi: 10.2174/156802610790410983

Hayslett, R. L., and Tizabi, Y. (2003). Effects of donepezil on DOI-induced head twitch response in mice: implications for Tourette syndrome. *Pharmacol. Biochem. Behav.* 76, 409–415. doi: 10.1016/j.pbb.2003.08.015

Hebb, C. O., and Silver, A. (1961). Gradient of choline acetylase activity. *Nature* 189, 123–125. doi: 10.1038/189123a0

Hellström-Lindahl, E., and Court, J. A. (2000). Nicotinic acetylcholine receptors during prenatal development and brain pathology in human aging. *Behav. Brain Res.* 113, 159–168. doi: 10.1016/S0166-4328(00)00210-2

Henny, P., Brown, M. T. C., Northrop, A., Faunes, M., Ungless, M. A., Magill, P. J., et al. (2012). Structural correlates of heterogeneous *in vivo* activity of midbrain dopaminergic neurons. *Nat. Neurosci.* 15, 613–619. doi: 10.1038/nn.3048

Hernández-Echeagaray, E., Galarraga, E., and Bargas, J. (1998). 3-Alpha-chloroimperialine, a potent blocker of cholinergic presynaptic modulation of glutamatergic afferents in the rat neostriatum. *Neuropharmacology* 37, 1493–1502. doi: 10.1016/S0028-3908(98)00131-2

Hersch, S. M., Gutekunst, C. A., Rees, H. D., Heilman, C. J., and Levey, A. I. (1994). Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies. *J. Neurosci.* 14, 3351–3363.

Higashi, S., Biskup, S., West, A. B., Trinkaus, D., Dawson, V. L., Faull, R. L. M., et al. (2007). Localization of Parkinson's disease-associated LRRK2 in normal and pathological human brain. *Brain Res.* 1155, 208–219. doi: 10.1016/j.brainres.2007.04.034

Higley, M. J., Gittis, A. H., Oldenburg, I. A., Balthasar, N., Seal, R. P., Edwards, R. H., et al. (2011). Cholinergic interneurons mediate fast VGluT3-dependent glutamatergic transmission in the striatum. *PLoS ONE* 6:e19155. doi: 10.1371/journal.pone.0019155

Hiley, C. R., and Bird, E. D. (1974). Decreased muscarinic receptor concentration in post-mortem brain in Huntington's chorea. *Brain Res.* 80, 355–358. doi: 10.1016/0006-8993(74)90700-8

Holt, D. J., Herman, M. M., Hyde, T. M., Kleinman, J. E., Sinton, C. M., German, D. C., et al. (1999). Evidence for a deficit in cholinergic interneurons in the striatum in schizophrenia. *Neuroscience* 94, 21–31. doi: 10.1016/S0306-4522(99)00279-1

Hong, C.-J., Lai, I.-C., Liou, L.-L., and Tsai, S.-J. (2004). Association study of the human partially duplicated alpha7 nicotinic acetylcholine receptor genetic variant with bipolar disorder. *Neurosci. Lett.* 355, 69–72. doi: 10.1016/j.neulet.2003.10.043

Howe, A. R., and Surmeier, D. J. (1995). Muscarinic receptors modulate N-, P-, and L-type Ca2+ currents in rat striatal neurons through parallel pathways. *J. Neurosci.* 15, 458–469.

Hristova, A. H., and Koller, W. C. (2000). Early Parkinson's disease: what is the best approach to treatment. *Drugs Aging* 17, 165–181. doi: 10.2165/00002512-200017030-00002

Hsu, K. S., Yang, C. H., Huang, C. C., and Gean, P. W. (1996). Carbachol induces inward current in neostriatal neurons through M1-like muscarinic receptors. *Neuroscience* 73, 751–760. doi: 10.1016/0306-4522(96)00066-8

Hu, M., Liu, Q., Chang, K. T., and Berg, D. K. (2002). Nicotinic regulation of CREB activation in hippocampal neurons by glutamatergic and nonglutamatergic pathways. *Mol. Cell. Neurosci.* 21, 616–625. doi: 10.1006/mcne.2002.1202

Huot, P., Lévesque, M., and Parent, A. (2007). The fate of striatal dopaminergic neurons in Parkinson's disease and Huntington's chorea. *Brain J. Neurol.* 130, 222–232. doi: 10.1093/brain/awl332

Hyland, B. I., Reynolds, J. N. J., Hay, J., Perk, C. G., and Miller, R. (2002). Firing modes of midbrain dopamine cells in the freely moving rat. *Neuroscience* 114, 475–492. doi: 10.1016/S0306-4522(02)00267-1

Ibáñez-Sandoval, O., Tecuapetla, F., Unal, B., Shah, F., Koós, T., and Tepper, J. M. (2010). Electrophysiological and morphological characteristics and synaptic connectivity of tyrosine hydroxylase-expressing neurons in adult mouse striatum. *J. Neurosci.* 30, 6999–7016. doi: 10.1523/JNEUROSCI.5996-09.2010

Innis, R. B., and Aghajanian, G. K. (1987). Pertussis toxin blocks 5-HT1A and GABAB receptor-mediated inhibition of serotonergic neurons. *Eur. J. Pharmacol.* 143, 195–204. doi: 10.1016/0014-2999(87)90533-4

Inokawa, H., Yamada, H., Matsumoto, N., Muranishi, M., and Kimura, M. (2010). Juxtacellular labeling of tonically active neurons and phasically active neurons in the rat striatum. *Neuroscience* 168, 395–404. doi: 10.1016/j.neuroscience.2010.03.062

Jabourian, M., Venance, L., Bourgoin, S., Ozon, S., Pérez, S., Godeheu, G., et al. (2005). Functional mu opioid receptors are expressed in cholinergic interneurons of the rat dorsal striatum: territorial specificity and diurnal variation. *Eur. J. Neurosci.* 21, 3301–3309. doi: 10.1111/j.1460-9568.2005.04154.x

Jackisch, R., Hotz, H., and Hertting, G. (1993). No evidence for presynaptic opioid receptors on cholinergic, but presence of kappa-receptors on dopaminergic neurons in the rabbit caudate nucleus: involvement of endogenous opioids. *Naunyn. Schmiedebergs Arch. Pharmacol.* 348, 234–241. doi: 10.1007/BF00169150

Jin, S., and Fredholm, B. B. (1997). Adenosine A2A receptor stimulation increases release of acetylcholine from rat hippocampus but not striatum, and does not affect catecholamine release. *Naunyn. Schmiedebergs Arch. Pharmacol.* 355, 48–56. doi: 10.1007/PL00004917

Johnson, M. D., and Yee, A. G. (1995). Ultrastructure of electrophysiologically-characterized synapses formed by serotonergic raphe neurons in culture. *Neuroscience* 67, 609–623. doi: 10.1016/0306-4522(95)00010-G

Jollivet, C., Montero-Menei, C. N., Venier-Julienne, M.-C., Sapin, A., Benoit, J.-P., and Menei, P. (2004). Striatal tyrosine hydroxylase immunoreactive neurons are induced by L-dihydroxyphenylalanine and nerve growth factor treatment in 6-hydroxydopamine lesioned rats. *Neurosci. Lett.* 362, 79–82. doi: 10.1016/j.neulet.2004.02.068

Jones, I. W., Bolam, J. P., and Wonnacott, S. (2001). Presynaptic localisation of the nicotinic acetylcholine receptor beta2 subunit immunoreactivity in rat nigrostriatal dopaminergic neurones. *J. Comp. Neurol.* 439, 235–247. doi: 10.1002/cne.1345

Jucaite, A., Öhd, J., Potter, A. S., Jaeger, J., Karlsson, P., Hannesdottir, K., et al. (2014). A randomized, double-blind, placebo-controlled crossover study of α4β 2* nicotinic acetylcholine receptor agonist AZD1446 (TC-6683) in adults with attention-deficit/hyperactivity disorder. *Psychopharmacology (Berl.)* 231, 1251–1265. doi: 10.1007/s00213-013-3116-7

Kaiser, S., and Wonnacott, S. (2000). Alpha-bungarotoxin-sensitive nicotinic receptors indirectly modulate [(3)H]dopamine release in rat striatal slices via glutamate release. *Mol. Pharmacol.* 58, 312–318. doi: 10.1124/mol.58.2.312

Kataoka, Y., Kalanithi, P. S. A., Grantz, H., Schwartz, M. L., Saper, C., Leckman, J. F., et al. (2010). Decreased number of parvalbumin and cholinergic interneurons in the striatum of individuals with Tourette syndrome. *J. Comp. Neurol.* 518, 277–291. doi: 10.1002/cne.22206

Katzenschlager, R., Sampaio, C., Costa, J., and Lees, A. (2003). Anticholinergics for symptomatic management of Parkinson's disease. *Cochrane Database Syst. Rev.* CD003735. doi: 10.1002/14651858.CD003735

Kawaguchi, Y. (1993). Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. *J. Neurosci.* 13, 4908–4923.

Keath, J. R., Iacoviello, M. P., Barrett, L. E., Mansvelder, H. D., and McGehee, D. S. (2007). Differential modulation by nicotine of substantia nigra versus ventral tegmental area dopamine neurons. *J. Neurophysiol.* 98, 3388–3396. doi: 10.1152/jn.00760.2007

Kelley, A. E. (2004). Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neurosci. Biobehav. Rev.* 27, 765–776. doi: 10.1016/j.neubiorev.2003.11.015

Kimura, M., Rajkowski, J., and Evarts, E. (1984). Tonically discharging putamen neurons exhibit set-dependent responses. *Proc. Natl. Acad. Sci. U.S.A.* 81, 4998–5001. doi: 10.1073/pnas.81.15.4998

Klink, R., de Kerchove d'Exaerde, A., Zoli, M., and Changeux, J. P. (2001). Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J. Neurosci.* 21, 1452–1463.

Koob, G. F. (1992). Neural mechanisms of drug reinforcement. *Ann. N.Y. Acad. Sci.* 654, 171–191. doi: 10.1111/j.1749-6632.1992.tb25966.x

Koranda, J. L., Cone, J. J., McGehee, D. S., Roitman, M. F., Beeler, J. A., and Zhuang, X. (2014). Nicotinic receptors regulate the dynamic range of dopamine release *in vivo*. *J. Neurophysiol.* 111, 103–111. doi: 10.1152/jn.00269.2013

Kostic, V., Przedborski, S., Flaster, E., and Sternic, N. (1991). Early development of levodopa-induced dyskinesias and response fluctuations in young-onset Parkinson's disease. *Neurology* 41, 202–205. doi: 10.1212/WNL.41.2_Part_1.202

Kravitz, A. V., Freeze, B. S., Parker, P. R. L., Kay, K., Thwin, M. T., Deisseroth, K., et al. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* 466, 622–626. doi: 10.1038/nature09159

Kubota, Y., Inagaki, S., Shimada, S., Kito, S., Eckenstein, F., and Tohyama, M. (1987). Neostriatal cholinergic neurons receive direct synaptic inputs from dopaminergic axons. *Brain Res.* 413, 179–184. doi: 10.1016/0006-8993(87)90167-3

Kulak, J. M., Musachio, J. L., McIntosh, J. M., and Quik, M. (2002). Declines in different beta2* nicotinic receptor populations in monkey striatum after nigrostriatal damage. *J. Pharmacol. Exp. Ther.* 303, 633–639. doi: 10.1124/jpet.102.039347

Kumar, N., Van Gerpen, J. A., Bower, J. H., and Ahlskog, J. E. (2005). Levodopa-dyskinesia incidence by age of Parkinson's disease onset. *Mov. Disord. Off. J. Mov. Disord. Soc.* 20, 342–344. doi: 10.1002/mds.20360

Kumar, P., and Kumar, A. (2009). Protective effect of rivastigmine against 3-nitropropionic acid-induced Huntington's disease like symptoms: possible behavioural, biochemical and cellular alterations. *Eur. J. Pharmacol.* 615, 91–101. doi: 10.1016/j.ejphar.2009.04.058

Künzle, H. (1975). Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study in *Macaca fascicularis*. *Brain Res.* 88, 195–209. doi: 10.1016/0006-8993(75)90384-4

Kurokawa, M., Kirk, I. P., Kirkpatrick, K. A., Kase, H., and Richardson, P. J. (1994). Inhibition by KF17837 of adenosine A2A receptor-mediated modulation of striatal GABA and ACh release. *Br. J. Pharmacol.* 113, 43–48. doi: 10.1111/j.1476-5381.1994.tb16171.x

Landwehrmeyer, G. B., Standaert, D. G., Testa, C. M., Penney, J. B. Jr., and Young, A. B. (1995). NMDA receptor subunit mRNA expression by projection neurons and interneurons in rat striatum. *J. Neurosci.* 15, 5297–5307.

Lange, K. W., Wells, F. R., Jenner, P., and Marsden, C. D. (1993). Altered muscarinic and nicotinic receptor densities in cortical and subcortical brain regions in Parkinson's disease. *J. Neurochem.* 60, 197–203. doi: 10.1111/j.1471-4159.1993.tb05838.x

Lanska, D. J. (2010). Chapter 33: the history of movement disorders. *Handb. Clin. Neurol.* 95, 501–546. doi: 10.1016/S0072-9752(08)02133-7

Lapper, S. R., and Bolam, J. P. (1992). Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. *Neuroscience* 51, 533–545. doi: 10.1016/0306-4522(92)90293-B

Lee, J., Laurin, N., Crosbie, J., Ickowicz, A., Pathare, T., Malone, M., et al. (2008). Association study of the nicotinic acetylcholine receptor alpha4 subunit gene, CHRNA4, in attention-deficit hyperactivity disorder. *Genes Brain Behav.* 7, 53–60. doi: 10.1111/j.1601-183X.2007.00325.x

Lee, K., Dixon, A. K., Freeman, T. C., and Richardson, P. J. (1998). Identification of an ATP-sensitive potassium channel current in rat striatal cholinergic interneurones. *J. Physiol.* 510(Pt 2), 441–453. doi: 10.1111/j.1469-7793.1998.441bk.x

Lehmann, J., and Langer, S. Z. (1982). Muscarinic receptors on dopamine terminals in the cat caudate nucleus: neuromodulation of [3H]dopamine release *in vitro* by endogenous acetylcholine. *Brain Res.* 248, 61–69. doi: 10.1016/0006-8993(82)91147-7

Lehmann, J., and Langer, S. Z. (1983). The striatal cholinergic interneuron: synaptic target of dopaminergic terminals? *Neuroscience* 10, 1105–1120. doi: 10.1016/0306-4522(83)90102-1

Le Moine, C., Kieffer, B., Gaveriaux-Ruff, C., Befort, K., and Bloch, B. (1994). Delta-opioid receptor gene expression in the mouse forebrain: localization in cholinergic neurons of the striatum. *Neuroscience* 62, 635–640. doi: 10.1016/0306-4522(94)90464-2

Le Novère, N., Zoli, M., and Changeux, J. P. (1996). Neuronal nicotinic receptor alpha 6 subunit mRNA is selectively concentrated in catecholaminergic nuclei of the rat brain. *Eur. J. Neurosci.* 8, 2428–2439. doi: 10.1111/j.1460-9568.1996.tb01206.x

Leonard, S., Gault, J., Hopkins, J., Logel, J., Vianzon, R., Short, M., et al. (2002). Association of promoter variants in the alpha7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. *Arch. Gen. Psychiatry* 59, 1085–1096. doi: 10.1001/archpsyc.59.12.1085

Lesser, R. P., Fahn, S., Snider, S. R., Cote, L. J., Isgreen, W. P., and Barrett, R. E. (1979). Analysis of the clinical problems in parkinsonism and the complications of long-term levodopa therapy. *Neurology* 29, 1253–1260. doi: 10.1212/WNL.29.9_Part_1.1253

Levey, A. I., Kitt, C. A., Simonds, W. F., Price, D. L., and Brann, M. R. (1991). Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J. Neurosci.* 11, 3218–3226.

Li, J.-L., Kaneko, T., and Mizuno, N. (2002). Synaptic association of dopaminergic axon terminals and neurokinin-1 receptor-expressing intrinsic neurons in the striatum of the rat. *Neurosci. Lett.* 324, 9–12. doi: 10.1016/S0304-3940(02)00138-6

Lin, J. Y., Chung, K. K. H., de Castro, D., Funk, G. D., and Lipski, J. (2004). Effects of muscarinic acetylcholine receptor activation on membrane currents and intracellular messengers in medium spiny neurones of the rat striatum. *Eur. J. Neurosci.* 20, 1219–1230. doi: 10.1111/j.1460-9568.2004.03576.x

Linazasoro, G. (1994). Anticholinergics and dyskinesia. *Mov. Disord. Off. J. Mov. Disord. Soc.* 9, 689. doi: 10.1002/mds.870090618

Linden, D. J., Dickinson, M. H., Smeyne, M., and Connor, J. A. (1991). A long-term depression of AMPA currents in cultured cerebellar Purkinje neurons. *Neuron* 7, 81–89. doi: 10.1016/0896-6273(91)90076-C

Liu, Z., Otsu, Y., Vasuta, C., Nawa, H., and Murphy, T. H. (2007). Action-potential-independent GABAergic tone mediated by nicotinic stimulation of immature striatal miniature synaptic transmission. *J. Neurophysiol.* 98, 581–593. doi: 10.1152/jn.00768.2006

Luo, R., Janssen, M. J., Partridge, J. G., and Vicini, S. (2013). Direct and GABA-mediated indirect effects of nicotinic ACh receptor agonists on striatal neurones. *J. Physiol.* 591, 203–217. doi: 10.1113/jphysiol.2012.241786

MacDermott, A. B., Role, L. W., and Siegelbaum, S. A. (1999). Presynaptic ionotropic receptors and the control of transmitter release. *Annu. Rev. Neurosci.* 22, 443–485. doi: 10.1146/annurev.neuro.22.1.443

Macintosh, F. C. (1941). The distribution of acetylcholine in the peripheral and the central nervous system. *J. Physiol.* 99, 436–442.

Mahon, S., Deniau, J.-M., and Charpier, S. (2004). Corticostriatal plasticity: life after the depression. *Trends Neurosci.* 27, 460–467. doi: 10.1016/j.tins.2004.06.010

Malenka, R. C., and Kocsis, J. D. (1988). Presynaptic actions of carbachol and adenosine on corticostriatal synaptic transmission studied *in vitro*. *J. Neurosci.* 8, 3750–3756.

Mansour, A., Fox, C. A., Burke, S., Meng, F., Thompson, R. C., Akil, H., et al. (1994). Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an *in situ* hybridization study. *J. Comp. Neurol.* 350, 412–438. doi: 10.1002/cne.903500307

Manyam, B. V. (1990). Paralysis agitans and levodopa in "Ayurveda": ancient Indian medical treatise. *Mov. Disord. Off. J. Mov. Disord. Soc.* 5, 47–48. doi: 10.1002/mds.870050112

Manyam, B. V., and Sánchez-Ramos, J. R. (1999). Traditional and complementary therapies in Parkinson's disease. *Adv. Neurol.* 80, 565–574.

Marchi, M., Risso, F., Viola, C., Cavazzani, P., and Raiteri, M. (2002). Direct evidence that release-stimulating alpha7* nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. *J. Neurochem.* 80, 1071–1078. doi: 10.1046/j.0022-3042.2002.00805.x

Marchi, M., Sanguineti, P., and Raiteri, M. (1990). Muscarinic receptors mediate direct inhibition of GABA release from rat striatal nerve terminals. *Neurosci. Lett.* 116, 347–351. doi: 10.1016/0304-3940(90)90099-U

Markram, H., Lübke, J., Frotscher, M., and Sakmann, B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* 275, 213–215. doi: 10.1126/science.275.5297.213

Martella, G., Platania, P., Vita, D., Sciamanna, G., Cuomo, D., Tassone, A., et al. (2009). Enhanced sensitivity to group II mGlu receptor activation at corticostriatal synapses in mice lacking the familial parkinsonism-linked genes PINK1 or Parkin. *Exp. Neurol.* 215, 388–396. doi: 10.1016/j.expneurol.2008.11.001

Martin-Schild, S., Gerall, A. A., Kastin, A. J., and Zadina, J. E. (1999). Differential distribution of endomorphin 1- and endomorphin 2-like immunoreactivities in the CNS of the rodent. *J. Comp. Neurol.* 405, 450–471.

Martone, M. E., Armstrong, D. M., Young, S. J., and Groves, P. M. (1992). Ultrastructural examination of enkephalin and substance P input to cholinergic neurons within the rat neostriatum. *Brain Res.* 594, 253–262. doi: 10.1016/0006-8993(92)91132-X

Matsubayashi, H., Amano, T., Amano, H., and Sasa, M. (2001). Excitation of rat striatal large neurons by dopamine and/or glutamate released from nerve terminals via presynaptic nicotinic receptor (A4beta2 type) stimulation. *Jpn. J. Pharmacol.* 86, 429–436. doi: 10.1254/jjp.86.429

Matsuda, W., Furuta, T., Nakamura, K. C., Hioki, H., Fujiyama, F., Arai, R., et al. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *J. Neurosci.* 29, 444–453. doi: 10.1523/JNEUROSCI.4029-08.2009

Maurin, Y., Banrezes, B., Menetrey, A., Mailly, P., and Deniau, J. M. (1999). Three-dimensional distribution of nigrostriatal neurons in the rat: relation to the topography of striatonigral projections. *Neuroscience* 91, 891–909. doi: 10.1016/S0306-4522(98)00681-2

McEvoy, J. P., and Allen, T. B. (2002). The importance of nicotinic acetylcholine receptors in schizophrenia, bipolar disorder and Tourette's syndrome. *Curr. Drug Targets CNS Neurol. Disord.* 1, 433–442. doi: 10.2174/1568007023339210

McGeer, P. L., and McGeer, E. G. (1977). Possible changes in striatal and limbic cholinergic systems in schizophrenia. *Arch. Gen. Psychiatry* 34, 1319–1323. doi: 10.1001/archpsyc.1977.01770230061003

McGehee, D. S., Heath, M. J., Gelber, S., Devay, P., and Role, L. W. (1995). Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science* 269, 1692–1696. doi: 10.1126/science.7569895

McGehee, D. S., and Role, L. W. (1995). Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annu. Rev. Physiol.* 57, 521–546. doi: 10.1146/annurev.ph.57.030195.002513

Mirenowicz, J., and Schultz, W. (1996). Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature* 379, 449–451. doi: 10.1038/379449a0

Morens, D. M., Grandinetti, A., Reed, D., White, L. R., and Ross, G. W. (1995). Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? *Neurology* 45, 1041–1051. doi: 10.1212/WNL.45.6.1041

Mori, F., Tanji, K., Zhang, H., Kakita, A., Takahashi, H., and Wakabayashi, K. (2008). alpha-Synuclein pathology in the neostriatum in Parkinson's disease. *Acta Neuropathol. (Berl.)* 115, 453–459. doi: 10.1007/s00401-007-0316-4

Moss, J., and Bolam, J. P. (2008). A dopaminergic axon lattice in the striatum and its relationship with cortical and thalamic terminals. *J. Neurosci.* 28, 11221–11230. doi: 10.1523/JNEUROSCI.2780-08.2008

Mulder, A. H., Burger, D. M., Wardeh, G., Hogenboom, F., and Frankhuyzen, A. L. (1991). Pharmacological profile of various kappa-agonists at kappa-, mu- and delta-opioid receptors mediating presynaptic inhibition of neurotransmitter release in the rat brain. *Br. J. Pharmacol.* 102, 518–522. doi: 10.1111/j.1476-5381.1991.tb12203.x

Mulder, A. H., Wardeh, G., Hogenboom, F., and Frankhuyzen, A. L. (1984). Kappa- and delta-opioid receptor agonists differentially inhibit striatal dopamine and acetylcholine release. *Nature* 308, 278–280. doi: 10.1038/308278a0

Mulle, C., Choquet, D., Korn, H., and Changeux, J. P. (1992). Calcium influx through nicotinic receptor in rat central neurons: its relevance to cellular regulation. *Neuron* 8, 135–143. doi: 10.1016/0896-6273(92)90115-T

Nelson, A. B., Bussert, T. G., Kreitzer, A. C., and Seal, R. P. (2014a). Striatal cholinergic neurotransmission requires VGLUT3. *J. Neurosci.* 34, 8772–8777. doi: 10.1523/JNEUROSCI.0901-14.2014

Nelson, A. B., Hammack, N., Yang, C. F., Shah, N. M., Seal, R. P., and Kreitzer, A. C. (2014b). Striatal cholinergic interneurons drive GABA release from dopamine terminals. *Neuron* 82, 63–70. doi: 10.1016/j.neuron.2014.01.023

Nicholas, A. P., Pieribone, V. A., Arvidsson, U., and Hökfelt, T. (1992). Serotonin-, substance P- and glutamate/aspartate-like immunoreactivities in medullo-spinal pathways of rat and primate. *Neuroscience* 48, 545–559. doi: 10.1016/0306-4522(92)90401-M

Nishiyama, M., Hong, K., Mikoshiba, K., Poo, M. M., and Kato, K. (2000). Calcium stores regulate the polarity and input specificity of synaptic modification. *Nature* 408, 584–588. doi: 10.1038/35046067

Norgren, R., Hajnal, A., and Mungarndee, S. S. (2006). Gustatory reward and the nucleus accumbens. *Physiol. Behav.* 89, 531–535. doi: 10.1016/j.physbeh.2006.05.024

Ovallath, S., and Deepa, P. (2013). The history of parkinsonism: descriptions in ancient Indian medical literature. *Mov. Disord. Off. J. Mov. Disord. Soc.* 28, 566–568. doi: 10.1002/mds.25420

Pakhotin, P., and Bracci, E. (2007). Cholinergic interneurons control the excitatory input to the striatum. *J. Neurosci.* 27, 391–400. doi: 10.1523/JNEUROSCI.3709-06.2007

Pancani, T., Bolarinwa, C., Smith, Y., Lindsley, C. W., Conn, P. J., and Xiang, Z. (2014). M4 mAChR-mediated modulation of glutamatergic transmission at corticostriatal synapses. *ACS Chem. Neurosci.* 5, 318–324. doi: 10.1021/cn500003z

Panula, P., Yang, H. Y., and Costa, E. (1984). Histamine-containing neurons in the rat hypothalamus. *Proc. Natl. Acad. Sci. U.S.A.* 81, 2572–2576. doi: 10.1073/pnas.81.8.2572

Parkinson, J. (1817). *An Essay on the Shaking Palsy.* London: Whittingham and Rowland for Sherwood, Needly and Jones.

Parsons, M. E., and Ganellin, C. R. (2006). Histamine and its receptors. *Br. J. Pharmacol.* 147(Suppl. 1), S127–S135. doi: 10.1038/sj.bjp.0706440

Patrick, J., Séquéla, P., Vernino, S., Amador, M., Luetje, C., and Dani, J. A. (1993). Functional diversity of neuronal nicotinic acetylcholine receptors. *Prog. Brain Res.* 98, 113–120. doi: 10.1016/S0079-6123(08)62387-0

Pérez-Garci, E., Bargas, J., and Galarraga, E. (2003). The role of Ca2+ channels in the repetitive firing of striatal projection neurons. *Neuroreport* 14, 1253–1256. doi: 10.1097/01.wnr.0000081861.45938.71

Perez-Rosello, T., Figueroa, A., Salgado, H., Vilchis, C., Tecuapetla, F., Guzman, J. N., et al. (2005). Cholinergic control of firing pattern and neurotransmission in rat neostriatal projection neurons: role of CaV2.1 and CaV2.2 Ca2+ channels. *J. Neurophysiol.* 93, 2507–2519. doi: 10.1152/jn.00853.2004

Perez, X. A., Ly, J., McIntosh, J. M., and Quik, M. (2012). Long-term nicotine exposure depresses dopamine release in nonhuman primate nucleus accumbens. *J. Pharmacol. Exp. Ther.* 342, 335–344. doi: 10.1124/jpet.112.194084

Perez, X. A., McIntosh, J. M., and Quik, M. (2013). Long-term nicotine treatment down-regulates α6β2* nicotinic receptor expression and function in nucleus accumbens. *J. Neurochem.* 127, 762–771. doi: 10.1111/jnc.12442

Perreault, M. L., Fan, T., Alijaniaram, M., O'Dowd, B. F., and George, S. R. (2012). Dopamine D1-D2 receptor heteromer in dual phenotype GABA/glutamate-coexpressing striatal medium spiny neurons: regulation of BDNF, GAD67 and VGLUT1/2. *PLoS ONE* 7:e33348. doi: 10.1371/journal.pone.0033348

Persohn, E., Malherbe, P., and Richards, J. G. (1992). Comparative molecular neuroanatomy of cloned GABAA receptor subunits in the rat CNS. *J. Comp. Neurol.* 326, 193–216. doi: 10.1002/cne.903260204

Picconi, B., Passino, E., Sgobio, C., Bonsi, P., Barone, I., Ghiglieri, V., et al. (2006). Plastic and behavioral abnormalities in experimental Huntington's disease: a crucial role for cholinergic interneurons. *Neurobiol. Dis.* 22, 143–152. doi: 10.1016/j.nbd.2005.10.009

Piggott, M. A., Owens, J., O'Brien, J., Colloby, S., Fenwick, J., Wyper, D., et al. (2003). Muscarinic receptors in basal ganglia in dementia with Lewy bodies, Parkinson's disease and Alzheimer's disease. *J. Chem. Neuroanat.* 25, 161–173. doi: 10.1016/S0891-0618(03)00002-4

Pisani, A., Bonsi, P., Catania, M. V., Giuffrida, R., Morari, M., Marti, M., et al. (2002). Metabotropic glutamate 2 receptors modulate synaptic inputs and calcium signals in striatal cholinergic interneurons. *J. Neurosci.* 22, 6176–6185.

Pisani, A., Bonsi, P., Centonze, D., Bernardi, G., and Calabresi, P. (2001). Functional coexpression of excitatory mGluR1 and mGluR5 on striatal cholinergic interneurons. *Neuropharmacology* 40, 460–463. doi: 10.1016/S0028-3908(00)00184-2

Pisani, A., Bonsi, P., Centonze, D., Martorana, A., Fusco, F., Sancesario, G., et al. (2003). Activation of beta1-adrenoceptors excites striatal cholinergic interneurons through a cAMP-dependent, protein kinase-independent pathway. *J. Neurosci.* 23, 5272–5282.

Plotkin, J. L., Shen, W., Rafalovich, I., Sebel, L. E., Day, M., Chan, C. S., et al. (2013). Regulation of dendritic calcium release in striatal spiny projection neurons. *J. Neurophysiol.* 110, 2325–2336. doi: 10.1152/jn.00422.2013

Poewe, W., Wolters, E., Emre, M., Onofrj, M., Hsu, C., Tekin, S., et al. (2006). Long-term benefits of rivastigmine in dementia associated with Parkinson's disease: an active treatment extension study. *Mov. Disord. Off. J. Mov. Disord. Soc.* 21, 456–461. doi: 10.1002/mds.20700

Ponterio, G., Tassone, A., Sciamanna, G., Riahi, E., Vanni, V., Bonsi, P., et al. (2013). Powerful inhibitory action of mu opioid receptors (MOR) on cholinergic interneuron excitability in the dorsal striatum. *Neuropharmacology* 75, 78–85. doi: 10.1016/j.neuropharm.2013.07.006

Potter, A. S., Dunbar, G., Mazzulla, E., Hosford, D., and Newhouse, P. A. (2014). AZD3480, a novel nicotinic receptor agonist, for the treatment of attention-deficit/hyperactivity disorder in adults. *Biol. Psychiatry* 75, 207–214. doi: 10.1016/j.biopsych.2013.06.002

Pourcher, E., Bonnet, A. M., Kefalos, J., Dubois, B., and Agid, Y. (1989). Effects of etybenzatropine and diazepam on levodopa-induced diphasic dyskinesias in Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc.* 4, 195–201. doi: 10.1002/mds.870040301

Prast, H., Tran, M. H., Fischer, H., Kraus, M., Lamberti, C., Grass, K., et al. (1999a). Histaminergic neurons modulate acetylcholine release in the ventral striatum: role of H3 histamine receptors. *Naunyn. Schmiedebergs Arch. Pharmacol.* 360, 558–564. doi: 10.1007/s002109900097

Prast, H., Tran, M. H., Lamberti, C., Fischer, H., Kraus, M., Grass, K., et al. (1999b). Histaminergic neurons modulate acetylcholine release in the ventral striatum: role of H1 and H2 histamine receptors. *Naunyn. Schmiedebergs Arch. Pharmacol.* 360, 552–557. doi: 10.1007/s002109900098

Preston, Z., Lee, K., Widdowson, L., Richardson, P. J., and Pinnock, R. D. (2000). Tachykinins increase [3H]acetylcholine release in mouse striatum through multiple receptor subtypes. *Neuroscience* 95, 367–376. doi: 10.1016/S0306-4522(99)00440-6

Puttfarcken, P. S., Jacobs, I., and Faltynek, C. R. (2000). Characterization of nicotinic acetylcholine receptor-mediated [(3)H]-dopamine release from rat cortex and striatum. *Neuropharmacology* 39, 2673–2680. doi: 10.1016/S0028-3908(00)00131-3

Quik, M., Bordia, T., and O'Leary, K. (2007). Nicotinic receptors as CNS targets for Parkinson's disease. *Biochem. Pharmacol.* 74, 1224–1234. doi: 10.1016/j.bcp.2007.06.015

Quik, M., and McIntosh, J. M. (2006). Striatal alpha6* nicotinic acetylcholine receptors: potential targets for Parkinson's disease therapy. *J. Pharmacol. Exp. Ther.* 316, 481–489. doi: 10.1124/jpet.105.094375

Quik, M., Perez, X. A., and Bordia, T. (2012). Nicotine as a potential neuroprotective agent for Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc.* 27, 947–957. doi: 10.1002/mds.25028

Quik, M., Sum, J. D., Whiteaker, P., McCallum, S. E., Marks, M. J., Musachio, J., et al. (2003). Differential declines in striatal nicotinic receptor subtype function after nigrostriatal damage in mice. *Mol. Pharmacol.* 63, 1169–1179. doi: 10.1124/mol.63.5.1169

Quik, M., and Wonnacott, S. (2011). α6β2* and α4β2* nicotinic acetylcholine receptors as drug targets for Parkinson's disease. *Pharmacol. Rev.* 63, 938–966. doi: 10.1124/pr.110.003269

Radek, R. J., Kohlhaas, K. L., Rueter, L. E., and Mohler, E. G. (2010). Treating the cognitive deficits of schizophrenia with alpha4beta2 neuronal nicotinic receptor agonists. *Curr. Pharm. Des.* 16, 309–322. doi: 10.2174/138161210790170166

Raedler, T. J., Knable, M. B., Jones, D. W., Urbina, R. A., Gorey, J. G., Lee, K. S., et al. (2003). *In vivo* determination of muscarinic acetylcholine receptor availability in schizophrenia. *Am. J. Psychiatry* 160, 118–127. doi: 10.1176/appi.ajp.160.1.118

Ragozzino, M. E. (2003). Acetylcholine actions in the dorsomedial striatum support the flexible shifting of response patterns. *Neurobiol. Learn. Mem.* 80, 257–267. doi: 10.1016/S1074-7427(03)00077-7

Raudino, F. (2012). The parkinson disease before james parkinson. *Neurol. Sci. Off. J. Ital. Neurol. Soc. Ital. Soc. Clin. Neurophysiol.* 33, 945–948. doi: 10.1007/s10072-011-0816-9

Ravel, S., Legallet, E., and Apicella, P. (1999). Tonically active neurons in the monkey striatum do not preferentially respond to appetitive stimuli. *Exp. Brain Res.* 128, 531–534. doi: 10.1007/s002210050876

Reynolds, J. N. J., Hyland, B. I., and Wickens, J. R. (2004). Modulation of an afterhyperpolarization by the substantia nigra induces pauses in the tonic firing of striatal cholinergic interneurons. *J. Neurosci.* 24, 9870–9877. doi: 10.1523/JNEUROSCI.3225-04.2004

Rezvani, A. H., Cauley, M. C., Johnson, E. C., Gatto, G. J., and Levin, E. D. (2012). Effects of AZD3480, a neuronal nicotinic acetylcholine receptor agonist, and donepezil on dizocilpine-induced attentional impairment in rats. *Psychopharmacology (Berl.)* 223, 251–258. doi: 10.1007/s00213-012-2712-2

Rice, M. E., and Cragg, S. J. (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nat. Neurosci.* 7, 583–584. doi: 10.1038/nn1244

Richardson, P. J., Dixon, A. K., Lee, K., Bell, M. I., Cox, P. J., Williams, R., et al. (2000). Correlating physiology with gene expression in striatal cholinergic neurones. *J. Neurochem.* 74, 839–846. doi: 10.1046/j.1471-4159.2000.740839.x

Rinne, J. O., Laihinen, A., Lönnberg, P., Marjamäki, P., and Rinne, U. K. (1991). A post-mortem study on striatal dopamine receptors in Parkinson's disease. *Brain Res.* 556, 117–122. doi: 10.1016/0006-8993(91)90554-9

Robinson, T. E., and Berridge, K. C. (2000). The psychology and neurobiology of addiction: an incentive-sensitization view. *Addict. Abingdon Engl.* 95(Suppl. 2), S91–S117. doi: 10.1080/09652140050111681

Rodríguez, M., and González-Hernández, T. (1999). Electrophysiological and morphological evidence for a GABAergic nigrostriatal pathway. *J. Neurosci.* 19, 4682–4694.

Rodríguez-Pallares, J., Labandeira-García, J. L., Muñoz, A., and Caruncho, H. J. (2000). Morphology and neurochemistry of two striatal neuronal subtypes expressing the GABA(A) receptor alpha3-subunit in the rat. *Brain Res.* 876, 124–130. doi: 10.1016/S0006-8993(00)02636-6

Rojas, A., and Dingledine, R. (2013). Ionotropic glutamate receptors: regulation by G-protein-coupled receptors. *Mol. Pharmacol.* 83, 746–752. doi: 10.1124/mol.112.083352

Sanberg, P. R., Shytle, R. D., and Silver, A. A. (1998). Treatment of Tourette's syndrome with mecamylamine. *Lancet* 352, 705–706. doi: 10.1016/S0140-6736(05)60822-7

Sandor, N. T., Zelles, T., Kiss, J., Sershen, H., Torocsik, A., Lajtha, A., et al. (1991). Effect of nicotine on dopaminergic-cholinergic interaction in the striatum. *Brain Res.* 567, 313–316. doi: 10.1016/0006-8993(91)90810-I

Santangelo, G., Barone, P., Trojano, L., and Vitale, C. (2013a). Pathological gambling in Parkinson's disease. A comprehensive review. *Parkinsonism Relat. Disord.* 19, 645–653. doi: 10.1016/j.parkreldis.2013.02.007

Santangelo, G., Trojano, L., Barone, P., Errico, D., Grossi, D., and Vitale, C. (2013b). Apathy in Parkinson's disease: diagnosis, neuropsychological correlates, pathophysiology and treatment. *Behav. Neurol.* 27, 501–513. doi: 10.3233/BEN-129025

Sato, A., Sasaoka, T., Nishijo, T., and Momiyama, T. (2014). GABAergic synaptic transmission onto striatal cholinergic interneurons in dopamine D2 receptor knock-out mice. *Neuroscience* 263, 138–147. doi: 10.1016/j.neuroscience.2014.01.010

Scheibler, P., Pesic, M., Franke, H., Reinhardt, R., Wirkner, K., Illes, P., et al. (2004). P2X2 and P2Y1 immunofluorescence in rat neostriatal medium-spiny projection neurones and cholinergic interneurones is not linked to respective purinergic receptor function. *Br. J. Pharmacol.* 143, 119–131. doi: 10.1038/sj.bjp.0705916

Schoffelmeer, A. N., Hogenboom, F., and Mulder, A. H. (1997). Kappa1- and kappa2-opioid receptors mediating presynaptic inhibition of dopamine and acetylcholine release in rat neostriatum. *Br. J. Pharmacol.* 122, 520–524. doi: 10.1038/sj.bjp.0701394

Schwartz, J. C., Arrang, J. M., Garbarg, M., and Korner, M. (1986). Properties and roles of the three subclasses of histamine receptors in brain. *J. Exp. Biol.* 124, 203–224.

Sharples, C. G., Kaiser, S., Soliakov, L., Marks, M. J., Collins, A. C., Washburn, M., et al. (2000). UB-165: a novel nicotinic agonist with subtype selectivity implicates the alpha4beta2* subtype in the modulation of dopamine release from rat striatal synaptosomes. *J. Neurosci.* 20, 2783–2791.

Shen, W., Hamilton, S. E., Nathanson, N. M., and Surmeier, D. J. (2005). Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. *J. Neurosci.* 25, 7449–7458. doi: 10.1523/JNEUROSCI.1381-05.2005

Shiflett, M. W., Brown, R. A., and Balleine, B. W. (2010). Acquisition and performance of goal-directed instrumental actions depends on ERK signaling in

distinct regions of dorsal striatum in rats. *J. Neurosci.* 30, 2951–2959. doi: 10.1523/JNEUROSCI.1778-09.2010

Shimohama, S., Taniguchi, T., Fujiwara, M., and Kameyama, M. (1985). Biochemical characterization of the nicotinic cholinergic receptors in human brain: binding of (-)-[3H]nicotine. *J. Neurochem.* 45, 604–610. doi: 10.1111/j.1471-4159.1985.tb04029.x

Shytle, R. D., Silver, A. A., Philipp, M. K., McConville, B. J., and Sanberg, P. R. (1996). Transdermal nicotine for Tourette's syndrome. *Drug Dev. Res.* 38, 290–298. doi: 10.1002/(SICI)1098-2299(199607/08)38:3/4<290::AID-DDR18>3.0.CO;2-2

Silver, A. A., Shytle, R. D., and Sanberg, P. R. (2000). Mecamylamine in Tourette's syndrome: a two-year retrospective case study. *J. Child Adolesc. Psychopharmacol.* 10, 59–68. doi: 10.1089/cap.2000.10.59

Simón-Sánchez, J., Schulte, C., Bras, J. M., Sharma, M., Gibbs, J. R., Berg, D., et al. (2009). Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.* 41, 1308–1312. doi: 10.1038/ng.487

Sirviö, J., Rinne, J. O., Valjakka, A., Rinne, U. K., Riekkinen, P. J., and Paljärvi, L. (1989). Different forms of brain acetylcholinesterase and muscarinic binding in Parkinson's disease. *J. Neurol. Sci.* 90, 23–32. doi: 10.1016/0022-510X(89)90042-7

Smiley, J. F., Levey, A. I., and Mesulam, M. M. (1999). m2 muscarinic receptor immunolocalization in cholinergic cells of the monkey basal forebrain and striatum. *Neuroscience* 90, 803–814. doi: 10.1016/S0306-4522(98)00527-2

Smith, R., Chung, H., Rundquist, S., Maat-Schieman, M. L. C., Colgan, L., Englund, E., et al. (2006). Cholinergic neuronal defect without cell loss in Huntington's disease. *Hum. Mol. Genet.* 15, 3119–3131. doi: 10.1093/hmg/ddl252

Smith, Y., and Kieval, J. Z. (2000). Anatomy of the dopamine system in the basal ganglia. *Trends Neurosci.* 23, S28–S33. doi: 10.1016/S1471-1931(00)00023-9

Smolders, I., Bogaert, L., Ebinger, G., and Michotte, Y. (1997). Muscarinic modulation of striatal dopamine, glutamate, and GABA release, as measured with *in vivo* microdialysis. *J. Neurochem.* 68, 1942–1948. doi: 10.1046/j.1471-4159.1997.68051942.x

Sprouse, J. S., Bradberry, C. W., Roth, R. H., and Aghajanian, G. K. (1989). MDMA (3,4-methylenedioxymethamphetamine) inhibits the firing of dorsal raphe neurons in brain slices via release of serotonin. *Eur. J. Pharmacol.* 167, 375–383. doi: 10.1016/0014-2999(89)90446-9

Standaert, D. G., Friberg, I. K., Landwehrmeyer, G. B., Young, A. B., and Penney, J. B. Jr. (1999). Expression of NMDA glutamate receptor subunit mRNAs in neurochemically identified projection and interneurons in the striatum of the rat. *Brain Res. Mol. Brain Res.* 64, 11–23. doi: 10.1016/S0169-328X(98)00293-9

Stephenson-Jones, M., Samuelsson, E., Ericsson, J., Robertson, B., and Grillner, S. (2011). Evolutionary conservation of the basal ganglia as a common vertebrate mechanism for action selection. *Curr. Biol.* 21, 1081–1091. doi: 10.1016/j.cub.2011.05.001

Straub, C., Tritsch, N. X., Hagan, N. A., Gu, C., and Sabatini, B. L. (2014). Multiphasic modulation of cholinergic interneurons by nigrostriatal afferents. *J. Neurosci.* 34, 8557–8569. doi: 10.1523/JNEUROSCI.0589-14.2014

Stuber, G. D., Hnasko, T. S., Britt, J. P., Edwards, R. H., and Bonci, A. (2010). Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J. Neurosci.* 30, 8229–8233. doi: 10.1523/JNEUROSCI.1754-10.2010

Sugita, S., Uchimura, N., Jiang, Z. G., and North, R. A. (1991). Distinct muscarinic receptors inhibit release of gamma-aminobutyric acid and excitatory amino acids in mammalian brain. *Proc. Natl. Acad. Sci. U.S.A.* 88, 2608–2611. doi: 10.1073/pnas.88.6.2608

Sullivan, M. A., Chen, H., and Morikawa, H. (2008). Recurrent inhibitory network among striatal cholinergic interneurons. *J. Neurosci.* 28, 8682–8690. doi: 10.1523/JNEUROSCI.2411-08.2008

Surmeier, D. J., Ding, J., Day, M., Wang, Z., and Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci.* 30, 228–235. doi: 10.1016/j.tins.2007.03.008

Suzuki, M., Desmond, T. J., Albin, R. L., and Frey, K. A. (2001a). Vesicular neurotransmitter transporters in Huntington's disease: initial observations and comparison with traditional synaptic markers. *Synapse* 41, 329–336. doi: 10.1002/syn.1089

Suzuki, T., Miura, M., Nishimura, K., and Aosaki, T. (2001b). Dopamine-dependent synaptic plasticity in the striatal cholinergic interneurons. *J. Neurosci.* 21, 6492–6501.

Szydlowski, S. N., Pollak Dorocic, I., Planert, H., Carlén, M., Meletis, K., and Silberberg, G. (2013). Target selectivity of feedforward inhibition by striatal fast-spiking interneurons. *J. Neurosci.* 33, 1678–1683. doi: 10.1523/JNEUROSCI.3572-12.2013

Tallaksen-Greene, S. J., Kaatz, K. W., Romano, C., and Albin, R. L. (1998). Localization of mGluR1a-like immunoreactivity and mGluR5-like immunoreactivity in identified populations of striatal neurons. *Brain Res.* 780, 210–217. doi: 10.1016/S0006-8993(97)01141-4

Tashiro, Y., Sugimoto, T., Hattori, T., Uemura, Y., Nagatsu, I., Kikuchi, H., et al. (1989). Tyrosine hydroxylase-like immunoreactive neurons in the striatum of the rat. *Neurosci. Lett.* 97, 6–10. doi: 10.1016/0304-3940(89)90130-4

Tepper, J. M., Tecuapetla, F., Koós, T., and Ibáñez-Sandoval, O. (2010). Heterogeneity and diversity of striatal GABAergic interneurons. *Front. Neuroanat.* 4:150. doi: 10.3389/fnana.2010.00150

Testa, C. M., Standaert, D. G., Young, A. B., and Penney, J. B. Jr. (1994). Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. *J. Neurosci.* 14, 3005–3018.

Thomas, T. M., Smith, Y., Levey, A. I., and Hersch, S. M. (2000). Cortical inputs to m2-immunoreactive striatal interneurons in rat and monkey. *Synapse* 37, 252–261. doi: 10.1002/1098-2396(20000915)37:4<252::AID-SYN2>3.0.CO;2-A

Threlfell, S., Clements, M. A., Khodai, T., Pienaar, I. S., Exley, R., Wess, J., et al. (2010). Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *J. Neurosci.* 30, 3398–3408. doi: 10.1523/JNEUROSCI.5620-09.2010

Threlfell, S., and Cragg, S. J. (2011). Dopamine signaling in dorsal versus ventral striatum: the dynamic role of cholinergic interneurons. *Front. Syst. Neurosci.* 5:11. doi: 10.3389/fnsys.2011.00011

Threlfell, S., Lalic, T., Platt, N. J., Jennings, K. A., Deisseroth, K., and Cragg, S. J. (2012). Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. *Neuron* 75, 58–64. doi: 10.1016/j.neuron.2012.04.038

Tian, Y., Gunther, J. R., Liao, I. H., Liu, D., Ander, B. P., Stamova, B. S., et al. (2011). GABA- and acetylcholine-related gene expression in blood correlate with tic severity and microarray evidence for alternative splicing in Tourette syndrome: a pilot study. *Brain Res.* 1381, 228–236. doi: 10.1016/j.brainres.2011.01.026

Timmerman, H. (1989). Histamine receptors in the central nervous system. *Pharm. Weekbl. Sci.* 11, 146–150. doi: 10.1007/BF01959461

Todd, R. D., Lobos, E. A., Sun, L.-W., and Neuman, R. J. (2003). Mutational analysis of the nicotinic acetylcholine receptor alpha 4 subunit gene in attention deficit/hyperactivity disorder: evidence for association of an intronic polymorphism with attention problems. *Mol. Psychiatry* 8, 103–108. doi: 10.1038/sj.mp.4001257

Tritsch, N. X., Ding, J. B., and Sabatini, B. L. (2012). Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. *Nature* 490, 262–266. doi: 10.1038/nature11466

Tso, P. H., and Wong, Y. H. (2003). Molecular basis of opioid dependence: role of signal regulation by G-proteins. *Clin. Exp. Pharmacol. Physiol.* 30, 307–316. doi: 10.1046/j.1440-1681.2003.03835.x

Vilaró, M. T., Palacios, J. M., and Mengod, G. (1990). Localization of m5 muscarinic receptor mRNA in rat brain examined by *in situ* hybridization histochemistry. *Neurosci. Lett.* 114, 154–159. doi: 10.1016/0304-3940(90)90064-G

Voon, V., Fernagut, P.-O., Wickens, J., Baunez, C., Rodriguez, M., Pavon, N., et al. (2009). Chronic dopaminergic stimulation in Parkinson's disease: from dyskinesias to impulse control disorders. *Lancet Neurol.* 8, 1140–1149. doi: 10.1016/S1474-4422(09)70287-X

Voorn, P., Vanderschuren, L. J. M. J., Groenewegen, H. J., Robbins, T. W., and Pennartz, C. M. A. (2004). Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci.* 27, 468–474. doi: 10.1016/j.tins.2004.06.006

Vorobjev, V. S., Sharonova, I. N., Haas, H. L., and Sergeeva, O. A. (2000). Differential modulation of AMPA receptors by cyclothiazide in two types of striatal neurons. *Eur. J. Neurosci.* 12, 2871–2880. doi: 10.1046/j.1460-9568.2000.00175.x

Vuillet, J., Dimova, R., Nieoullon, A., and Kerkerian-Le Goff, L. (1992). Ultrastructural relationships between choline acetyltransferase- and neuropeptide y-containing neurons in the rat striatum. *Neuroscience* 46, 351–360. doi: 10.1016/0306-4522(92)90057-9

Wallis, D., Arcos-Burgos, M., Jain, M., Castellanos, F. X., Palacio, J. D., Pineda, D., et al. (2009). Polymorphisms in the neural nicotinic acetylcholine receptor α4

subunit (CHRNA4) are associated with ADHD in a genetic isolate. *Atten. Deficit Hyperact. Disord.* 1, 19–24. doi: 10.1007/s12402-009-0003-5

Wan, J., and Poo, M. (1999). Activity-induced potentiation of developing neuromuscular synapses. *Science* 285, 1725–1728. doi: 10.1126/science.285.5434.1725

Watanabe, K., and Kimura, M. (1998). Dopamine receptor-mediated mechanisms involved in the expression of learned activity of primate striatal neurons. *J. Neurophysiol.* 79, 2568–2580.

Weiner, D. M., Levey, A. I., and Brann, M. R. (1990). Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. *Proc. Natl. Acad. Sci. U.S.A.* 87, 7050–7054. doi: 10.1073/pnas.87.18.7050

Wess, J. (1996). Molecular biology of muscarinic acetylcholine receptors. *Crit. Rev. Neurobiol.* 10, 69–99. doi: 10.1615/CritRevNeurobiol.v10.i1.40

West, A. B., Cowell, R. M., Daher, J. P. L., Moehle, M. S., Hinkle, K. M., Melrose, H. L., et al. (2014). Differential LRRK2 expression in the cortex, striatum, and substantia nigra in transgenic and nontransgenic rodents. *J. Comp. Neurol.* 522, 2465–2480. doi: 10.1002/cne.23583

Whyte, R. K., Hunter, K. R., Laurence, D. R., Stern, G. M., and Armitage, P. (1971). Levodopa and orphenadrine hydrochloride in Parkinsonism. *Eur. J. Clin. Pharmacol.* 4, 18–21. doi: 10.1007/BF00568893

Wilens, T. E., and Decker, M. W. (2007). Neuronal nicotinic receptor agonists for the treatment of attention-deficit/hyperactivity disorder: focus on cognition. *Biochem. Pharmacol.* 74, 1212–1223. doi: 10.1016/j.bcp.2007.07.002

Wilson, C. J., Chang, H. T., and Kitai, S. T. (1990). Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. *J. Neurosci.* 10, 508–519.

Wilson, C. J., and Groves, P. M. (1981). Spontaneous firing patterns of identified spiny neurons in the rat neostriatum. *Brain Res.* 220, 67–80. doi: 10.1016/0006-8993(81)90211-0

Won, L., Ding, Y., Singh, P., and Kang, U. J. (2014). Striatal cholinergic cell ablation attenuates L-DOPA induced dyskinesia in Parkinsonian mice. *J. Neurosci.* 34, 3090–3094. doi: 10.1523/JNEUROSCI.2888-13.2014

Wonnacott, S., Kaiser, S., Mogg, A., Soliakov, L., and Jones, I. W. (2000). Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. *Eur. J. Pharmacol.* 393, 51–58. doi: 10.1016/S0014-2999(00)00005-4

Woolf, N. J., and Butcher, L. L. (1981). Cholinergic neurons in the caudate-putamen complex proper are intrinsically organized: a combined Evans blue and acetylcholinesterase analysis. *Brain Res. Bull.* 7, 487–507. doi: 10.1016/0361-9230(81)90004-6

Woolf, N. J., Eckenstein, F., and Butcher, L. L. (1984). Cholinergic systems in the rat brain: I. projections to the limbic telencephalon. *Brain Res. Bull.* 13, 751–784. doi: 10.1016/0361-9230(84)90236-3

Wu, J. C. F., Chruscinski, A., De Jesus Perez, V. A., Singh, H., Pitsiouni, M., Rabinovitch, M., et al. (2009). Cholinergic modulation of angiogenesis: role of the 7 nicotinic acetylcholine receptor. *J. Cell. Biochem.* 108, 433–446. doi: 10.1002/jcb.22270

Xiao, C., Nashmi, R., McKinney, S., Cai, H., McIntosh, J. M., and Lester, H. A. (2009). Chronic nicotine selectively enhances alpha4beta2* nicotinic acetylcholine receptors in the nigrostriatal dopamine pathway. *J. Neurosci.* 29, 12428–12439. doi: 10.1523/JNEUROSCI.2939-09.2009

Yan, Z., Flores-Hernandez, J., and Surmeier, D. J. (2001). Coordinated expression of muscarinic receptor messenger RNAs in striatal medium spiny neurons. *Neuroscience* 103, 1017–1024. doi: 10.1016/S0306-4522(01)00039-2

Yan, Z., Song, W. J., and Surmeier, J. (1997). D2 dopamine receptors reduce N-type Ca2+ currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein-kinase-C-insensitive pathway. *J. Neurophysiol.* 77, 1003–1015.

Yasuda, R. P., Ciesla, W., Flores, L. R., Wall, S. J., Li, M., Satkus, S. A., et al. (1993). Development of antisera selective for m4 and m5 muscarinic cholinergic receptors: distribution of m4 and m5 receptors in rat brain. *Mol. Pharmacol.* 43, 149–157.

Yin, H. H. (2010). The sensorimotor striatum is necessary for serial order learning. *J. Neurosci.* 30, 14719–14723. doi: 10.1523/JNEUROSCI.3989-10.2010

Zhang, D., Mallela, A., Sohn, D., Carroll, F. I., Bencherif, M., Letchworth, S., et al. (2013). Nicotinic receptor agonists reduce L-DOPA-induced dyskinesias in a monkey model of Parkinson's disease. *J. Pharmacol. Exp. Ther.* 347, 225–234. doi: 10.1124/jpet.113.207639

Zhang, H., and Sulzer, D. (2004). Frequency-dependent modulation of dopamine release by nicotine. *Nat. Neurosci.* 7, 581–582. doi: 10.1038/nn1243

Zhang, W., Yamada, M., Gomeza, J., Basile, A. S., and Wess, J. (2002). Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1-M5 muscarinic receptor knock-out mice. *J. Neurosci.* 22, 6347–6352.

Zhang, Z.-X., Dong, Z.-H., and Román, G. C. (2006). Early descriptions of Parkinson disease in ancient China. *Arch. Neurol.* 63, 782–784. doi: 10.1001/archneur.63.5.782

Zhou, F. M., Liang, Y., and Dani, J. A. (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nat. Neurosci.* 4, 1224–1229. doi: 10.1038/nn769

Zhou, F.-W., Xu, J.-J., Zhao, Y., LeDoux, M. S., and Zhou, F.-M. (2006). Opposite functions of histamine H1 and H2 receptors and H3 receptor in substantia nigra pars reticulata. *J. Neurophysiol.* 96, 1581–1591. doi: 10.1152/jn.00148.2006

Zugno, A. I., Julião, R. F., Budni, J., Volpato, A. M., Fraga, D. B., Pacheco, F. D., et al. (2013). Rivastigmine reverses cognitive deficit and acetylcholinesterase activity induced by ketamine in an animal model of schizophrenia. *Metab. Brain Dis.* 28, 501–508. doi: 10.1007/s11011-013-9417-z

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Cocaine inhibition of nicotinic acetylcholine receptors influences dopamine release

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Nicotinic acetylcholine receptors (nAChRs) potently regulate dopamine (DA) release in the striatum and alter cocaine's ability to reinforce behaviors. Since cocaine is a weak nAChR inhibitor, we hypothesized that cocaine may alter DA release by inhibiting the nAChRs in DA terminals in the striatum and thus contribute to cocaine's reinforcing properties primarily associated with the inhibition of DA transporters. We found that biologically relevant concentrations of cocaine can mildly inhibit nAChR-mediated currents in midbrain DA neurons and consequently alter DA release in the dorsal and ventral striatum. At very high concentrations, cocaine also inhibits voltage-gated Na channels in DA neurons. Furthermore, our results show that partial inhibition of nAChRs by cocaine reduces evoked DA release. This diminution of DA release via nAChR inhibition more strongly influences release evoked at low or tonic stimulation frequencies than at higher (phasic) stimulation frequencies, particularly in the dorsolateral striatum. This cocaine-induced shift favoring phasic DA release may contribute to the enhanced saliency and motivational value of cocaine-associated memories and behaviors.

Keywords: substantia nigra, ventral tegmental area, addiction, mesolimbic, voltammetry, nAChRs

INTRODUCTION

Midbrain dopamine (DA) projections to the striatum comprise an important neuronal system mediating the initiation of drug addiction (Bonci et al., 2003; Wise, 2004; Hyman et al., 2006). The most well known action of the addictive drug cocaine is its inhibition of monoamine transporters, such as the DA transporter (DAT), with an affinity of about 500 nM (Ritz et al., 1987; Pristupa et al., 1994; Jones et al., 1995). In the brains of abusers, however, cocaine often reaches concentrations of 5–10 μM for considerable durations, and higher concentrations are achieved for shorter times depending on the route of administration and other factors (Mittleman and Wetli, 1984; Evans et al., 1996; Ward et al., 1997; Fowler et al., 1998). In addition, cocaine inhibits α4β2-containing (α4β2*) nicotinic acetylcholine receptors (nAChRs) with an IC_{50} in the range of 5–15 μM (Damaj et al., 1999; Francis et al., 2000). This inhibition may be significant because α4β2* nAChRs (often in combination with α6) are highly expressed in DA neuron somata and terminals (Mansvelder and McGehee, 2000; Jones et al., 2001; Champtiaux et al., 2003; Wooltorton et al., 2003; Quik and McIntosh, 2006; Zanetti et al., 2006), and nAChRs have been shown to regulate the frequency dependence of DA release (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley and Cragg, 2008; Zhang et al., 2009a). Intertwined with the DA neurons and their axons, cholinergic neurons in the midbrain and brainstem project to the substantia nigra and ventral tegmental area (VTA) (Woolf and Butcher, 1986; Gould et al., 1989; Oakman et al., 1995), while cholinergic interneurons in the striatum innervate locally (Woolf and Butcher, 1981; Zhou et al., 2001; Nelson et al., 2014), providing the endogenous neurotransmitter ACh to the nAChRs in these DA areas.

In the striatum, DA release is normally dependent on both afferent spikes along DA fibers and nAChR activity at axonal and presynaptic locations (Zhou et al., 2001; Grady et al., 2002; Salminen et al., 2004; Zhang et al., 2009a). The nAChRs normally increase the initial DA release probability, and regulate the frequency dependence of DA release (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley and Cragg, 2008; Zhang et al., 2009a). Cholinergic interneuron activity may directly facilitate DA release from DA axon terminals in the dorsal and ventral striatum (Cachope et al., 2012; Threlfell et al., 2012). Inactivation of nAChRs on the DA fibers decreases the DA release probability and increases the phasic to tonic DA ratio (Rice and Cragg, 2004; Exley and Cragg, 2008; Zhang et al., 2009a). Based on those published findings, we hypothesized that cocaine acts via nAChRs to regulate DA signals beyond the expected inhibition of DATs.

MATERIALS AND METHODS

MICE

Male and female C57BL/6J mice from The Jackson Laboratory (Bar Harbor, Maine), β2-subunit knockout (KO) mice (Xu et al., 1999), DAT knockin mice having DATs that are insensitive to

cocaine (Chen et al., 2006), and their wild-type (W-T) littermates were used in our present study. The mutant mice were generated, maintained, euthanized and genotyped according to established procedures (Xu et al., 1999; Chen et al., 2006) and in accordance with national and institutional guidelines. Experiments on the mutant mice and their W-T littermates were performed double blind.

FAST-SCAN CYCLIC VOLTAMMETRY (FCV) IN STRIATAL BRAIN SLICES

For these studies, horizontal brain slices (**Figure 1**) containing the striatum (400 μm in thickness) from mice 1–3 months old were cut on a Leica VT1000 or Leica VT1200s vibratome (Zhang et al., 2009a). Anesthesia, handling, and experimental procedures followed our established techniques at 30°C (Zhou et al., 2001).

FCV was performed with home-made carbon-fiber electrodes constructed from P55S carbon fibers of 10 μm diameter (Amoco Polymers, Greenville, SC). Axopatch 200B amplifier, pClamp 9 software, and Digidata 1320A interface (Axon Instruments) were used to acquire and analyze data. The holding potential was 0 mV between scans. Scans of 20 ms duration were applied at 10 or 20 Hz. The scans were from 0 mV to −400 to 1000 to −400 to 0 at a rate of 300 mV/ms and were sampled at 50 kHz. The peaks of the voltammograms were plotted over time and converted to concentrations by post-experiment calibration with 0.1–5 μM DA standards.

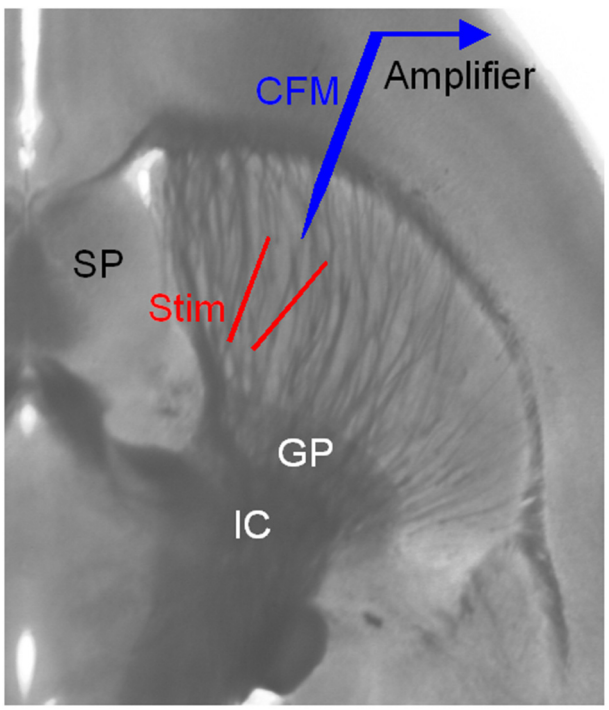

FIGURE 1 | Arrangement for electrical stimulation and FCV recording in striatal brain slices. The striatum is easily identified by its anatomical location and the distinct fiber bundles in horizontal brain slices. Local electrical stimulation in the striatum was delivered using a bipolar tungsten electrode. The two tips of the stimulating electrode were ∼150 μm away from each other and from the carbon fiber microelectrode (CFM) tip. GP, globus pallidus; IC, internal capsule; SP, septum.

Local electrical stimulation in the striatum was delivered using a bipolar tungsten electrode (**Figure 1**). The two tips of the stimulating electrode were ∼150 μm away from each other. The tip of the carbon-fiber recording electrode was 100–200 μm away from the two tips of the stimulating electrode. The stimuli were relatively weak (0.1–0.2 mA for 0.1 ms) helping to keep the recording stable and minimize local interactions. The interval between single pulses was usually 100 s. Additional types of stimulation were used: tonic stimulation of 4 pulses at 4 Hz, phasic stimulation of 4 pulses at 20 Hz, and paired pulses separated by 50 ms. Cocaine was bath-applied for 10 min and then was washed out.

PATCH CLAMP RECORDING OF MIDBRAIN DA NEURONS

Coronal or horizontal midbrain slices, 200–300 μm in thickness and containing the substantia nigra pars compacta (SNc) and/or the VTA, were prepared from 15 to 30 day old mice according to established procedures (Pidoplichko et al., 1997). Visualized recordings were made at 30°C using an Axopatch 200 and pClamp data acquisition and analysis software.

nAChR-mediated currents in putative DA neurons were induced by pressure application of 1 mM ACh using a Picospritzer (General Valve) attached to a puffer pipette (Pidoplichko and Dani, 2005). The puffer was ∼30 μm from the neuron while ACh was applied (25 psi for 100 ms). Then, the puffer was retracted 100–200 μm between ACh applications by a computer-controlled manipulator to prevent leak-induced desensitization (Wooltorton et al., 2003; Pidoplichko and Dani, 2005). For this experiment, the brain slices were bathed in a normal extracellular solution containing (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1.3 $MgCl_2$, and 10 D-glucose that were continuously bubbled with 95% O_2 and 5% CO_2. The intracellular solution contained (in mM): 135 KCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, and 4 Na_2-phosphocreatine. pH 7.25, 280–290 mOsm. To record the fast voltage-activated sodium current (I_{Na}), the presumed nigral DA neuron (based on its slow firing rate at ∼1 Hz in cell-attached mode, see Ding et al., 2011) was held at −90 mV and then stepped to 0 mV for 10 ms. For this experiment, 2.5 mM $CaCl_2$ were substituted by 2.5 mM $MgCl_2$, and extracellular NaCl was reduced to 25 mM, 20 mM TEA and 5 mM 4-AP were used to inhibit K currents; the following Cs-based intracellular solution was also used that contained (in mM): 135 CsCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, 4 Na_2-phosphocreatine with pH was adjusted to 7.25 with CsOH.

Neurobiotin (0.2%) was included in the recording electrode and allowed to diffuse into the cell using approaches described previously (Pidoplichko et al., 1997). After recording, the brain slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Double immunostaining was performed according to established procedures (Pidoplichko et al., 1997; Neuhoff et al., 2002). Tyrosine hydroxylase (TH, a marker for midbrain DA neurons) was detected with a sheep anti-TH primary antibody and rhodamine red-X-tagged donkey anti-sheep secondary antibodies (**Figure 2A2**). Neurobiotin was detected with red Cy2-tagged

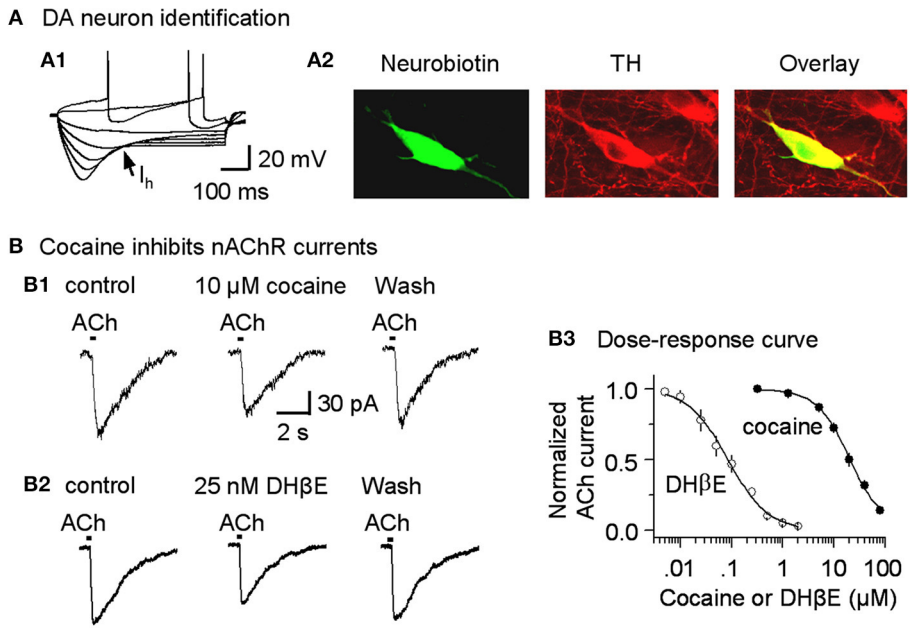

FIGURE 2 | Cocaine inhibits nicotinic currents in midbrain DA neurons from W-T mice. (A,A1) The membrane properties of presumed DA neurons from the lateral VTA or SNc were typical of DA neurons with a strong I_h sag (arrow). Injected current was 40 pA/step. Spikes were partially truncated for display. **(A,A2)** These midbrain DA neurons were backfilled with neurobiotin (green) and labeled for TH (red). The co-incidence of neurobiotin and TH is shown as yellow (Overlay), confirming the DA neuron identification. **(B,B1)** Three traces show nicotinic currents induced in a DA neuron by pressure application of ACh (1 mM, 100 ms pulse) under control conditions (left), with 10 μM cocaine present (middle), or with 25 nM DHβE present (right), which was applied after recovering from cocaine inhibition. The recordings were made in the presence of 1 μM atropine at a holding potential of −70 mV. **(B,B2)** Examples showing DHβE inhibits nicotinic currents in DA neurons. **(B,B3)** The dose-response relationships for inhibition of nAChR currents. The curves through the data were produced with $IC_{50} = 19.8$ μM and Hill coefficient = 1.3 for cocaine, and $IC_{50} = 82$ nM and Hill coefficient = 1.1 for DHβE with $n = 3$–9 per data point.

strepavidin antibody (**Figure 2A2**). Sections were examined on a Bio-Rad confocal laser-scanning microscope.

CHEMICALS

All chemicals including cocaine-HCl were purchased from Sigma Aldrich (St. Louis, MO) or Tocris (Ellisville, MO). Cocaine was also obtained from the NIH/NIDA's Drug Supply Program.

STATISTICS

The data are displayed as the mean and the standard error. Paired *t*-test was used to compare measurements before and during various pharmacological treatments. $P < 0.05$ was considered statistically significant. When applicable, the averaged data points in dose-response plots were fitted with the Hill equation to estimate IC_{50} and Hill coefficient. These and other computations were performed using the Origin analysis and plotting program (Northampton, MA).

RESULTS

COCAINE DIRECTLY INHIBITS nAChRs ON DA NEURONS

Because cocaine inhibits cloned neuronal nAChRs in heterologous expression systems (Damaj et al., 1999; Francis et al., 2000), we tested whether cocaine inhibits nAChRs expressed on DA neurons of the SNc and the lateral VTA (Picciotto et al., 1995; Pidoplichko et al., 1997; Mansvelder and McGehee, 2000; Wooltorton et al., 2003). DA neurons were initially identified by their characteristic membrane properties. The presumed DA neurons within the lateral midbrain fired spontaneously (2.1 ± 0.3 Hz, $n = 9$), displayed prominent I_h currents (**Figure 2A1**) and had relatively long spike durations (2.7 ± 0.3 ms at the base, $n = 9$). These membrane properties were consistent with commonly recognized DA neuron properties from this area (Neuhoff et al., 2002; Ford et al., 2006; Beckstead and Williams, 2007; Zhang et al., 2010; Ding et al., 2011; Li et al., 2011). These electrophysiologically identified DA neurons were further confirmed by back filling with neurobiotin and subsequently staining for TH (**Figure 2A2**).

In these DA neurons, pressure application of ACh (100 ms pulse of 1 mM ACh, 1 μM atropine was always present) induced currents (**Figure 2B1**, left trace) that have previously been characterized as predominantly β2* nAChR currents, and these currents can be inhibited by 1 μM dihydro-β-erythroidine (DHβE) (Alkondon and Albuquerque, 1993; Wooltorton et al., 2003). In the presence of a cocktail of antagonists to inhibit DA transporters (2 μM GBR12909), D_2-like autoreceptors (1 μM sulpiride), and muscarinic receptors (1 μM atropine), bath application of 10 μM cocaine inhibited the nAChR currents (**Figure 2B1**, middle trace). This concentration (10 μM cocaine) is within the range achieved by cocaine abusers and by animals during self-administration experiments (Mittleman and Wetli, 1984; Evans et al., 1996; Ward

et al., 1997; Fowler et al., 1998; Nicola and Deadwyler, 2000). The dose-response curve for cocaine inhibition of nAChR currents from SNc DA neurons had an apparent IC_{50} of 19.8 ± 1.5 μM and Hill coefficient of 1.3 ± 0.3 ($n = 3$–9, **Figure 2B3**), and the results were statistically the same for nAChRs in the VTA (IC_{50} of 19.5 ± 1.8 μM, Hill coefficient of 1.3 ± 0.2). To indicate the effectiveness of cocaine inhibition, the nicotinic antagonist, DHβE, was characterized with an apparent IC_{50} of 82.3 ± 4.3 nM and Hill coefficient of 1.1 ± 0.4 ($n = 3$–7, **Figures 2B2,B3**). These results suggest that cocaine directly inhibits nAChRs on DA neurons and, thus, acts as a weak nicotinic antagonist. Because the predominant β2-containing nAChRs on the cell body and axon terminals appear to be qualitatively similar (Champtiaux et al., 2003; Salminen et al., 2004; Quik and McIntosh, 2006), cocaine's nicotinic antagonism likely also occurs at DA axon terminals. Since the selective DAT inhibitor GBR12909 was also used in this study, we tested the potential effect of GBR12909 on nAChR-mediated currents as a separate control experiment. We found that GBR12909 (5 μM, the highest concentration we used) did not inhibit nAChR currents in 5 DA neurons tested. Since nAChRs facilitate DA release (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhang et al., 2009a; Threlfell et al., 2012), our result on cocaine inhibition of nAChRs leads to this question: can cocaine also affect DA release at DA axon terminals?

COCAINE INHIBITS DA RELEASE EVOKED BY A SINGLE PULSE

Cocaine is known to inhibit DA reuptake with a K_i of about 0.5 μM (Ritz et al., 1987). This effect is also well documented in the FCV literature (e.g., Jones et al., 1995) and readily observed under our current experimental conditions (see **Figures 6A1,B1**), but is not the focus of our present study. Our focus here is to determine if cocaine, at concentrations above what is needed to inhibit DATs, can affect DA release, because at the relatively high concentrations attained by abusers, the previous section suggests that cocaine inhibits nAChRs on DA fibers and terminals that normally regulate DA release. Therefore, we reasoned that cocaine at different concentrations alters the DA signal in qualitatively different manners via inhibition of DATs vs. nAChRs.

To examine cocaine's nAChR-mediated influence over DA signals, we electrically stimulated DA release and monitored the DA concentration using FCV in brain slices containing the striatum. The DA signal was evoked every 100 s in the dorsolateral striatum and was commonly stable for >2 h during our recordings (Zhou et al., 2001; Zhang et al., 2009a). Confounding mechanisms were minimized in all of the experiments by using pharmacological treatments. Sulpiride (1 μM) was used to inhibit DA autoinhibition by D_2-like receptors. SKF83566 (1 μM) was used to inhibit D_1-like receptors to prevent changes in cholinergic tone that affects DA release (Zhou et al., 2001). GBR12909 (1–5 μM), which has an IC_{50} ∼10 nM vs. cocaine's IC_{50} ∼0.5 μM, was used to minimize DA reuptake by DATs. Therefore, pretreatment with GBR12909 (1–5 μM) occludes cocaine's effect on DA reuptake by DATs.

Under these pharmacological conditions (with DATs inhibited), cocaine at concentrations that would normally inhibit DATs (0.1–1 μM) did not enhance the amplitude or duration of the already prolonged DA signal, as is indicated in the dose-response curve (**Figure 3B**, filled symbols). In other words, cocaine no longer enhanced the DA signal because DATs were already inhibited by GBR12909. However, at the same concentrations that inhibited nAChRs (**Figure 2B1**), cocaine (10 μM) and DHβE (25 nM) substantially inhibited DA release evoked by a single-pulse (1 p) stimulus (**Figures 3A,B**). Cocaine inhibited the DA signal with an estimated IC_{50} of 4.3 ± 0.3 μM ($n = 8$) and a Hill coefficient of 3 (**Figure 3B**, filled symbols). The high Hill coefficient is indicative of a cocaine effect on Ca^{2+}-triggered vesicular DA release, which has high cooperativity (Lou et al., 2005). Under the same pharmacological conditions, DA release evoked by 1 p also was inhibited by the nicotinic antagonist DHβE with an apparent IC_{50} of 22.3 ± 1.7 nM and Hill coefficient of 3 (**Figure 3B**, open symbols). The influence of cocaine over the DA release evoked by 1 p was comparable in the dorsolateral striatum (**Figures 3A,B**) and in the NAc shell of the ventral striatum (**Figures 3C,D**).

To verify that cocaine was not acting via DAT inhibition when GBR12909 was present in these experiments, we repeated the experiments using mutant mice possessing DATs that are insensitive to cocaine (Chen et al., 2006) instead of using GBR12909. A similar effect was seen when cocaine (10 μM) was applied while stimulating DA release in the presence of 1 μM sulpiride and 1 μM SKF83566 (**Figure 4A**, dorsolateral striatum; **Figure 4B**, NAc shell). The DA release to a 1 p stimulation was inhibited by cocaine (10 μM), and the dose-response relationships for cocaine inhibition of DA release is shown to the right in **Figure 4**. These results verify that cocaine inhibits DA release separately from its influence over DA reuptake or D2-like receptors.

COCAINE REDUCES DA RELEASE BY INHIBITING nAChRs

The results to this point show that cocaine can decrease DA release evoked by a 1 p stimulus. Cocaine's ability to inhibit nAChRs suggests that the mechanism of action is via nAChRs on DA fibers or terminals. The nAChRs that normally facilitate DA release contain the β2 subunit (Zhou et al., 2001; Salminen et al., 2004; Zhang et al., 2009a; Drenan et al., 2010; Exley et al., 2011). In nAChR β2-subunit KO mice, DA release does not depend on nAChR activation (Zhou et al., 2001; Zhang et al., 2009a). Therefore, if cocaine decreases DA release in response to a 1 p stimulus via nAChR inhibition, then β2-subunit KO mice should not show this cocaine-mediated effect.

In the dorsolateral striatum of β2-subunit KO mice, 1 p stimulation evoked nAChR-independent DA release that had an amplitude 70% smaller than that observed in W-T mice, as has been shown previously (Zhou et al., 2001). The smaller evoke DA signals are observable by comparing **Figure 3A** (left trace compared to the right trace after inhibition of nAChRs) to **Figure 5A** (left trace, note the change in scale). In the presence of GBR12909 (1–5 μM) to inhibit DATs, cocaine doses ranging from 50 nM to 5 μM did not affect the amplitude or duration of the evoked DA signal in β2-subunit KO mice (**Figure 5B**). A concentration of cocaine (10 μM) that caused a 74.9 ± 7.8% decrease in DA release in W-T mice (**Figures 3A,B**), produces only 7.6 ± 2.4% inhibition in nAChR β2 KO mice (**Figures 5A,B**). In W-T mice, cocaine reduced DA release with an IC_{50} of 4.3 μM (**Figure 5B**,

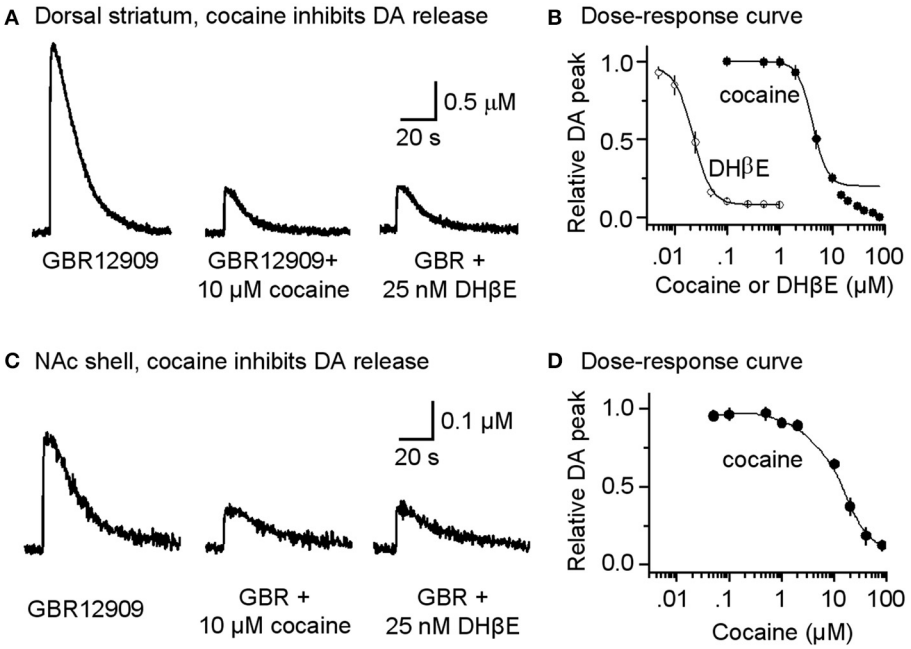

FIGURE 3 | Cocaine reduces DA release in the striatum from W-T mice. **(A)** Cocaine inhibits DA release from the dorsolateral striatum evoked by single-pulse (1 p) stimulation measured using fast-scan cyclic voltammetry. The recordings show the prolonged DA signal after DAT inhibition with 5 μM GBR12909 (left trace). DA release was substantially reduced by 10 μM cocaine (middle trace). After recovery from cocaine inhibition, 25 nM DHβE induced a similar inhibition of the DA signal (right trace). 1 μM sulpiride (D_2-like antagonist) and SKF83566 (D_1-like antagonist) were used to block local interactions in the striatum. **(B)** The dose-response relationships for inhibition of DA release. The curves through the data were produced with $IC_{50} = 4.3\,\mu M$ for cocaine and 22 nM for DHβE both with a Hill coefficient estimated to be 3 ($n = 4$–8 per data point). Data points with cocaine concentrations higher than 15 μM were not included in the fitting because a component arising from a local anesthetic effect was also present. **(C)** Likewise, cocaine inhibits DA release evoked by 1 p stimulation from the NAc shell measured using fast-scan cyclic voltammetry in the presence of 5 μM GBR12909, 1 μM sulpiride, and 1 μM SKF83566. **(D)** The dose-response relationship for inhibition of DA release in the NAc shell.

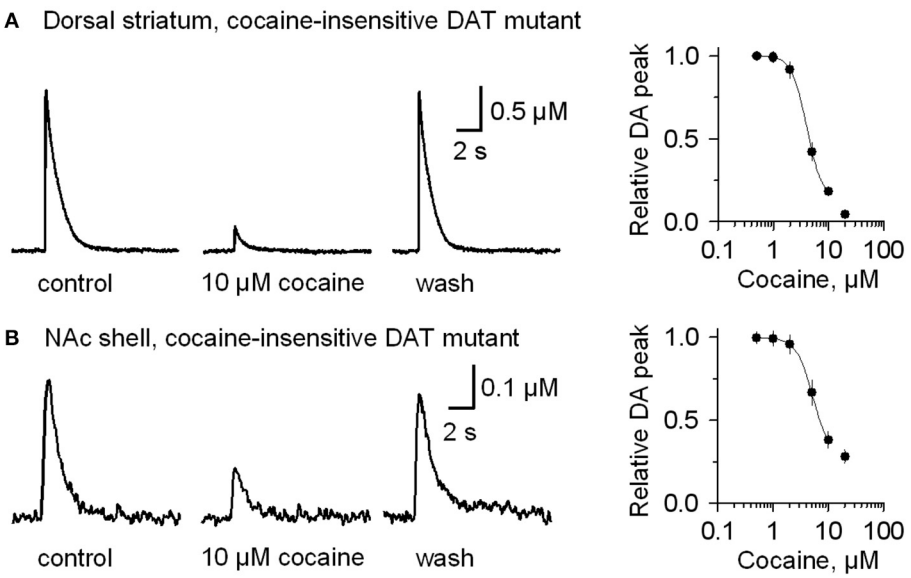

FIGURE 4 | In mutant mice having cocaine-insensitive DATs, DA release evoked by 1 p stimulation was still inhibited by cocaine (10 μM). **(A)** In 10 μM cocaine (middle trace) the DA signal was inhibited when compared to the control (left) or after washout of cocaine (right, wash). The dose-response relationship for cocaine inhibition (extreme right) was fitted with a curve through the data having $IC_{50} = 4\,\mu M$ and Hill coefficient = 3.2. **(B)** Likewise, cocaine (10 μM) inhibits DA release evoked by 1 p stimulation from the NAc shell. The dose-response relationship for inhibition of DA release in the NAc shell by cocaine (extreme right) was fitted by a curve with IC50 = 5.2 μM and the Hill coefficient = 3.0. The traces were collected in the presence of 1 μM sulpiride, and 1 μM SKF83566.

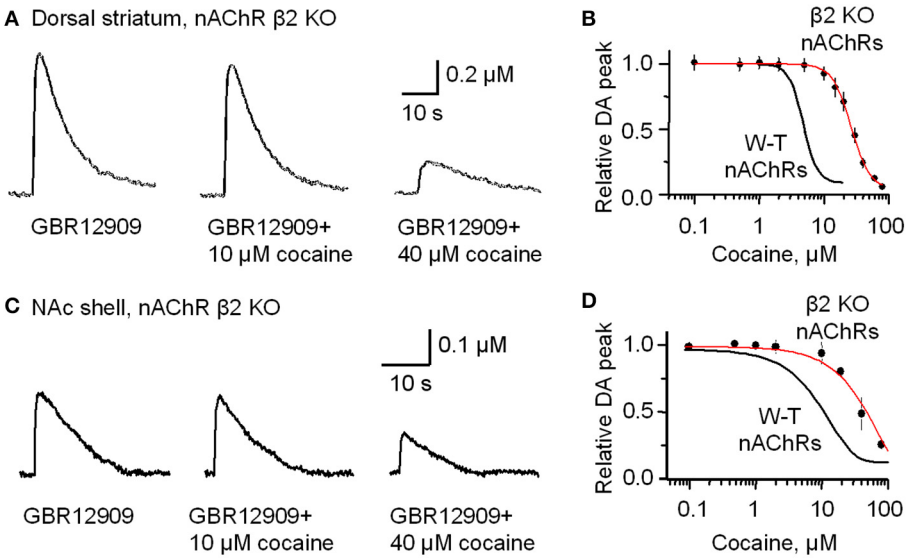

FIGURE 5 | In β2-subunit KO mice DA release evoked by 1 p stimulation was resistant to inhibition by cocaine (10 μM). (A) All the slices were bathed in 2 μM GBR12909 to block DATs and to remove complications arising from changes in DA reuptake. The control DA signal (left) evoked by 1 p stimulation shows a prolonged duration caused by inhibiting DA reuptake. In 10 μM cocaine (middle trace) there was slight inhibition of the DA signal. In 40 μM cocaine (right trace) there was a much greater inhibition of the DA signal. 1 μM sulpiride and SKF83566 were used to block local DA receptor influences within the striatum. **(B)** The dose-response relationship for cocaine inhibition of DA release in the dorsolateral striatum of β2-nAChR KO mice. The curve through the data was produced with $IC_{50} = 26\,\mu M$ and the Hill coefficient = 3 (red curve) ($n = 4$–6 slices). For comparison, the black curve from **Figure 1** obtained with W-T mice is given to show the substantial decrease in cocaine-mediated inhibition when β2* nAChRs were absent. **(C)** Likewise, cocaine (10 μM) did not, but cocaine (40 μM) did, inhibit DA release evoked by 1 p stimulation from the NAc shell again in the presence of 2 μM GBR12909, 1 μM sulpiride, and 1 μM SKF83566. **(D)** The dose-response relationships for inhibition of DA release in the NAc shell by cocaine in β2-subunit KO mice (red curve) ($n = 4$–5 slices) compared to the W-T mice (black curve).

smooth black curve), but in nAChR β2 KO mice the IC_{50} shifts to 26.4 ± 2.3 μM with a Hill coefficient of 3.0 (**Figure 5B**, red curve) ($n = 4$–6 slices for each data points). Comparable results (that may be similarly interpreted) were also obtained in the NAc shell (**Figures 5C,D**) from nAChR β2 KO mice.

For completeness, the whole range of cocaine's influence over the 1 p stimulated DA release is shown in the absence of GBR12909 in W-T mice normally expressing nAChRs in the dorsolateral striatum (**Figure 6A**) or the NAc shell (**Figure 6C**). At low cocaine concentrations, DATs are inhibited by cocaine, and the DA peak becomes larger (DAT effect, **Figures 6A,A1,C**). At cocaine concentrations above 1 μM, the DA peak decreases. As indicated by the earlier results, cocaine begins to inhibit nAChRs at this concentration (nAChR effect, **Figures 6A,C**). When nAChRs are absent (i.e., nAChR β2 KO mice), cocaine does not begin to inhibit the DA peak amplitude until ≥20 μM (**Figures 6B,B1,D**). The overall effect of cocaine acting via nAChRs is depicted in **Figure 6B**. The inhibition of the DA peak by ≥20 μM cocaine likely also involves the local anesthetic effect (Anesthetic effect, **Figure 6**), arising from cocaine inhibition of voltage-activated channels (e.g., sodium and/or calcium channels) and action potentials (O'Leary and Chahine, 2002). We verified that cocaine inhibited the fast sodium current in DA neuron somata in a dose-dependent manner (**Figures 7A,B**) that likely contributed to the decreased DA release seen at very high cocaine concentrations (Anesthetic effect, **Figure 6**). Because DA release depends of the 4th power of the intraterminal calcium concentration that arises from the depolarization caused by voltage-gated ion channels, cocaine's inhibition of voltage-gated channels (e.g., I_{Na} currents in **Figure 7**) is magnified when observing the effect over DA release (**Figure 6**).

COCAINE REDUCES PAIRED-PULSE DEPRESSION OF DA RELEASE DEPENDENT ON nAChRs

The results presented above indicate that cocaine acts as a nAChR antagonist at DA fibers and terminals in the striatum and via this mechanism reduces DA release evoked by a single pulse or low frequency stimulation. This reduction in DA release is, of course, relative to the release that would be seen if cocaine did not slightly inhibit nAChRs. Inhibition of nAChRs also has been shown to reduce paired-pulsed depression of DA release (Rice and Cragg, 2004; Zhang and Sulzer, 2004). Paired-pulse depression largely depends on the initial neurotransmitter release probability. The higher the initial release probability, the stronger the paired-pulse depression (Zucker and Regehr, 2002). Therefore, if cocaine is inhibiting nAChRs, it should decrease the initial DA release probability and reduce paired-pulse depression.

To test this idea, we performed paired-pulse experiments using two stimuli separated by 50 ms (i.e., 20 Hz). The experiments were conducted in the presence of sulpiride (1 μM) to block autoinhibition by D_2-like receptors and SKF83566 (1 μM) to block D_1-like receptors on cholinergic interneurons to prevent changes in cholinergic tone in the striatum. The DA signal was quantified by the area under the curve because the 2 pulse

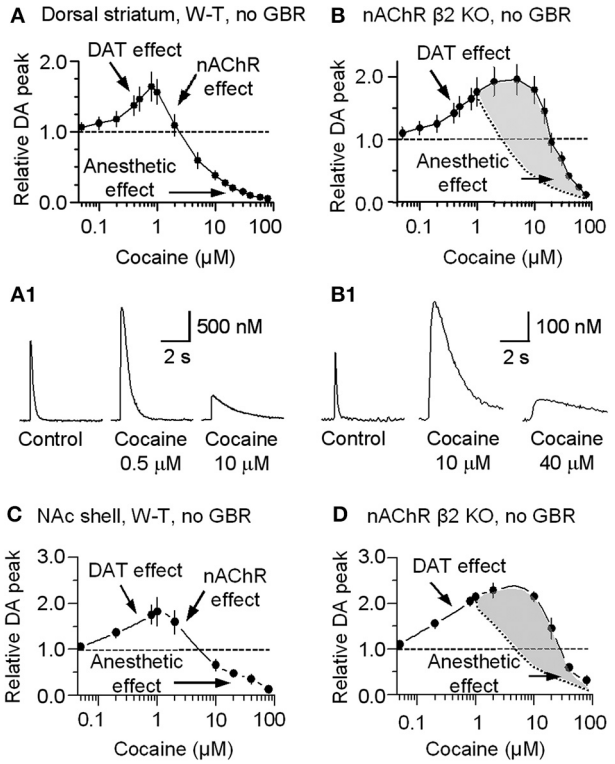

FIGURE 6 | Concentration-dependent multiple cocaine effects on the DA-release signal evoked by 1 p stimulation in the striatum measured by FCV. (A) In W-T mice DA signals were measured in the dorsolateral striatum while we bath applied cocaine ranging from 50 nM to 80 μM in the absence of GBR12909. At 50 nM to 1 μM, cocaine monotonically increased the amplitude and prolonged the duration of the evoked DA signal, as expected from cocaine's known inhibition of DATs (DAT effect). At cocaine concentrations above 1 μM, the DA peak decreased although the duration was further prolonged. This decrease is hypothesized to arise from cocaine inhibition of nAChRs (nAChR effect). **(B)** In β2-nAChR knockout (KO) mice in the absence of GBR12909, the DA signal is not dependent on nAChRs. Note that the mid-range cocaine inhibition of the DA amplitude is absent in these measurements (i.e., the nAChR effect is absent). The difference between cocaine's influences in the absence of β2-nAChRs is shaded in gray with the dotted curve representing the falling phase in **(A)**. **(A1,B1)** Examples of the multiple effects induced by different concentrations of cocaine in WT and in β2-nAChR KO mice. **(C)** In W-T mice, DA signaling in the NAc shell showed qualitatively similar cocaine effects as seen in the dorsal striatum. **(D)** In β2-nAChR KO mice, the DA signal is not dependent on nAChRs. The difference between cocaine's influences in the absence of β2-nAChRs is shaded in gray with the dotted curve representing the falling phase in **(C)**. In **(A–D)**, $n = 4$–7 slices for each data points.

protocol spreads the DA signal in time. The paired-pulse ratio (PPR) was defined as P_2/P_1. In the dorsolateral striatum of W-T mice, DA release displayed strong paired-pulse depression with a PPR of 0.12 ± 0.01, $n = 7$ (**Figures 8A1,A3**), consistent with published reports (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhang et al., 2009a; Cachope et al., 2012; Threlfell et al., 2012). Although a high initial release probability is probably a key factor, the mechanism for this severe depletion of DA release is not established. In the presence of 10 μM cocaine, the PPR increased to 0.43 ± 0.06 ($n = 5$, $p < 0.05$; **Figures 8A1–A3**). Qualitatively similar results were obtained in the NAc shell: PPR was 0.47 ± 0.07, $n = 5$ in the control and 0.65 ± 0.08, $n = 5$ in 10 μM cocaine (not shown). These PPR numbers reflect the lower probability of release under control conditions in the NAc shell compared to the dorsal striatum, which was shown previously (Zhang et al., 2009a).

To examine further whether cocaine's mechanism of action on PPR was via nicotinic antagonism, we repeated the paired-pulse experiment in nAChR β2 KO mice. In the absence of the β2-containing nAChRs, the probability of release decreases especially for P_1 (Zhou et al., 2001). Thus, in β2 KO mice with no cocaine, the PPR was higher than in W-T mice, 0.38 ± 0.05, ($n = 5$, **Figures 8B1,B3**). More importantly, in the absence of functional β2-containing nAChRs, 10 μM cocaine did not significantly affect the PPR consistent with cocaine mechanistically acting via nAChR inhibition: 0.39 ± 0.06 ($n = 5$, **Figures 8B1–B3**). Qualitatively similar results were obtained in the NAc shell: PPR was 0.68 ± 0.06 ($n = 5$) in the β2 KO mice and 0.65 ± 0.06 ($n = 5$) when cocaine (10 μM) was applied (not shown). In the absence of β2-containing nAChRs, cocaine did not influence the PPR.

To further demonstrate that cocaine is altering the PPR via nAChRs not owing to its inhibition of DATs, we repeated the experiments in the presence of GBR12909 (5 μM) to inhibit DATs as a control for cocaine's inhibition of DATs. Just as seen in **Figures 8A,B**, cocaine caused an increase in the PPR when nAChRs are present (**Figures 8C1–C3**) but not in β2 KO mice (**Figures 8D1–D3**).

COCAINE ENHANCES PHASIC RELATIVE TO TONIC DA SIGNALS DEPENDENT ON nAChRs

The earlier results showed that cocaine decreases DA release evoked by a 1 p stimulus. A low release probability can be overcome by the residual Ca^{2+} in axon terminals produced by high frequency stimulation (Abbott and Regehr, 2004). Therefore, we tested whether cocaine favors phasic DA release evoked by high frequency stimulation.

Tonic DA release was evoked by 4 stimulation pulses given at 4 Hz, and phasic DA release was mimicked by 4 pulses given at 20 Hz because rodent DA neurons fire bursting spikes with an intraburst frequency of ∼20 Hz and with 3–5 spikes per burst, and the averaged DA neuron firing rate is about 4 Hz (Hyland et al., 2002; Schultz, 2002; Zhang et al., 2009b). Under control conditions in the dorsolateral striatum, phasic and tonic DA release was very similar (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhang et al., 2009a), with the ratio of phasic to tonic DA release being 1.10 ± 0.05, $n = 10$ (**Figures 9A1,C**). Inhibition of DATs by GBR12909 (up to 5 μM) did not affect the phasic to tonic ratio (**Figures 9A2,C**). In contrast, 10 μM cocaine reduced the tonic DA release more strongly than the phasic DA signal increasing the phasic to tonic DA ratio to 1.38 ± 0.15 ($n = 8$, $p < 0.05$, **Figures 9A3,C**). To test whether cocaine was acting via inhibition of nAChRs to influence the phasic to tonic ratio, we repeated the experiment in nAChR β2 KO mice. Under control conditions in β2 KO mice, the phasic to tonic DA release ratio was 1.58 ± 0.14 ($n = 6$, **Figures 9B1,C**), which is higher than in W-T mice. Equally important, cocaine (up to 10 μM) did not alter

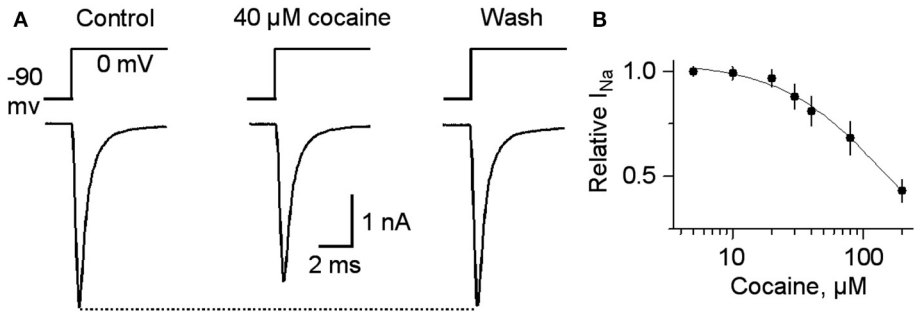

FIGURE 7 | Cocaine at high concentrations inhibits voltage-gated I_{Na}. **(A)** In 40 µM cocaine, I_{Na} was inhibited compared to the control or after cocaine washout. Holding potential was −90 mV and the testing potential was 0 mV for 10 ms given every 20 s. Extracellular and intracellular solutions are described in the Materials and Methods section. **(B)** The dose-response curve for cocaine inhibition of I_{Na} is shown on the right with a curve through the data with an $IC_{50} = 144$ and a Hill coefficient of 1.

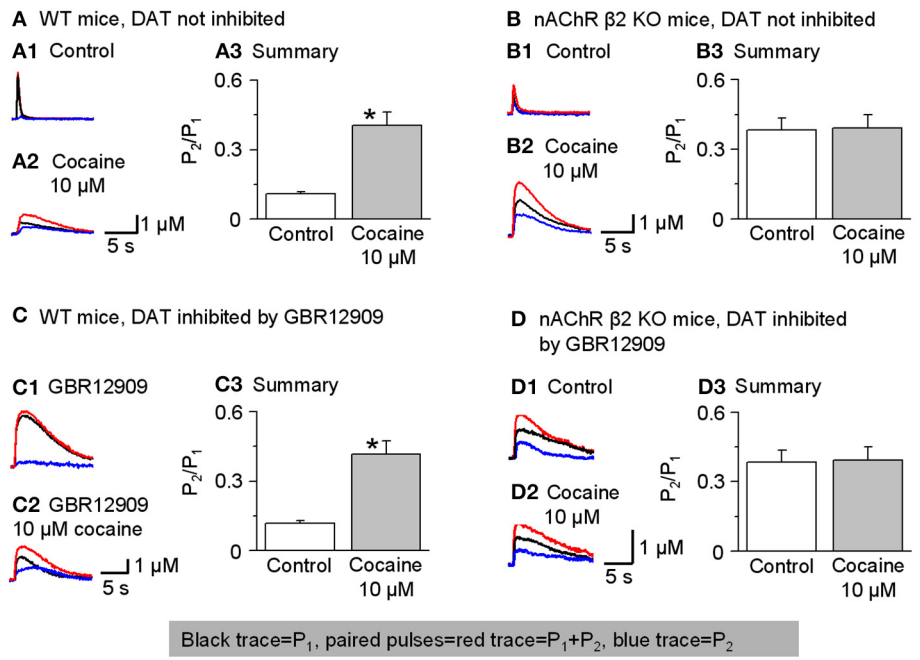

FIGURE 8 | Cocaine reduces paired-pulse depression of DA release in W-T but not in β2-nAChR KO mice. In this figure, P_1 was the 1 stimulus pulse-evoked DA signal shown in black and P_2 (shown in blue) was obtained by subtraction: the two paired pulses-evoked DA (red traces). **(A,A1–A3)** In W-T mice under control conditions (without inhibition of DATs), paired-pulse depression was strong, and the paired-pulse ratio (PPR, defined as P_2/P_1) was small. In 10 µM cocaine, the PPR significantly increased ($n = 6$). 1 µM sulpiride and SKF83566 were used in all these experiments to block local DA receptor interactions within the dorsolateral striatum. **(B,B1–B3)** In β2-nAChR KO mice (without inhibition of DATs), 10 µM cocaine did not affect the PPR ($n = 5$). **(C,C1–C3)** In W-T mice with DAT inhibition by GBR12909 (5 µM) cocaine (10 µM) still enhances the PPR ($n = 5$). **(D,D1–D3)** In β2-nAChR KO mice with inhibition of DATs, 10 µM cocaine did not affect the PPR ($n = 5$). $^*p < 0.01$.

the phasic to tonic DA ratio in β2 KO mice, 1.65 ± 0.17 ($n = 6$, **Figures 9B2,C**). These data support the hypothesis that cocaine acts via inhibition of nAChRs to increase the phasic to tonic DA ratio.

DISCUSSION

The results from our present study indicate that at concentrations achieved by cocaine abusers, cocaine inhibits nAChRs and increases the ratio of phasic to tonic DA release. For example, cocaine (10 µM) decreased DA release evoked by a single, isolated stimulation (1 p) by ∼70%, but that DA signaling loss was partially recovered during a stimulus train (4 p at 20 Hz). Because this relative enhancement of phasic signaling is occurring while cocaine also inhibits DA reuptake (i.e., inhibits DATs), the phasic DA signals are larger during cocaine abuse. Salient, reward-related DA signals arising from phasic DA neuron firing (Grace, 2000) will be highly exaggerated in cocaine concentrations greater than about 2 µM, which are easily achieved by cocaine abusers

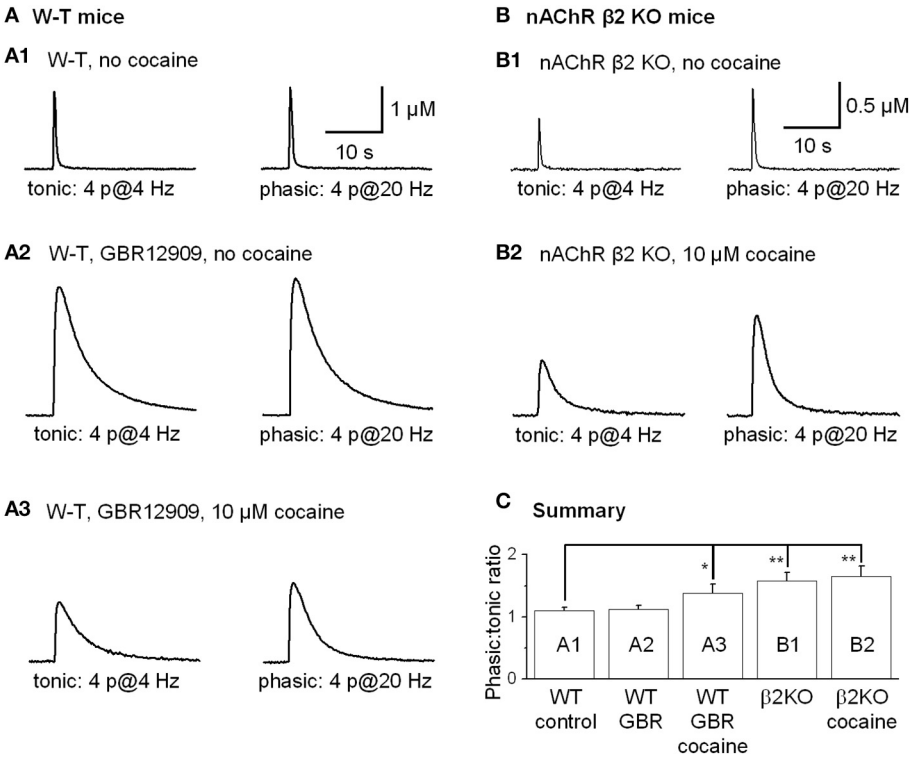

FIGURE 9 | Cocaine increased the phasic to tonic DA ratio in the dorsal striatum of W-T and nAChR KO mice. Sulpiride and SKF83566 both at 1 μM were used to block DA receptor interactions within the striatum. **(A,A1)** The FCV measurements of DA signals evoked by lower frequency tonic stimulation (4 Hz, 4-pulses, left trace) and by phasic stimulation (20 Hz, 4-pulses, right trace) under control conditions had a phasic to tonic DA signal ratio close to 1. **(A,A2)** In 5 μM GBR12909 to inhibit DATs selectively (no cocaine), the DA signals evoked by tonic stimulation (left trace) and by phasic stimulation (right trace) showed a phasic to tonic ratio near 1. GBR12909 prolonged the DA signal duration but did not enhance the phasic to tonic DA ratio. **(A,A3)** In 10 μM cocaine, the DA signals evoked by tonic stimulation (left trace) and phasic stimulation (right trace) showed a larger phasic to tonic ratio. The DA signals were broader because cocaine inhibited DATs and, thus, prolonged the DA signal duration. **(B,B1)** Cocaine did not increase the phasic to tonic DA ratio in the dorsal striatum in β2-nAChR KO Mice. The DA signal evoked by a tonic stimulation (4 Hz, 4-pulses, left trace) and phasic stimulation (20 Hz, 4-pulses, right trace) under control conditions. In β2-nAChR KO mice the phasic to tonic DA signal ratio was larger than 1 and larger than in W-T mice. **(B,B2)** In 10 μM cocaine, the DA signals evoked by a tonic stimulation (left trace) and by a phasic stimulation (right trace) were prolonged, but the ratio of phasic to tonic signal was unchanged compared to the no cocaine condition. Cocaine did not dose-dependently enhance the phasic to tonic DA ratio in β2-nAChR KO mice. Sulpiride and SKF83566 both at 1 μM were used to block DA receptor interactions within the striatum. **(C)** Summary graph showing the PPR under different conditions. $^{*}p < 0.05$, $^{**}p < 0.01$.

(Mittleman and Wetli, 1984; Evans et al., 1996; Ward et al., 1997; Fowler et al., 1998).

It must be kept in mind, however, that the striatal brain slice preparation and the exogenous electrical stimulation of the striatal tissue to evoke DA release is a reduced experimental model system that allows us to examine the different aspects of cocaine's effect on the DA signal in the striatum. As seen in **Figure 6B**, under these experimental conditions, cocaine's influence acting exclusively via nAChRs is mainly to diminish the DA release that would be even larger owing to cocaine's inhibition of DATs. By inhibiting nAChRs, the stimulus evoked DA release is not as large as would be achieved if the nAChRs were not partially inhibited by cocaine (Zhang et al., 2009a). In intact animals, cocaine's effects are more complex. For example, cocaine's inhibition of DA uptake increases the basal extracellular DA level that may activate the inhibitory D2 autoreceptors that in turn reduce DA release (Schmitz et al., 2002), complicating the determination of the physiological functions of the individual receptors and neurotransmitter systems.

NICOTINIC ANTAGONISM BY COCAINE REGULATES DA RELEASE IN THE STRIATUM

The results from this study indicate three different cocaine concentration ranges that induce different antagonist actions (best seen in **Figure 6**). At low concentrations, below 1 μM ($IC_{50} \cong 0.5\,\mu M$) (Ritz et al., 1987), cocaine inhibits DATs and elevates extracellular DA by reducing DA reuptake. This is probably cocaine's most common effect in cocaine abusers for the simple reason that low cocaine concentrations are more common and last longer than the high cocaine peak (Evans et al., 1996; Ward et al., 1997; Fowler et al., 1998). At intermediate concentrations above 2 μM (IC_{50} of 4.3 μM), also commonly achieved by cocaine abusers, cocaine begins to inhibit nAChRs (**Figure 3B**) and, thereby, alters DA signaling via nicotinic mechanisms (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004). At

high concentrations above 10 μM ($IC_{50} \cong 26.4$ μM) that can be reached during a large cocaine dosing, local anesthetic-like effects begin (Mittleman and Wetli, 1984; O'Leary and Chahine, 2002). The mechanism for cocaine's stronger inhibition of DA release than that of voltage-gated Na current see in our data is not known; we speculate that the Na current at the DA axon fibers and terminals may be more sensitive to cocaine; the extensive DA axon fiber bifurcations may also increase the sensitivity of the action potential generation and propagation to cocaine inhibition (Matsuda et al., 2009; Debanne et al., 2011).

Our results show that in striatal slices from W-T mice, cocaine inhibited β2-containing nAChR-dependent DA release (from 1 p stimulation) with an IC_{50} of 4.3 μM (**Figure 3B**). In contrast, a six time greater cocaine concentration is needed to depress 1 p-stimulation DA release from β2-containing nAChR KO mice with an IC_{50} of 26.4 μM (**Figure 5B**). The data indicate that cocaine's inhibition of DA release is mediated by β2 nAChRs on DA fibers and terminals that normally regulate DA release (Zhou et al., 2001; Grady et al., 2002; Rice and Cragg, 2004; Salminen et al., 2004; Zhang and Sulzer, 2004; Zhang et al., 2009a). This conclusion is also supported by our finding that in DA neuron somata, cocaine inhibited the β2* nAChR current (**Figure 2B**), consistent with previous studies of cloned α4β2 nAChRs in expression systems (Damaj et al., 1999; Francis et al., 2000). The inhibition of nAChR currents and DA release was quantitatively mimicked by the selective nAChR antagonist DHβE (**Figure 2B2**). Because cocaine often reaches greater than 2 μM in the brains of abusers (Mittleman and Wetli, 1984; Evans et al., 1996; Ward et al., 1997; Fowler et al., 1998), our results indicate that biologically relevant cocaine levels directly alter DA release.

The observation that weak cocaine inhibition of nAChRs (**Figure 2B**) leads to a greatly amplified inhibition of DA release (**Figure 3A**) likely arises from the site of nAChR action. nAChRs on DA fibers and terminals regulate action potential propagation and presynaptic calcium signals. For example, nAChRs mediate direct and indirect Ca^{2+} elevation within axon terminals (Vernino et al., 1992, 1994; Lena et al., 1993; McGehee et al., 1995; Gray et al., 1996; Rathouz et al., 1996). Neurotransmitter release is related to a high power (≅4th) of intra-terminal Ca^{2+} (Zucker and Regehr, 2002; Lou et al., 2005). Consequently, even a small decrease in the nAChR-initiated presynaptic Ca^{2+} signal or depolarization would induce a larger decrease in DA release. This conclusion is supported by the quantitatively similar data obtained with the specific nAChR inhibitor, DHβE, which decreases nAChR-dependent DA release more strongly than nAChR-mediated currents (**Figures 2B**, **3A**).

A number of recent studies have shown that nAChR activity on DA fibers and terminals regulates the relationship between afferent action potentials and DA release. The nAChRs on DA fibers and terminals increase the initial DA release probability, enhancing tonic DA signals (Grady et al., 2002; Rice and Cragg, 2004; Salminen et al., 2004; Zhang and Sulzer, 2004; Exley and Cragg, 2008; Zhang et al., 2009a). nAChR activation may also directly evoke action potentials in DA axon terminals and thus DA release (Cachope et al., 2012; Threlfell et al., 2012). Inhibiting or desensitizing striatal nAChRs decreases tonic DA release, but phasic DA release arising from stimulus trains is not inhibited. This nicotinic effect increases the ratio of phasic to tonic DA release arising from the biologically complex series of action potentials along DA fibers (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhang et al., 2009a). By inhibiting nAChRs, cocaine influences this nicotinic mechanism that alters the frequency dependence of DA release favoring phasic signals.

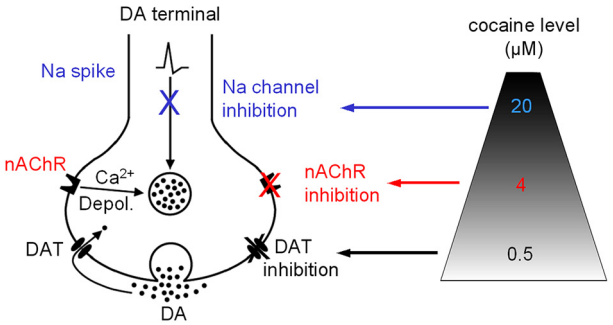

FIGURE 10 | Diagram showing that cocaine may inhibit DAT-mediated DA uptake and DA release via nAChRs and Na channels in a dose-dependent manner. Depol., depolarization.

BIOLOGICAL IMPLICATIONS

Our data indicate that besides inhibiting DAT, the most common effect on the DA system, cocaine, at concentrations (around 4 μM) readily achievable in cocaine abusers (Evans et al., 1996; Ward et al., 1997; Fowler et al., 1998), may also, via inhibiting nAChRs, alter DA release property. On rare occasions when the abuser uses very high doses of cocaine leading to a cocaine level ≥20 μM, cocaine's anesthetic effect may be triggered (**Figure 10**). The functional importance of our data obtained in brain slices is reflected in several behavioral studies showing that nicotinic agonism increases the abusive potential of cocaine whereas inhibition of nAChRs decreases cocaine reinforced behaviors (Reid et al., 1998; Zachariou et al., 2001; Schoffelmeer et al., 2002; Blokhina et al., 2005; Champtiaux et al., 2006; Zanetti et al., 2006). Particularly, pharmacological or genetic inactivation of nAChRs before exposure to cocaine was reported to disrupt place preference to cocaine, whereas low doses of nicotine were able to lower the threshold for cocaine induced place preference, and nAChR β2 KO mice showed decreased cocaine induced place preference (Zachariou et al., 2001). It was also reported that nAChR inhibition by mecamylamine dose dependently suppressed cocaine self-administration (Blokhina et al., 2005). These literature data clearly indicate that nAChRs are involved in cocaine's addictive processes, although the underlying neural mechanisms are likely to be complex. Therefore, it is reasonable to conclude that the mechanistic effects reported in this study contribute to those *in vivo* findings: acting via inhibition of nAChRs, cocaine increases the ratio of phasic to tonic DA release and thus potentially enhances its reinforcing abilities (Goto and Grace, 2005).

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REFERENCES

Abbott, L. F., and Regehr, W. G. (2004). Synaptic computation. *Nature* 431, 796–803. doi: 10.1038/nature03010

Alkondon, M., and Albuquerque, E. X. (1993). Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. *J. Pharmacol. Exp. Ther.* 265, 1455–1473.

Beckstead, M. J., and Williams, J. T. (2007). Long-term depression of a dopamine IPSC. *J. Neurosci.* 27, 2074–2080. doi: 10.1523/JNEUROSCI.3251-06.2007

Blokhina, E. A., Kashkin, V. A., Zvartau, E. E., Danysz, W., and Bespalov, A. Y. (2005). Effects of nicotinic and NMDA receptor channel blockers on intravenous cocaine and nicotine self-administration in mice. *Eur. Neuropsychopharmacol.* 15, 219–225. doi: 10.1016/j.euroneuro.2004.07.005

Bonci, A., Bernardi, G., Grillner, P., and Mercuri, N. B. (2003). The dopamine-containing neuron: maestro or simple musician in the orchestra of addiction? *Trends Pharmacol. Sci.* 24, 172–177. doi: 10.1016/S0165-6147(03)00068-3

Cachope, R., Mateo, Y., Mathur, B. N., Irving, J., Wang, H. L., Morales, M., et al. (2012). Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: setting the tone for reward processing. *Cell Rep.* 2, 33–41. doi: 10.1016/j.celrep.2012.05.011

Champtiaux, N., Gotti, C., Cordero-Erausquin, M., David, D. J., Przybylski, C., Lena, C., et al. (2003). Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. *J. Neurosci.* 23, 7820–7829.

Champtiaux, N., Kalivas, P. W., and Bardo, M. T. (2006). Contribution of dihydro-beta-erythroidine sensitive nicotinic acetylcholine receptors in the ventral tegmental area to cocaine-induced behavioral sensitization in rats. *Behav. Brain Res.* 168, 120–126. doi: 10.1016/j.bbr.2005.10.017

Chen, R., Tilley, M. R., Wei, H., Zhou, F., Zhou, F. M., Ching, S., et al. (2006). Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9333–9338. doi: 10.1073/pnas.0600905103

Damaj, M. I., Slemmer, J. E., Carroll, F. I., and Martin, B. R. (1999). Pharmacological characterization of nicotine's interaction with cocaine and cocaine analogs. *J. Pharmacol. Exp. Ther.* 289, 1229–1236.

Debanne, D., Campanac, E., Bialowas, A., Carlier, E., and Alcaraz, G. (2011). Axon physiology. *Physiol. Rev.* 91, 555–602. doi: 10.1152/physrev.00048.2009

Ding, S., Wei, W., and Zhou, F. M. (2011). Molecular and functional differences in voltage-activated sodium currents between GABA projection neurons and dopamine neurons in the substantia nigra. *J. Neurophysiol.* 106, 3019–3034. doi: 10.1152/jn.00305.2011

Drenan, R. M., Grady, S. R., Steele, A. D., McKinney, S., Patzlaff, N. E., McIntosh, J. M., et al. (2010). Cholinergic modulation of locomotion and striatal dopamine release is mediated by alpha6alpha4* nicotinic acetylcholine receptors. *J. Neurosci.* 30, 9877–9889. doi: 10.1523/JNEUROSCI.2056-10.2010

Evans, S. M., Cone, E. J., and Henningfield, J. E. (1996). Arterial and venous cocaine plasma concentrations in humans: relationship to route of administration, cardiovascular effects and subjective effects. *J. Pharmacol. Exp. Ther.* 279, 1345–1356.

Exley, R., and Cragg, S. J. (2008). Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. *Br. J. Pharmacol.* 153(Suppl. 1), S283–S297. doi: 10.1038/sj.bjp.0707510

Exley, R., Maubourguet, N., David, V., Eddine, R., Evrard, A., Pons, S., et al. (2011). Distinct contributions of nicotinic acetylcholine receptor subunit {alpha}4 and subunit {alpha}6 to the reinforcing effects of nicotine. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7577–7582. doi: 10.1073/pnas.1103000108

Ford, C. P., Mark, G. P., and Williams, J. T. (2006). Properties and opioid inhibition of mesolimbic dopamine neurons vary according to target location. *J. Neurosci.* 26, 2788–2797. doi: 10.1523/JNEUROSCI.4331-05.2006

Fowler, J. S., Volkow, N. D., Logan, J., Gatley, S. J., Pappas, N., King, P., et al. (1998). Measuring dopamine transporter occupancy by cocaine *in vivo*: radiotracer considerations. *Synapse* 28, 111–116. doi: 10.1002/(SICI)1098-2396(199802)28:2<111::AID-SYN1>3.0.CO;2-E

Francis, M. M., Vazquez, R. W., Papke, R. L., and Oswald, R. E. (2000). Subtype-selective inhibition of neuronal nicotinic acetylcholine receptors by cocaine is determined by the alpha4 and beta4 subunits. *Mol. Pharmacol.* 58, 109–119.

Goto, Y., and Grace, A. A. (2005). Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nat. Neurosci.* 8, 805–812. doi: 10.1038/nn1471

Gould, E., Woolf, N. J., and Butcher, L. L. (1989). Cholinergic projections to the substantia nigra from the pedunculopontine and laterodorsal tegmental nuclei. *Neuroscience* 28, 611–623.

Grace, A. A. (2000). The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. *Addiction* 95(Suppl. 2), S119–S128. doi: 10.1046/j.1360-0443.95.8s2.1.x

Grady, S. R., Murphy, K. L., Cao, J., Marks, M. J., McIntosh, J. M., and Collins, A. C. (2002). Characterization of nicotinic agonist-induced [(3)H]dopamine release from synaptosomes prepared from four mouse brain regions. *J. Pharmacol. Exp. Ther.* 301, 651–660. doi: 10.1124/jpet.301.2.651

Gray, R., Rajan, A. S., Radcliffe, K. A., Yakehiro, M., and Dani, J. A. (1996). Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature* 383, 713–716. doi: 10.1038/383713a0

Hyland, B. I., Reynolds, J. N., Hay, J., Perk, C. G., and Miller, R. (2002). Firing modes of midbrain dopamine cells in the freely moving rat. *Neuroscience* 114, 475–492. doi: 10.1016/S0306-4522(02)00267-1

Hyman, S. E., Malenka, R. C., and Nestler, E. J. (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu. Rev. Neurosci.* 29, 565–598. doi: 10.1146/annurev.neuro.29.051605.113009

Jones, I. W., Bolam, J. P., and Wonnacott, S. (2001). Presynaptic localisation of the nicotinic acetylcholine receptor beta2 subunit immunoreactivity in rat nigrostriatal dopaminergic neurones. *J. Comp. Neurol.* 439, 235–247. doi: 10.1002/cne.1345

Jones, S. R., Garris, P. A., Kilts, C. D., and Wightman, R. M. (1995). Comparison of dopamine uptake in the basolateral amygdaloid nucleus, caudate-putamen, and nucleus accumbens of the rat. *J. Neurochem.* 64, 2581–2589. doi: 10.1046/j.1471-4159.1995.64062581.x

Lena, C., Changeux, J. P., and Mulle, C. (1993). Evidence for "preterminal" nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. *J. Neurosci.* 13, 2680–2688.

Li, W., Doyon, W. M., and Dani, J. A. (2011). Acute *in vivo* nicotine administration enhances synchrony among dopamine neurons. *Biochem. Pharmacol.* 82, 977–983. doi: 10.1016/j.bcp.2011.06.006

Lou, X., Scheuss, V., and Schneggenburger, R. (2005). Allosteric modulation of the presynaptic Ca^{2+} sensor for vesicle fusion. *Nature* 435, 497–501. doi: 10.1038/nature03568

Mansvelder, H. D., and McGehee, D. S. (2000). Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* 27, 349–357. doi: 10.1016/S0896-6273(00)00042-8

Matsuda, W., Furuta, T., Nakamura, K. C., Hioki, H., Fujiyama, F., Arai, R., et al. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *J. Neurosci.* 29, 444–453. doi: 10.1523/JNEUROSCI.4029-08.2009

McGehee, D. S., Heath, M. J., Gelber, S., Devay, P., and Role, L. W. (1995). Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science* 269, 1692–1696. doi: 10.1126/science.7569895

Mittleman, R. E., and Wetli, C. V. (1984). Death caused by recreational cocaine use. An update. *JAMA* 252, 1889–1893.

Nelson, A. B., Hammack, N., Yang, C. F., Shah, N. M., Seal, R. P., and Kreitzer, A. C. (2014). Striatal cholinergic interneurons drive GABA release from dopamine terminals. *Neuron* 82, 63–70. doi: 10.1016/j.neuron.2014.01.023

Neuhoff, H., Neu, A., Liss, B., and Roeper, J. (2002). I(h) channels contribute to the different functional properties of identified dopaminergic subpopulations in the midbrain. *J. Neurosci.* 22, 1290–1302.

Nicola, S. M., and Deadwyler, S. A. (2000). Firing rate of nucleus accumbens neurons is dopamine-dependent and reflects the timing of cocaine-seeking behavior in rats on a progressive ratio schedule of reinforcement. *J. Neurosci.* 20, 5526–5537.

Oakman, S. A., Faris, P. L., Kerr, P. E., Cozzari, C., and Hartman, B. K. (1995). Distribution of pontomesencephalic cholinergic neurons projecting to substantia nigra differs significantly from those projecting to ventral tegmental area. *J. Neurosci.* 15, 5859–5869.

O'Leary, M. E., and Chahine, M. (2002). Cocaine binds to a common site on open and inactivated human heart (Na(v)1.5) sodium channels. *J. Physiol. (Lond.)* 541, 701–716. doi: 10.1113/jphysiol.2001.016139

Picciotto, M. R., Zoli, M., Lena, C., Bessis, A., Lallemand, Y., Le Novere, N., et al. (1995). Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. *Nature* 374, 65–67. doi: 10.1038/374065a0

Pidoplichko, V. I., and Dani, J. A. (2005). Applying small quantities of multiple compounds to defined locations of *in vitro* brain slices. *J. Neurosci. Methods* 142, 55–66. doi: 10.1016/j.jneumeth.2004.07.012

Pidoplichko, V. I., De Biasi, M., Williams, J. T., and Dani, J. A. (1997). Nicotine activates and desensitizes midbrain dopamine neurons. *Nature* 390, 401–404. doi: 10.1038/37120

Pristupa, Z. B., Wilson, J. M., Hoffman, B. J., Kish, S. J., and Niznik, H. B. (1994). Pharmacological heterogeneity of the cloned and native human dopamine transporter: disassociation of [3H]WIN 35,428 and [3H]GBR 12,935 binding. *Mol. Pharmacol.* 45, 125–135.

Quik, M., and McIntosh, J. M. (2006). Striatal alpha6* nicotinic acetylcholine receptors: potential targets for parkinson's disease therapy. *J. Pharmacol. Exp. Ther.* 316, 481–489. doi: 10.1124/jpet.105.094375

Rathouz, M. M., Vijayaraghavan, S., and Berg, D. K. (1996). Elevation of intracellular calcium levels in neurons by nicotinic acetylcholine receptors. *Mol. Neurobiol.* 12, 117–131. doi: 10.1007/BF02740649

Reid, M. S., Mickalian, J. D., Delucchi, K. L., Hall, S. M., and Berger, S. P. (1998). An acute dose of nicotine enhances cue-induced cocaine craving. *Drug Alcohol Depend.* 49, 95–104. doi: 10.1016/S0376-8716(97)00144-0

Rice, M. E., and Cragg, S. J. (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nat. Neurosci.* 7, 583–584. doi: 10.1038/nn1244

Ritz, M. C., Lamb, R. J., Goldberg, S. R., and Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237, 1219–1223. doi: 10.1126/science.2820058

Salminen, O., Murphy, K. L., McIntosh, J. M., Drago, J., Marks, M. J., Collins, A. C., et al. (2004). Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol. Pharmacol.* 65, 1526–1535. doi: 10.1124/mol.65.6.1526

Schmitz, Y., Schmauss, C., and Sulzer, D. (2002). Altered dopamine release and uptake kinetics in mice lacking D2 receptors. *J. Neurosci.* 22, 8002–8009.

Schoffelmeer, A. N., De Vries, T. J., Wardeh, G., Van De Ven, H. W., and Vanderschuren, L. J. (2002). Psychostimulant-induced behavioral sensitization depends on nicotinic receptor activation. *J. Neurosci.* 22, 3269–3276.

Schultz, W. (2002). Getting formal with dopamine and reward. *Neuron* 36, 241–263. doi: 10.1016/S0896-6273(02)00967-4

Threlfell, S., Lalic, T., Platt, N. J., Jennings, K. A., Deisseroth, K., and Cragg, S. J. (2012). Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. *Neuron* 75, 58–64. doi: 10.1016/j.neuron.2012.04.038

Vernino, S., Amador, M., Luetje, C. W., Patrick, J., and Dani, J. A. (1992). Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. *Neuron* 8, 127–134. doi: 10.1016/0896-6273(92)90114-S

Vernino, S., Rogers, M., Radcliffe, K. A., and Dani, J. A. (1994). Quantitative measurement of calcium flux through muscle and neuronal nicotinic acetylcholine receptors. *J. Neurosci.* 14, 5514–5524.

Ward, A. S., Haney, M., Fischman, M. W., and Foltin, R. W. (1997). Binge cocaine self-administration in humans: intravenous cocaine. *Psychopharmacology (Berl.)* 132, 375–381. doi: 10.1007/s002130050358

Wise, R. A. (2004). Dopamine, learning and motivation. *Nat. Rev. Neurosci.* 5, 483–494. doi: 10.1038/nrn1406

Woolf, N. J., and Butcher, L. L. (1981). Cholinergic neurons in the caudate-putamen complex proper are intrinsically organized: a combined Evans blue and acetylcholinesterase analysis. *Brain Res. Bull.* 7, 487–507.

Woolf, N. J., and Butcher, L. L. (1986). Cholinergic systems in the rat brain: III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia, and basal forebrain. *Brain Res. Bull.* 16, 603–637.

Wooltorton, J. R., Pidoplichko, V. I., Broide, R. S., and Dani, J. A. (2003). Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. *J. Neurosci.* 23, 3176–3185.

Xu, W., Orr-Urtreger, A., Nigro, F., Gelber, S., Sutcliffe, C. B., Armstrong, D., et al. (1999). Multiorgan autonomic dysfunction in mice lacking the beta2 and the beta4 subunits of neuronal nicotinic acetylcholine receptors. *J. Neurosci.* 19, 9298–9305.

Zachariou, V., Caldarone, B. J., Weathers-Lowin, A., George, T. P., Elsworth, J. D., Roth, R. H., et al. (2001). Nicotine receptor inactivation decreases sensitivity to cocaine. *Neuropsychopharmacology* 24, 576–589. doi: 10.1016/S0893-133X(00)00224-4

Zanetti, L., De Kerchove D'exaerde, A., Zanardi, A., Changeux, J. P., Picciotto, M. R., and Zoli, M. (2006). Inhibition of both alpha7* and beta2* nicotinic acetylcholine receptors is necessary to prevent development of sensitization to cocaine-elicited increases in extracellular dopamine levels in the ventral striatum. *Psychopharmacology (Berl.)* 187, 181–188. doi: 10.1007/s00213-006-0419-y

Zhang, H., and Sulzer, D. (2004). Frequency-dependent modulation of dopamine release by nicotine. *Nat. Neurosci.* 7, 581–582. doi: 10.1038/nn1243

Zhang, L., Doyon, W. M., Clark, J. J., Phillips, P. E., and Dani, J. A. (2009a). Controls of tonic and phasic dopamine transmission in the dorsal and ventral striatum. *Mol. Pharmacol.* 76, 396–404. doi: 10.1124/mol.109.056317

Zhang, T., Zhang, L., Liang, Y., Siapas, A. G., Zhou, F. M., and Dani, J. A. (2009b). Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine. *J. Neurosci.* 29, 4035–4043. doi: 10.1523/JNEUROSCI.0261-09.2009

Zhang, T. A., Placzek, A. N., and Dani, J. A. (2010). *In vitro* identification and electrophysiological characterization of dopamine neurons in the ventral tegmental area. *Neuropharmacology* 59, 431–436. doi: 10.1016/j.neuropharm.2010.06.004

Zhou, F. M., Liang, Y., and Dani, J. A. (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nat. Neurosci.* 4, 1224–1229. doi: 10.1038/nn769

Zucker, R. S., and Regehr, W. G. (2002). Short-term synaptic plasticity. *Annu. Rev. Physiol.* 64, 355–405. doi: 10.1146/annurev.physiol.64.092501.114547

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Oxotremorine treatment reduces repetitive behaviors in BTBR T+ tf/J mice

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Repetitive behaviors with restricted interests is one of the core criteria for the diagnosis of autism spectrum disorder (ASD). Current pharmacotherapies that target the dopaminergic or serotonergic systems have limited effectiveness in treating repetitive behaviors. Previous research has demonstrated that administration of muscarinic cholinergic receptor (mAChR) antagonists can exacerbate motor stereotypies while mAChR agonists reduce stereotypies. The present study determined whether the mAChR agonist, oxotremorine affected repetitive behaviors in the BTBR T+ tf/J (BTBR) mouse model of autism. To test the effects of oxotremorine on repetitive behaviors, marble burying and grooming behavior were measured in BTBR mice and compared to that in C57BL/6J (B6) mice. The effects of oxotremorine on locomotor activity was also measured. Thirty minutes before each test, mice received an intraperitoneal (ip) injection of saline, 0.001 mg or 0.01 mg of oxotremorine methiodide. Saline- treated BTBR mice exhibited increased marble burying and self-grooming behavior compared to that of saline-treated B6 mice. Oxotremorine significantly reduced marble burying and self-grooming behavior in BTBR mice, but had no significant effect in B6 mice. In addition, oxotremorine did not affect locomotor activity in BTBR mice, but significantly reduced locomotor activity in B6 mice at the 0.01 mg dose. These findings demonstrate that activation of mAChRs reduces repetitive behavior in the BTBR mouse and suggest that treatment with a mAChR agonist may be effective in reducing repetitive behaviors in ASD.

Keywords: acetylcholine, muscarinic receptors, autism, repetitive behaviors, marble burying, grooming

INTRODUCTION

Autism spectrum disorders (ASDs) represent a cluster of neurodevelopmental disorders characterized by social and communicative impairments, as well restricted interests and repetitive behaviors (RRBs). RRBs are subdivided into lower-order and high-order behaviors (Lam and Aman, 2007). Lower order RRBs involve repetitive manipulation of objects, stereotyped movements or repetitive self-injurious behavior (Lam and Aman, 2007). Higher order RRBs are characterized by an insistence on sameness, or rigid adherence to a rule or routine (Turner, 1999; Szatmari et al., 2006). RRBs are reported to be the most distressing aspect of ASD for patients and families that profoundly impact daily living (Bishop et al., 2007).

Current treatments for RRBs have limited effectiveness (Boyd et al., 2012). Most pharmacotherapies in ASD focus on treating symptoms by principally modifying dopaminergic and serotonergic signaling (McPheeters et al., 2011). Atypical antipsychotics have food and drug administration (FDA) indications for treating irritability but not the core features of ASD (McPheeters et al., 2011). Selective serotonin reuptake inhibitor (SSRI) medications, used to reduce restricted interest symptoms, have resulted in mixed improvements with irritability observed in some individuals (Hollander et al., 2005; Owley et al., 2005, 2010; Henry et al., 2006; King et al., 2009). An alternative possibility is to treat RRBs by targeting the cholinergic system. Accumulating evidence suggests that brain cholinergic abnormalities could explain some of the pathophysiology in ASD. Post-mortem studies indicate that there is altered expression of muscarinic and nicotinic cholinergic receptors (Perry et al., 2001; Deutsch et al., 2010). Recent gene networks that confer risk for ASD include genes related to cholinergic transmission and these are also highly expressed in the brain (Voineagu et al., 2011; Ben-David and Shifman, 2012; Lee et al., 2012). Moreover, anti-psychotic treatments which have significant muscarinic receptor antagonism, e.g., quetiapine, can exacerbate symptoms in ASD (Martin et al., 1999; Hardan et al., 2005). Thus, treatments that increase muscarinic cholinergic receptor (mAChR) transmission may reduce core symptoms in ASD.

Animal models are often an important initial step in evaluating new treatment approaches. The BTBR T+ tf/J (BTBR) mouse is one preclinical model employed to better understand ASD because the mouse exhibits a phenotype that is comparable to the core symptoms in ASD (see Meyza et al., 2013 for review). Compared to B6 mice, BTBR mice exhibit deficits in social interactions and communication (McFarlane et al., 2008; Scattoni et al., 2008, 2011; Pobbe et al., 2010; Silverman et al., 2010;

Chadman, 2011); restricted interests and behavioral inflexibility (Moy et al., 2008; Pearson et al., 2011; Amodeo et al., 2012; Karvat and Kimchi, 2012; Guariglia and Chadman, 2013); as well as repetitive or stereotyped behaviors, e.g., increased self-grooming and marble burying (McFarlane et al., 2008; Silverman et al., 2010; Amodeo et al., 2012; Babineau et al., 2013; McTighe et al., 2013; Reynolds et al., 2013). Grooming and digging can be viewed as part of the common behavioral repertoire exhibited by rodents (Garner and Mason, 2002). Studying these behaviors in BTBR mice is of particular interest related to ASD because of the excessive quantity in which these behaviors are expressed (Yang et al., 2007, 2009; McFarlane et al., 2008; Pobbe et al., 2010; Pearson et al., 2011; Amodeo et al., 2012), as well as being exhibited in various contexts and with repeated testing (Yang et al., 2007, 2009; McFarlane et al., 2008; Pobbe et al., 2010). For example, Yang et al. (2007) found that BTBR mice showed higher levels of repetitive grooming than B6 mice when raised with either a biological BTBR mother, a foster BTBR mother, or a low grooming B6 mother, demonstrating that excessive self-grooming in BTBR mice is not attenuated by an environmental influence such as caregiver.

In addition, recent findings indicate that BTBR mice exhibit decreased brain acetylcholine levels (McTighe et al., 2013) and infusion of an acetylcholinestase inhibitor into the dorsomedial striatum can alleviate a reversal learning deficit in BTBR mice (Karvat and Kimchi, 2014). However, unknown is whether cholinergic treatments may also be effective in reducing repetitive motor behaviors in BTBR mice. A past study reported that treatment with a mAChR agonist can reduce stereotyped behaviors in rodents (Wang and McGinty, 1997). Moreover, post-mortem studies report reduced mAChR signaling in various brain regions of ASD patients (Deutsch et al., 2010). Thus, treatment with a mAChR agonist may be effective in reducing repetitive behaviors in ASD.

To determine whether treatment with a mAChR agonist reduces repetitive behaviors, the present experiments examined whether the mAChR agonist, oxotremorine methoidine alleviates elevated self-grooming and marble burying in BTBR mice compared to that in B6 mice. To understand whether oxotremorine treatment has a more general effect on motor behavior, the effect of oxotremorine on horizontal locomotor activity was also measured in BTBR and B6 mice.

MATERIALS AND METHODS

ANIMALS

Male C57BL/6J and BTBR mice, 7–8 weeks old, were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were singly housed in plastic cages (28 cm wide × 17 cm long × 12 cm high) in humidity (30%) and temperature (22°C) controlled room with a 12-h light/dark cycle (lights on at 07:00 am). Ten to fourteen days after arrival behavioral testing procedures began. Animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and was approved by the Institutional Laboratory Animal Care and Use Committee at the University of Illinois at Chicago.

DRUGS

Oxotremorine methoidine (Tocris, Ellisville, MO) 0.001 and 0.01 mg/kg was dissolved in 0.9% physiological saline. Mice received an intraperitoneal (ip) injection at 10 ml/kg volume.

SPONTANEOUS SELF-GROOMING

The procedure used to measure spontaneous self-grooming behavior was modified from McFarlane et al. (2008). Mice were individually placed in a clear plastic cage (28 cm wide × 17 cm long × 12 cm high) for a total of 20 min. Twenty minutes before being placed in the plastic cage mice received an ip injection of either vehicle, 0.001 or 0.01 mg/kg of oxotremorine. The treatment groups included the following: B6-vehicle (n = 8), B6-0.001oxotremorine (n = 8), B6-0.01oxotremorine (n = 8), BTBR-vehicle (n = 9), BTBR-0.001oxotremorine (n = 9), BTBR-0.01oxotremorine (n = 9). These doses were chosen based on past studies measuring the effects of oxotremorine on activity in rodents (Yano et al., 2009; Koda et al., 2011). The plastic cage was placed in a room separate from the mouse housing room. Subjects were allowed to freely explore the cage for the entirety of the test. The first 10 min served as a habituation period. Therefore mice received injections 30 min prior to measurement of grooming behavior. During the second 10 min of testing a trained observer sat approximately 1.6 m from the test cage and recorded cumulative time spent grooming all body regions in real time with a stopwatch. Grooming behavior included head washing, body grooming, genital/tail grooming and paw and leg licking. Experimenters were blind to treatment but were not blind to strain because BTBR mice are dark brown with a cream colored ventral patch while B6 do not have this patch. After each mouse was tested, the cage was thoroughly cleaned with a 2% ammonium chloride solution.

MARBLE BURYING

Subjects tested for grooming behavior were also tested for marble burying. The marble burying test occurred 8 days following the grooming test to ensure there were no potential residual effects from the initial drug treatment (Birdsall et al., 1978). Subjects received a different treatment before marble burying from that administered during the spontaneous self-grooming experiment, with the exception of three B6 mice. One B6 received vehicle in both tests and two B6 mice received oxotremorine 0.01 mg in both tests. For all other mice, approximately half from each treatment group in the self-grooming test were assigned to one of the other two treatment groups. For example, for mice receiving 0.001 mg oxotremorine in the self-grooming test, approximately half were assigned to the vehicle group and half assigned to the 0.01 mg oxotremorine group. With this experimental design, half of the mice in the oxotremorine treatment groups (low or high dose) were receiving the drug for the first time. Seven days after the grooming test, mice were habituated to the plastic container used for the marble burying test. The same marble burying test procedure was used as in Amodeo et al. (2012). Mice were individually placed in a plastic container (46 cm long by 24 cm wide by 21 cm deep) with 3 cm of clean woodchip bedding (Northeastern Products, NY). The plastic container was placed in a room used for behavioral testing. Mice were allowed to

freely explore a container for 30 min undisturbed. This served to habituate mice to the chamber. Twenty-four hours later, 20 glass marbles (1.5 cm in diameter) were arranged in five rows of four. The marbles were placed on top of 3 cm of clean woodchip bedding. A template was used to ensure that there was a consistent positioning of marbles. Thirty minutes before being placed into the test container, mice received an injection of either vehicle, 0.001, or 0.01 mg of oxotremorine in 0.9% physiological saline. The treatment groups included the following: B6-vehicle (n = 8), B6-0.001oxo (n = 8), B6-0.01oxo (n = 8), BTBR-vehicle (n = 9), BTBR-0.001oxo (n = 9), BTBR-0.01oxo (n = 9). As in the grooming test, experimenters were blind to treatment but were not blind to strain. Once a mouse was placed into the test container a wire lid was placed on top. Mice were allowed to explore the container and marbles for 30 min. After 30 min, each mouse was removed from the testing container and returned to their home cage. Marbles were considered buried if $\geq$2/3 of the surface area was covered in woodchip bedding. The total number of buried marbles was recorded. Between testing, marbles were thoroughly cleaned and new bedding was used for each mouse.

LOCOMOTOR ACTIVITY

A separate group of naïve mice were used to measure locomotor activity. Testing of locomotor activity was conducted in a black acrylic rectangular-shaped chamber (76 cm long × 50 cm wide × 30 cm high). Mice were injected with vehicle, 0.001 or 0.01 mg of oxotremorine 30 min before being placed in the test chamber. Before mice were introduced to the testing chamber, the entire apparatus was cleaned with 2% ammonium chloride solution. Treatment groups included the following: B6-vehicle (n = 8), B6-0.001oxo (n = 8), B6-0.01oxo (n = 8), BTBR-vehicle (n = 8), BTBR-0.001oxo (n = 8), BTBR-0.01oxo (n = 8). The bottom of the chamber was divided into nine (25 × 16.5 cm) equally sized rectangles. After a mouse was placed into the chamber the experimenter exited the testing room for 20 min. Once the session ended mice were removed from the test chamber and returned to the vivarium. Locomotor activity was recorded via camcorder (Sony Handycam, model DCR-DVD650) stationed above the chamber. Once the testing session was complete, locomotor activity was measured by an observer blind to treatment conditions. The number of lines crossed was calculated. A line cross was defined as a mouse having all four paws cross a line. The number of lines crossed was calculated in two separate 10-min blocks.

STATISTICAL ANALYSIS

Separate two-way analysis of variance ANOVAs (*strain*: B6, BTBR × *treatment*: vehicle, 0.001, 0.01 mg/kg oxotremorine) were conducted for self-grooming and marble burying. A significant interaction was followed by Tukey HSD *post hoc* tests to determine significant treatment differences in both strains. A three-way ANOVA with repeated measures (*strain* × *treatment* × *block*) was conducted for locomotor activity. A significant interaction was followed by Tukey HSD *post hoc* tests.

RESULTS

SPONTANEOUS SELF-GROOMING

Figure 1 illustrates the findings for spontaneous self-grooming in BTBR and B6 mice. Vehicle-treated BTBR mice spent approximately 180 s grooming compared to 20 s in B6 mice. Oxotremorine decreased self-grooming behavior in BTBR mice with the largest effect at the 0.01 mg dose which reduced the time self-grooming to half that observed in vehicle-treated BTBR mice. In contrast, oxotremorine treatment tended to increase self-grooming behavior in B6 mice. The main effect of strain was significant ($F_{(1,45)} = 48.26$, $p < 0.01$), but there was no significant treatment effect ($F_{(2,45)} = 1.78$, $p > 0.05$). However there was a significant strain × treatment interaction ($F_{(2,45)} = 6.99$, $p < 0.01$). *Post hoc* tests indicated that in the vehicle-treated groups,

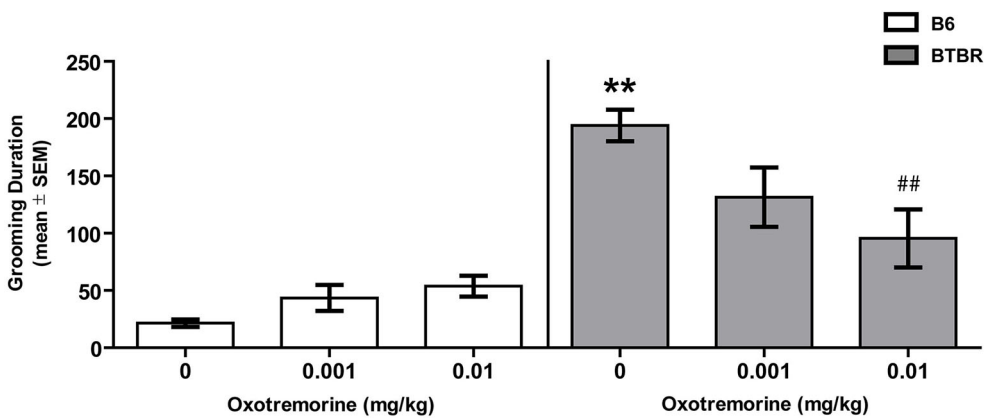

FIGURE 1 | Oxotremorine treatment attenuates spontaneous self-grooming behavior in BTBR mice. Mean (±SEM) time spent grooming all body regions. Self-grooming behavior was measured in BTBR and B6 mice. Each mouse received an i.p. injection of vehicle, 0.001 or 0.01 mg of oxotremorine 30 min before grooming behavior was measured. BTBR mice spent significantly more time grooming compared to that of B6 mice. Vehicle or oxotremorine treatment did not affect spontaneous grooming behavior in B6 mice. Oxotremorine at 0.01 mg significantly decreased spontaneous grooming in BTBR mice. B6: vehicle (n = 8), 0.001 (n = 8), 0.01 (n = 8), BTBR: vehicle (n = 9), 0.001 oxo (n = 9), 0.01 oxo (n = 9). ** $p < 0.01$ vs. B6-vehicle, ## $p < 0.01$ vs. BTBR-vehicle.

BTBR mice spent significantly more time self-grooming than that of B6 mice ($p < 0.01$). Oxotremorine 0.001 treatment in BTBR mice reduced self-grooming time, but the difference was not significantly different from that of vehicle-treated BTBR mice ($p > 0.05$). In contrast, oxotremorine 0.01 treatment significantly reduced self-grooming time compared to that of vehicle treatment in BTBR mice ($p < 0.01$). In B6 mice, oxotremorine treatment tended to increase in self-grooming time, but self-grooming time for both doses compared to that of vehicle treatment was not significant (p's > 0.05). Thus, oxotremorine treatment reduced self-grooming behavior in BTBR mice in a dose-dependent fashion.

MARBLE BURYING

The effects of oxotremorine treatment on marble burying behavior in BTBR and B6 mice are shown in **Figure 2**. Vehicle-treated BTBR buried approximately 10 marbles compared to 3 in B6 mice. Oxotremorine dose-dependently decreased marbles buried in BTBR mice. The oxotremorine 0.001 mg dose reduced marbles buried to approximately seven. The higher dose of oxotremorine reduce marble burying to approximately three. There was a significant main effect for strain ($F_{(1,42)} = 35.87$, $p < 0.01$) and treatment ($F_{(2,42)} = 13.12$, $p < 0.01$). Similarly, there was a significant strain × treatment interaction, ($F_{(2,42)} = 3.92$, $p < 0.05$). *Post hoc* tests indicated that in the vehicle-treated groups, BTBR mice buried significantly more marbles than that of B6 mice ($p < 0.01$). In BTBR mice, oxotremorine 0.001 mg treatment reduced marble burying, but the difference was not significantly different from that of vehicle treatment ($p > 0.05$). In contrast, oxotremorine 0.01 mg treatment significantly reduced marble burying compared to that of vehicle treatment in BTBR mice ($p < 0.01$). In B6 mice, there was a trend for oxotremorine treatment to reduce marble burying, although neither dose compared to that of vehicle treatment was significant (p's > 0.05). Thus, oxotremorine treatment reduced marble burying in BTBR mice in a dose-dependent manner.

One possibility is that the prior treatment received in the self-grooming test interacted with the treatment received in the marble burying test to affect performance. As each treatment group in marble burying included mice that received a mixture of treatments this could be examined within each treatment group. In both the low-dose and high-dose oxotremorine group, BTBR mice that previously received vehicle treatment compared to mice that previously received the drug exhibited comparable marble burying performance. Specifically, in the oxotremorine 0.001 mg group, mice that previously received vehicle had a mean marble burying score of 6.75 ± 2.7 SEM while mice that previously received the drug had a mean score of 7.75 ± 1.3. In the oxotremorine 0.01 mg group, previous vehicle treatment led to a mean score of 3.0 ± 1.5 while previous drug treatment led to a mean score of 2.5 ± 0.9. In the vehicle-treated group, mice that previously received the low dose of oxotremorine had a mean score of 11.0 ± 1.3 while mice that previously received the high dose of oxotremorine had a mean score of 10.75 ± 0.6. Thus, previous treatment in the self-grooming test did not affect performance in the vehicle-treated group or drug groups during the marble burying test.

A similar pattern was observed for B6 mice. In the oxotremorine 0.001 mg group, mice that previously received vehicle had a mean marble burying score of 2.33 ± 0.58 SEM while mice that previously received the drug had a mean score of 2.25 ± 1.44. In the oxotremorine 0.01 mg group, previous vehicle treatment led to a mean score of 1.0 ± 0.41 while previous drug treatment led to a mean score of 1.33 ± 1.15. In the vehicle-treated group, mice that previously received the low dose of oxotremorine had a mean score of 3.25 ± 0.53 while mice that previously received the high dose of oxotremorine had a mean score of 3.67 ± 0.38.

LOCOMOTOR ACTIVITY

Figures 3A,B illustrates the findings for locomotor activity in B6 and BTBR mice, respectively. The locomotor activity was analyzed

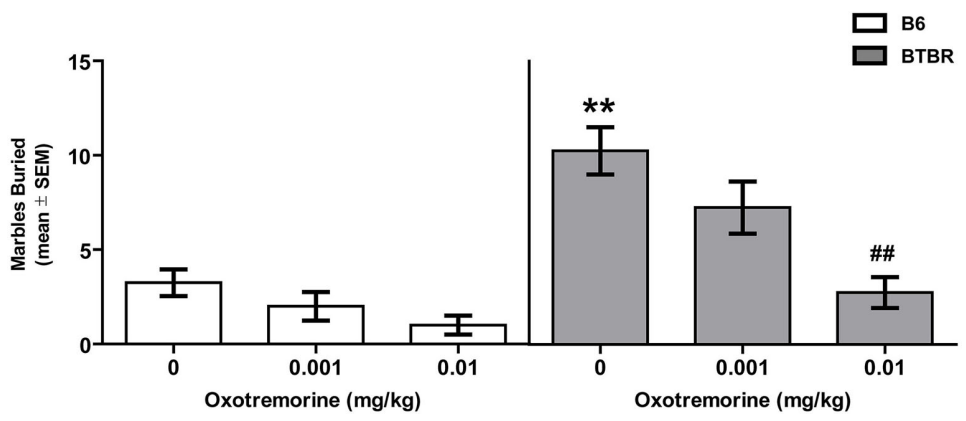

FIGURE 2 | Oxotremorine treatment attenuates marble burying in BTBR mice. Mean (±SEM) marbles buried. Marble burying was measured in BTBR and B6 mice. Each mouse received an i.p. injection of vehicle, 0.001 or 0.01 mg of oxotremorine 30 min prior to marble exposure. BTBR mice buried significantly more marbles compared to that of B6 mice. Vehicle or oxotremorine treatment did not significantly affect marble burying in B6 mice. Oxotremorine at 0.01 mg significantly decreased marble burying in BTBR mice. B6: vehicle ($n = 9$), 0.001 oxo ($n = 8$), 0.01 oxo ($n = 8$), BTBR: vehicle ($n = 9$), 0.001 oxo ($n = 9$), 0.01 oxo ($n = 9$). ** $p < 0.01$ vs. B6-vehicle, ## $p < 0.01$ vs. BTBR vehicle.

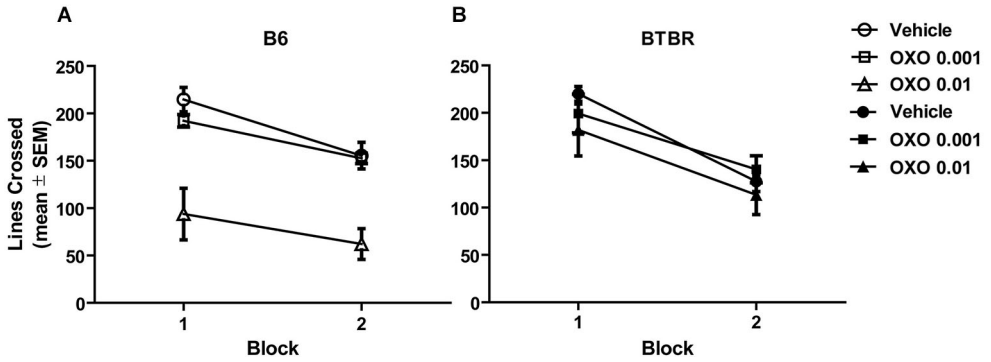

FIGURE 3 | Oxotremorine treatment did not affect locomotor activity in BTBR mice across two consecutive 10-min blocks. Mean (±SEM) lines crossed. Locomotor activity was measured in B6 and BTBR mice. Each mouse received an i.p. injection of vehicle, 0.001 or 0.01 mg of oxotremorine 30 min before locomotor activity was measured. **(A)** Locomotor activity in B6 mice. Activity decreased in the second 10-min block compared to the first. Oxotremorine 0.01 mg significantly reduced locomotor activity compared to that of other treatment groups. B6: vehicle (n = 8), 0.001 oxo (n = 8), 0.01 oxo (n = 8). **(B)** Locomotor activity in BTBR mice. Activity decreased in the second 10-min block compared to the first. Oxotremorine treatment did not affect activity compared to that of vehicle treatment. BTBR: vehicle (n = 8), 0.001 oxo (n = 8), 0.01 oxo (n = 8).

across two 10 min blocks. All groups exhibited similar locomotor activity, with the exception of oxotremorine 0.01 mg in B6 mice. There was a significant effect of treatment ($F_{(2,42)} = 18.82$, $p < 0.001$), but there was no significant strain effect ($F_{(1,42)} = 2.65$, $p > 0.05$). However, the strain × treatment interaction was significant ($F_{(2,42)} = 8.31$, $p < 0.01$). *Post hoc* analysis revealed that in B6 mice, the oxotremorine 0.01 mg treatment significantly lowered activity compared to that of all other treatment groups (p's < 0.05). The analysis further revealed that there was a significant effect for block ($F_{(1,40)} = 110.01$, $p < 0.001$), reflecting that mice decreased their activity in the second block compared to the first block. There was also a significant block × strain interaction ($F_{(1,40)} = 5.88$, $p < 0.05$). *Post hoc* tests revealed that block 2 activity in B6 mice was significantly reduced compared to block 1 activity in B6 and BTBR mice (p's < 0.05). In addition, block 2 activity was significantly reduced compared to block 1 activity in BTBR mice ($p < 0.05$). No other interactions were significant.

DISCUSSION

Individuals with ASD exhibit repetitive, stereotyped behaviors and cognitive inflexibility that can severely limit daily living (Bishop et al., 2007; Lam and Aman, 2007; D'Cruz et al., 2013). Comparable to that observed in ASD, BTBR mice exhibited increased repetitive behaviors compared to that of B6 mice. The increased repetitive behaviors in BTBR mice included both elevated self-grooming and marble burying as observed in past studies (Yang et al., 2007; Silverman et al., 2010; Gould et al., 2011; Pearson et al., 2011; Amodeo et al., 2012). Because past studies in rats indicated that mAChR antagonists increase stereotyped behavior while mAChR agonists reduce stereotyped behavior (Wang and McGinty, 1997; Laviolette et al., 2000; Aliane et al., 2011), these studies investigated whether treatment with the non-specific, mAChR agonist, oxotremorine reduced repetitive behaviors in BTBR mice. Acute oxotremorine treatment, dose-dependently, attenuated the elevated self-grooming and marble-burying behavior in BTBR mice. These findings suggest that activation of mAChR can attenuate certain repetitive behaviors.

A past study examined the self-grooming microstructure in BTBR mice (Pearson et al., 2011). This analysis showed that BTBR mice exhibit an increase in almost all grooming subtypes with the exception of paw licking. Examination of the self-grooming microstructure also revealed that BTBR mice display a decrease in the percentage of incorrect transitions across the different grooming subtypes. Mice commonly groom in a cephalocaudal fashion starting with head washing and concluding with tail/genital licking. The present study did not examine the self-grooming microstructure or the grooming sequence. Thus, unknown is whether oxotremorine preferentially affected grooming subtypes or broadly decreased grooming subtypes. Further, unclear from the present study is whether oxotremorine altered the grooming sequence in BTBR mice in any way. Future studies investigating the effects of mAChR treatment on repetitive behaviors can address how mAChR agonists may alter self-grooming subtypes and self-grooming sequence. However, the present results indicate that oxotremorine treatment decreases self-grooming duration in BTBR mice without the highest dose of oxotremorine having an effect on locomotor activity. Taken together, the results suggest that mAChR agonist treatment may be effective in reducing lower-order repetitive behaviors in ASD.

In contrast to BTBR mice, B6 mice exhibited minimal grooming behavior as reported previously (Yang et al., 2007; McFarlane et al., 2008; Silverman et al., 2010; Pearson et al., 2011; Amodeo et al., 2012). Oxotremorine treatment actually showed a trend toward increasing grooming behavior in B6 mice. This increase in grooming behavior may explain why an oxotremorine injection in B6 mice tended to decrease marble burying and locomotor activity. The opposite effects of oxotremorine on grooming behavior in BTBR and B6 mice may suggest that there is an inverted U-shaped curve for mAChR activation to minimize grooming behavior. In particular, B6 mice may typically exhibit the "optimal level" of mAChR activity, but when treated with a mAChR agonist, i.e.,

oxotremorine, this increases mAChR activation above the optimal levels leading to increased grooming. Conversely, BTBR mice may have lower levels of mAChR activation leading to increased grooming, but treatment with oxotremorine brings mAChR activity into the optimal range that then decreases grooming behavior. Therefore, either too little or too great mAChR activation may lead to increased grooming behavior.

Comparable to that observed with self-grooming, oxotremorine 0.01 mg significantly reduced marble burying in BTBR mice. In B6 mice, there was a trend for oxotremorine 0.01 mg to reduce marble burying. Relative to BTBR mice, B6 mice display low levels of marble burying. The lower level of marble burying in B6 mice is consistent with previous studies (Amodeo et al., 2012; Schwartzer et al., 2013). However, because B6 mice exhibit a low level of marble burying this may obscure a drug effect in reducing marble burying. Another potential issue in interpreting the marble burying results is that mice were tested on marble burying following a self-grooming test. One possibility is that a previous treatment in the self-grooming test affected marble burying behavior. However, examination of the previous treatment received indicated that this did not influence marble burying behavior. This was the case for both BTBR mice and B6 mice. Also worth noting is that the number of marbles buried by vehicle-treated BTBR mice was comparable to that buried by drug-naïve BTBR mice in previous studies (Gould et al., 2011, 2012; Amodeo et al., 2012; Schwartzer et al., 2013). Thus, despite vehicle-treated BTBR mice in the marble burying test receiving either the low or high dose of oxotremorine in the self-grooming test, this did not alter their marble burying behavior compared to past observations in BTBR mice. Again, because oxotremorine treatment had no effect on locomotor activity in BTBR mice, the drug-induced reduction in marble burying behavior can not be explained by a more general reduction in activity. Instead, the results suggest that activation of mAChRs selectively modulated repetitive behaviors in BTBR mice.

The current findings complements a recent study that demonstrated treatment with the acetylcholinesterase inhibitor, donepezil, can improve behavioral rigidity as measured by reversal learning in BTBR mice (Karvat and Kimchi, 2014). Because acetylcholinesterase inhibitors leads to a non-specific increase in acetylcholine levels unknown is whether specific cholinergic receptors mediate these behavioral effects. The current experiments investigating the effects of oxotremorine demonstrate that activation of mAChRs is sufficient to attenuate repetitive behaviors in BTBR mice. Although stimulation of mAChRs was able to attenuate repetitive behaviors, this does not rule out that nicotinic cholinergic receptors may also play a role in affecting repetitive behaviors. Nicotine treatment in rats has shown to reduce certain stereotyped or repetitive behaviors (Zarrindast et al., 1999; Tizabi et al., 2002). Moreover, oxotremorine is a non-specific mAChR agonist, therefore still to be determined is whether specific mAChR subtypes may be sufficient to alleviate repetitive behaviors.

Previous studies investigated the effects of a M1 mAChR agonist on drug-induced or spontaneous grooming behavior in rodents (Bhattacharya and Sen, 1991; Inan et al., 2011). In both studies, McN-A-343 significantly reduced grooming behavior. Unclear is whether other muscarinic receptor subtypes may also be sufficient to reduce repetitive behaviors. There is evidence that targeting M5 mAChR can affect locomotion (Wang et al., 2004; Steidl and Yeomans, 2009), but unknown is whether this is restricted to general ambulation or also to motor stereotyped behavior. Repetitive behaviors in ASD have been separated into lower-order and higher-order repetitive behaviors (Bodfish et al., 2000; Lam and Aman, 2007). Lower order repetitive behaviors can include stereotyped movements or repetitive self-injurious behavior. Higher order RRBs instead are characterized by an "insistence on sameness" or rigid adherence to a rule or routine (Lam and Aman, 2007; Boyd et al., 2012). The findings with McN-343 suggest that treatment with a M1 mAChR agonist may be effective in treating lower-order repetitive behaviors in ASD. However, a recent study reported that the partial M1 mAChR agonist, CDD-102A, enhances set-shifting in rats (Ragozzino et al., 2012). Thus, treatment with a M1 mAChR agonist may be effective in treating both lower-order and higher-order repetitive behaviors.

The present studies indicated that a systemic injection of oxotremorine reduced repetitive behaviors in BTBR mice. The dorsomedial striatum may be a key anatomical site in which oxotremorine acts to affect repetitive behaviors. This is because drug treatments that increase stereotyped behaviors decrease acetylcholine output from this region (Aliane et al., 2011). Furthermore, destruction of cholinergic interneurons or injection of a mAChR antagonist in the dorsomedial striatum leads to increased repetitive behaviors that is alleviated by drug treatments that increase dorsomedial striatal acetylcholine output (Aliane et al., 2011). Cholinergic signaling in the dorsomedial striatum may not only be important for minimizing repetitive motor behaviors, but also for enabling cognitive flexibility. Karvat and Kimchi (2014) showed that donepezil injections into the dorsomedial striatum also improved reversal learning in BTBR mice. This effect of donepezil is consistent with past results showing that enhancing acetylcholine efflux in the rat dorsomedial striatum improves reversal learning while blocking acetylcholine efflux in this region impairs reversal learning (Palencia and Ragozzino, 2006; Ragozzino et al., 2009). Moreover, recent findings suggest that activation of M1 mAChRs in the dorsomedial striatum may mediate acetylcholine effects on cognitive flexibility (Tzavos et al., 2004; McCool et al., 2008; Ragozzino et al., 2012). Thus, treatment with a mAChR agonist may be effective in alleviating stereotyped motor behaviors and cognitive flexibility deficits.

To date, there exists some evidence, but not extensive findings, suggesting altered brain cholinergic transmission in ASD. In particular, there are results from gene networks that confer risk of ASD that include genes related to cholinergic transmission (Voineagu et al., 2011; Ben-David and Shifman, 2012; Lee et al., 2012) and post-mortem studies indicating reduced brain mAChR expression in ASD individuals (Deutsch et al., 2010). However, there is not a definitive understanding of whether pathophysiology of the brain cholinergic system exists in ASD. Related, unknown is whether there are specific brain cholinergic systems that are altered in the disorder and/or if a brain acetylcholine pathophysiology exists and how it may relate to

particular symptoms in ASD. Addressing these issues can further our understanding of the etiology of ASD and help develop new effective therapeutics. The employment of animal models can help address these issues. The present findings in the BTBR mouse, a model of idiopathic autism, reveal that the non-specific mAChR agonist, oxotremorine attenuates repetitive motor behaviors without affecting general ambulation. Thus, treatment with a mAChR agonist may be effective in reducing repetitive behaviors in ASD.

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REFERENCES

Aliane, V., Pérez, S., Bohren, Y., Deniau, J. M., and Kemel, M. L. (2011). Key role of striatal cholinergic interneurons in processes leading to arrest of motor stereotypies. *Brain* 134, 110–118. doi: 10.1093/brain/awq285

Amodeo, D. A., Jones, J. H., Sweeney, J. A., and Ragozzino, M. E. (2012). Differences in BTBR T+ tf/J and C57BL/6J mice on probabilistic reversal learning and stereotyped behaviors. *Behav. Brain Res.* 227, 64–72. doi: 10.1016/j.bbr.2011.10.032

Babineau, B. A., Yang, M., Berman, R. F., and Crawley, J. N. (2013). Low home cage social behaviors in BTBR T+ tf/J mice during juvenile development. *Physiol. Behav.* 114, 49–54. doi: 10.1016/j.physbeh.2013.03.006

Ben-David, E., and Shifman, S. (2012). Networks of neuronal genes affected by common and rare variants in autism spectrum disorders. *PLoS Genet.* 8:e1002556. doi: 10.1371/journal.pgen.1002556

Bhattacharya, S. K., and Sen, A. P. (1991). Effects of muscarinic receptor agonists and antagonists on dopamine-mediated behavioural paradigms. *J. Neural Transm. Gen. Sect.* 84, 241–246. doi: 10.1007/bf01244975

Birdsall, N. J., Burgen, A. S., and Hulme, E. C. (1978). The binding of agonists to brain muscarinic receptors. *Mol. Pharmacol.* 14, 723–736.

Bishop, S. L., Richler, J., Cain, A. C., and Lord, C. (2007). Predictors of perceived negative impact in mothers of children with autism spectrum disorder. *Am. J. Ment. Retard.* 112, 450–461. doi: 10.1352/0895-8017(2007)112[450:popnii]2.0.co;2

Bodfish, J. W., Symons, F. J., Parker, D. E., and Lewis, M. H. (2000). Varieties of repetitive behavior in autism: comparisons to mental retardation. *J. Autism Dev. Disord.* 30, 237–243. doi: 10.1023/A:1005596502855

Boyd, B. A., McDonough, S. G., and Bodfish, J. W. (2012). Evidence-based behavioral interventions for repetitive behaviors in autism. *J. Autism Dev. Disord.* 42, 1236–1248. doi: 10.1007/s10803-011-1284-z

Chadman, K. K. (2011). Fluoxetine but not risperidone increases sociability in the BTBR mouse model of autism. *Pharmacol. Biochem. Behav.* 97, 586–594. doi: 10.1016/j.pbb.2010.09.012

D'Cruz, A. M., Ragozzino, M. E., Mosconi, M. W., Shrestha, S., Cook, E. H., and Sweeney, J. A. (2013). Reduced behavioral flexibility in autism spectrum disorders. *Neuropsychology* 27, 152–160. doi: 10.1037/a0031721

Deutsch, S. I., Urbano, M. R., Neumann, S. A., Burket, J. A., and Katz, E. (2010). Cholinergic abnormalities in autism: is there a rationale for selective nicotinic agonist interventions? *Clin. Neuropharmacol.* 33, 114–120. doi: 10.1097/wnf.0b013e3181d6f7ad

Garner, J. P., and Mason, G. J. (2002). Evidence for a relationship between cage stereotypies and behavioural disinhibition in laboratory rodents. *Behav. Brain Res.* 136, 83–92. doi: 10.1016/s0166-4328(02)00111-0

Gould, G. G., Hensler, J. G., Burke, T. F., Benno, R. H., Onaivi, E. S., and Daws, L. C. (2011). Density and function of central serotonin (5-HT) transporters, 5-HT1A and 5-HT2A receptors and effects of their targeting on, BTBR T+tf/J mouse social behavior. *J. Neurochem.* 116, 291–303. doi: 10.1111/j.1471-4159.2010.07104.x

Gould, G. G., Seillier, A., Weiss, G., Giuffrida, A., Burke, T. F., Hensler, J. G., et al. (2012). Acetaminophen differentially enhances social behavior and cortical cannabinoid levels in inbred mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 38, 260–269. doi: 10.1016/j.pnpbp.2012.04.011

Guariglia, S. R., and Chadman, K. K. (2013). Water T-maze: a useful assay for determination of repetitive behaviors in mice. *J. Neurosci. Methods* 220, 24–29. doi: 10.1016/j.jneumeth.2013.08.019

Hardan, A. Y., Jou, R. J., and Handen, B. L. (2005). Retrospective study of quetiapine in children and adolescents with pervasive developmental disorders. *J. Autism Dev. Disord.* 35, 387–391. doi: 10.1007/s10803-005-3306-1

Henry, C. A., Steingard, R., Venter, J., Guptill, J., Halpern, E. F., and Bauman, M. (2006). Treatment outcome and outcome associations in children with pervasive developmental disorders treated with selective serotonin reuptake inhibitors: a chart review. *J. Child Adolesc. Psychopharmacol.* 16, 187–195. doi: 10.1089/cap.2006.16.187

Hollander, E., Phillips, A., Chaplin, W., Zagursky, K., Novotny, S., Wasserman, S., et al. (2005). A placebo controlled crossover trial of liquid fluoxetine on repetitive behaviors in childhood and adolescent autism. *Neuropsychopharmacology* 30, 582–589. doi: 10.1038/sj.npp.1300627

Inan, S., Dun, N. J., and Cowan, A. (2011). Investigation of gastrin-releasing peptide as a mediator for 5′-guanidinonaltrindole-induced compulsive scratching in mice. *Peptides* 32, 286–292. doi: 10.1016/j.peptides.2010.11.022

Karvat, G., and Kimchi, T. (2012). Systematic autistic-like behavioral phenotyping of 4 mouse strains using a novel wheel-running assay. *Behav. Brain Res.* 233, 405–414. doi: 10.1016/j.bbr.2012.05.028

Karvat, G., and Kimchi, T. (2014). Acetylcholine elevation relieves cognitive rigidity and social deficiency in a mouse model of autism. *Neuropsychopharmacology* 39, 831–840. doi: 10.1038/npp.2013.274

King, B. H., Hollander, E., Sikich, L., McCracken, J. T., Scahill, L., Bregman, J. D., et al. (2009). Lack of efficacy of citalopram in children with autism spectrum disorders and high levels of repetitive behavior: citalopram ineffective in children with autism. *Arch. Gen. Psychiatry* 66, 583–590. doi: 10.1001/archgenpsychiatry.2009.30

Koda, K., Ago, Y., Yano, K., Nishimura, M., Kobayashi, H., Fukada, A., et al. (2011). Involvement of decreased muscarinic receptor function in prepulse inhibition deficits in mice reared in social isolation. *Br. J. Pharmacol.* 162, 763–772. doi: 10.1111/j.1476-5381.2010.01080.x

Lam, K. S. L., and Aman, M. G. (2007). The repetitive behavior scale—revised: independent validation in individuals with autism spectrum disorders. *J. Autism Dev. Disord.* 37, 855–866. doi: 10.1007/s10803-006-0213-z

Laviolette, S. R., Priebe, R. P., and Yeomans, J. S. (2000). Role of the laterodorsal tegmental nucleus in scopolamine- and amphetamine-induced locomotion and stereotypy. *Pharmacol. Biochem. Behav.* 65, 163–174. doi: 10.1016/s0091-3057(99)00195-1

Lee, T. L., Raygada, M. J., and Rennert, O. M. (2012). Integrative gene network analysis provides novel regulatory relationships, genetic contributions and susceptible targets in autism spectrum disorders. *Gene* 496, 88–96. doi: 10.1016/j.gene.2012.01.020

Martin, A., Koenig, K., Scahill, L., and Bregman, J. (1999). Open-label quetiapine in the treatment of children and adolescents with autistic disorder. *J. Child Adolesc. Psychopharmacol.* 9, 99–107. doi: 10.1089/cap.1999.9.99

McCool, M. F., Patel, S., Talati, R., and Ragozzino, M. E. (2008). Differential involvement of M1-type and M4-type muscarinic cholinergic receptors in the dorsomedial striatum in task switching. *Neurobiol. Learn. Mem.* 89, 114–124. doi: 10.1016/j.nlm.2007.06.005

McFarlane, H. G., Kusek, G. K., Yang, M., Phoenix, J. L., Bolivar, V. J., and Crawley, J. N. (2008). Autism-like behavioral phenotypes in, BTBR T+tf/J mice. *Genes Brain Behav.* 7, 152–163. doi: 10.1111/j.1601-183x.2007.00330.x

McPheeters, M. L., Warren, Z., Sathe, N., Bruzek, J. L., Krishnaswami, S., Jerome, R. N., et al. (2011). A systematic review of medical treatments for children with autism spectrum disorders. *Pediatrics* 127, e1312–e1321. doi: 10.1542/peds.2011-0427

McTighe, S. M., Neal, S. J., Lin, Q., Hughes, Z. A., and Smith, D. G. (2013). The BTBR mouse model of autism spectrum disorders has learning and attentional impairments and alterations in acetylcholine and kynurenic acid in prefrontal cortex. *PLoS One* 8:e62189. doi: 10.1371/journal.pone.0062189

Meyza, K. Z., Defensor, E. B., Jensen, A. L., Corley, M. J., Pearson, B. L., Pobbe, R. L., et al. (2013). The BTBR T+ tf/J mouse model for autism spectrum disorders-in search of biomarkers. *Behav. Brain Res.* 251, 25–34. doi: 10.1016/j.bbr.2012.07.021

Moy, S. S., Nadler, J. J., Poe, M. D., Nonneman, R. J., Young, N. B., Koller, B. H., et al. (2008). Development of a mouse test for repetitive, restricted behaviors: relevance to autism. *Behav. Brain Res.* 188, 178–194. doi: 10.1016/j.bbr.2007.10.029

Owley, T., Brune, C. W., Salt, J., Walton, L., Guter, S., Ayuyao, N., et al. (2010). A pharmacogenetic study of escitalopram in autism spectrum disorders. *Autism Res.* 3, 1–7. doi: 10.1002/aur.109

Owley, T., Walton, L., Salt, J., Guter, S. J. Jr., Winnega, M., Leventhal, B. L., et al. (2005). An open-label trial of escitalopram in pervasive developmental disorders. *J. Am. Acad. Child Adolesc. Psychiatry* 44, 343–348. doi: 10.1097/01.chi.0000153229.80215.a0

Palencia, C. A., and Ragozzino, M. E. (2006). The effect of N-methyl-D-aspartate receptor blockade on acetylcholine efflux in the dorsomedial striatum during response reversal learning. *Neuroscience* 143, 671–678. doi: 10.1016/j.neuroscience.2006.08.024

Pearson, B. L., Pobbe, R. L. H., Defensor, E. B., Oasay, L., Bolivar, V. J., Blanchard, D. C., et al. (2011). Motor and cognitive stereotypies in the BTBR T+tf/J mouse model of autism. *Genes Brain Behav.* 10, 228–235. doi: 10.1111/j.1601-183x.2010.00659.x

Perry, E. K., Lee, M. L., Martin-Ruiz, C. M., Court, J. A., Volsen, S. G., Merrit, J., et al. (2001). Cholinergic activity in autism: abnormalities in the cerebral cortex and basal forebrain. *Am. J. Psychiatry* 158, 1058–1066. doi: 10.1176/appi.ajp.158.7.1058

Pobbe, R. L. H., Pearson, B. L., Defensor, E. B., Bolivar, V. J., Blanchard, D. C., and Blanchard, R. J. (2010). Expression of social behaviors of C57BL/6J versus BTBR inbred mouse strains in the visible burrow system. *Behav. Brain Res.* 214, 443–449. doi: 10.1016/j.bbr.2010.06.025

Ragozzino, M. E., Artis, S., Singh, A., Twose, T. M., Beck, J. E., and Messer, W. S. Jr. (2012). The selective M1 muscarinic cholinergic agonist CDD-0102A enhances working memory and cognitive flexibility. *J. Pharmacol. Exp. Ther.* 340, 588–594. doi: 10.1124/jpet.111.187625

Ragozzino, M. E., Mohler, E. G., Prior, M., Palencia, C. A., and Rozman, S. (2009). Acetylcholine activity in selective striatal regions supports behavioral flexibility. *Neurobiol. Learn. Mem.* 91, 13–22. doi: 10.1016/j.nlm.2008.09.008

Reynolds, S., Urruela, M., and Devine, D. P. (2013). Effects of environmental enrichment on repetitive behaviors in the BTBR T+ tf/J mouse model of autism. *Autism Res.* 6, 337–343. doi: 10.1002/aur.1298

Scattoni, M. L., Gandhy, S. U., Ricceri, L., and Crawley, J. N. (2008). Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. *PLoS One* 3:e3067. doi: 10.1371/journal.pone.0003067

Scattoni, M. L., Ricceri, L., and Crawley, J. N. (2011). Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. *Genes Brain Behav.* 10, 44–56. doi: 10.1111/j.1601-183x.2010.00623.x

Schwartzer, J. J., Careaga, M., Onore, C. E., Rushakoff, J. A., Berman, R. F., and Ashwood, P. (2013). Maternal immune activation and strain specific interactions in the development of autism-like behaviors in mice. *Transl. Psychiatry* 3:e240. doi: 10.1038/tp.2013.16

Silverman, J. L., Tolu, S. S., Barkan, C. L., and Crawley, J. N. (2010). Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP. *Neuropsychopharmacology* 35, 976–989. doi: 10.1038/npp.2009.201

Steidl, S., and Yeomans, J. S. (2009). M5 muscarinic receptor knockout mice show reduced morphine-induced locomotion but increased locomotion after cholinergic antagonism in the ventral tegmental area. *J. Pharmacol. Exp. Ther.* 328, 263–275. doi: 10.1124/jpet.108.144824

Szatmari, P., Georgiades, S., Bryson, S., Zwaigenbaum, L., Roberts, W., Mahoney, W., et al. (2006). Investigating the structure of the restricted, repetitive behaviours and interests domain of autism. *J. Child Psychol. Psychiatry* 47, 582–590. doi: 10.1111/j.1469-7610.2005.01537.x

Tizabi, Y., Louis, V. A., Taylo, C. T., Waxman, D., Culver, K. E., and Szechtman, H. (2002). Effect of nicotine on quinpirole-induced checking behavior in rats: implications for obsessive-compulsive disorder. *Biol. Psychiatry* 51, 164–171. doi: 10.1016/s0006-3223(01)01207-0

Turner, M. (1999). Annotation: repetitive behaviour in autism: a review of psychological research. *J. Child Psychol. Psychiatry* 40, 839–849. doi: 10.1111/1469-7610.00502

Tzavos, A., Jih, J., and Ragozzino, M. E. (2004). Differential effects of M1 muscarinic receptor blockade and nicotinic receptor blockade in the dorsomedial striatum on response reversal learning. *Behav. Brain Res.* 154, 245–253. doi: 10.1016/j.bbr.2004.02.011

Voineagu, I., Wang, X., Johnston, P., Lowe, J. K., Tian, Y., Horvath, S., et al. (2011). Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474, 380–384. doi: 10.1038/nature10110

Wang, J. Q., and McGinty, J. F. (1997). Intrastriatal injection of a muscarinic receptor agonist and antagonist regulates striatal neuropeptide mRNA expression in normal and amphetamine-treated rats. *Brain Res.* 748, 62–70. doi: 10.1016/s0006-8993(96)01244-9

Wang, H., Ng, K., Hayes, D., Gao, X., Forster, G., Blaha, C., et al. (2004). Decreased amphetamine-induced locomotion and improved latent inhibition in mice mutant for the M5 muscarinic receptor gene found in the human 15q schizophrenia region. *Neuropsychopharmacology* 29, 2126–2139. doi: 10.1038/sj.npp.1300502

Yang, M., Clarke, A. M., and Crawley, J. N. (2009). Postnatal lesion evidence against a primary role for the corpus callosum in mouse sociability. *Eur. J. Neurosci.* 29, 1663–1677. doi: 10.1111/j.1460-9568.2009.06714.x

Yang, M., Zhodzishsky, V., and Crawley, J. N. (2007). Social deficits in BTBR T+tf/J mice are unchanged by cross-fostering with C57BL/6J mothers. *Int. J. Dev. Neurosci.* 25, 515–521. doi: 10.1016/j.ijdevneu.2007.09.008

Yano, K., Koda, K., Ago, Y., Kobayashi, H., Kawasaki, T., Takuma, K., et al. (2009). Galantamine improves apomorphine-induced deficits in prepulse inhibition via muscarinic ACh receptors in mice. *Br. J. Pharmacol.* 156, 173–180. doi: 10.1111/j.1476-5381.2008.00037.x

Zarrindast, M. R., Shekarchi, M., and Rezaya, T. M. (1999). Effect of nicotine on apomorphine-induced licking behaviour in rats. *Eur. Neuropsychopharmacol.* 9, 235–238. doi: 10.1016/s0924-977x(98)00027-3

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