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CORTICAL GABAERGIC NEURONS: STRETCHING IT

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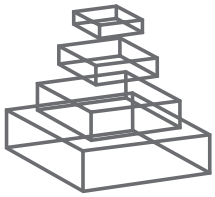

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CORTICAL GABAERGIC NEURONS: STRETCHING IT

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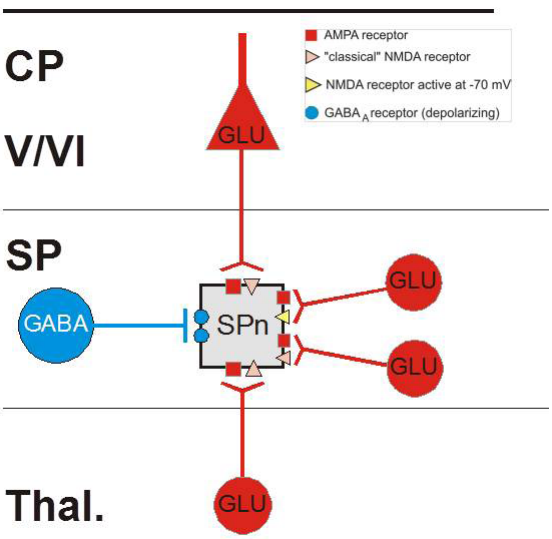

In the cerebellum and basal ganglia, projection neurons are GABAergic; but in the cerebral cortex, there has been a historically strong dichotomy between glutamatergic projection neurons and GABAergic local circuit neurons. While this dichotomy is overwhelmingly the case, it is now clear that a small population of long-distance projecting GABAergic neurons (positive for somatostatin and nNOS, and negative for parvalbumin) occurs in primates, as well as in cats and rodents. Beyond their well-documented existence, however, the functional significance, ontogeny, and connectivity of this intriguing subpopulation remain obscure.

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EDITORIAL
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Cortical GABAergic neurons: stretching it

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In the cerebellum and basal ganglia, projection neurons are GABAergic; but in the cerebral cortex, there has been a historically strong dichotomy between glutamatergic projection neurons and GABAergic local circuit neurons. While this dichotomy is overwhelmingly valid, it is now clear that a small population of long-distance projecting GABAergic neurons occurs in primates, as well as in cats and rodents. Beyond their well-documented existence, however, the functional significance, ontogeny, and connectivity of this intriguing subpopulation remain obscure.

This volume brings together nine articles that are intended to provide a summary of some of the current thinking regarding cortical GABAergic neurons with long-distance connections. These cover issues of nomenclature, subtype heterogeneity, species differences, and functional importance, in the adult brain, and in development. A final article (Clancy et al.) is organized as a series of extracts and commentaries on the individual articles, each section of which is followed by general comments and discussion. The order of articles in the present volume parallels the order in this final article. The topics in the other eight articles are briefly described below.

The first article (Clancy et al.) presents a database of neural developmental events in three commonly used experimental species: rat, mouse, and macaque, using data from the online database www.translatingtime.net, as well as GABAergic and subplate developmental data from the empirical literature. Striking similarities between the two rodent populations permit the identification of developmental dates for GABAergic and subplate neural events in rats that were previously identified only in mice, and proposed timing of development events in mouse, previously identified in rats. The authors also exploit their model to produce estimates of dates for GABAergic and subplate neural events that have not yet been, or cannot be determined empirically in humans.

The next two articles focus on projections and neurochemical profiles of long-distance projecting GABAergic neurons in several cortical areas (Higo et al.) and in the hippocampal system (Jinno). The authors use a variety of techniques to visualize and characterize in detail these scattered, hard to localize subpopulations: Golgi-like filling by NADPH-d (Higo et al.), GFP-labeling of GABAergic neurons in GAD67-GFP Cre-reporter mice (Higo et al.), and combined molecular, electrophysiological, and retrograde tracer approaches (Jinno).

A cluster of three articles address the identity of white matter neurons, their role in development, and variations across species (Kanold; Luhmann et al.; Petanjek et al.). Subplate neurons are a largely transient subpopulation, morphologically, and neurochemically heterogeneous, that resides in the neonatal cortical white matter. They play a key role in the segregation and functional maturation of thalamocortical and intracortical circuits and, especially as discussed in Luhmann et al., are postulated not only to serve as a transient relay station for afferent inputs, but also as an active element amplifying the afferent and intracortical activity. Local and long-distance projections arise from subpopulations of glutamatergic and GABAergic subplate neurons. Projections to more distant neocortical regions are thought to form a corticocortical synaptic network. Clinical evidence suggests that subplate cells in the mature cortex contribute to the manifestation of abnormal neuronal circuits, pathological activity, and long-term neurological deficits.

Espinoza et al. propose a novel classification of non-subplate pioneer neurons, with distinctive neurochemical phenotypes, projections, and localization within the preplate before its partition. They report the two populations as recognizable in rat and mouse, but with species-specific neurochemical features.

Finally, Zhang et al. report a subpopulation of immature, interneuron-like cells in the association cortex and amygdala of non-human primates, and infer that these are involved in a life-long role, at least partly associated with plasticity.

In summary, these articles present a range of data and issues that are under active investigation. Even since the short time since 2009 (the original publication of these articles), technical advances have occurred that promise significant new approaches to unraveling the structure and function of this subpopulation, not only as relates to basic cortical circuitry and function but also in relation to developmental and psychiatric disorders.

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REVIEW ARTICLE
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Cross-species analyses of the cortical GABAergic and subplate neural populations

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Cortical GABAergic (γ-aminobutyric acidergic) neurons include a recently identified subset whose projections extend over relatively long distances in adult rodents and primates. A number of these inhibitory projection neurons are located in and above the conventionally identified white matter, suggesting their persistence from, or a correspondence with, the developmental subplate. GABAergic and subplate neurons share some unique properties unlike those of the more prevalent pyramidal neurons. To better understand the GABAergic and subplate populations, we constructed a database of neural developmental events common to the three species most frequently used in experimental studies: rat, mouse, and macaque, using data from the online database www.translatingtime.net as well as GABAergic and subplate developmental data from the empirical literature. We used a general linear model to test for similarities and differences, a valid approach because the sequence of most neurodevelopmental events is remarkably conserved across mammalian species. Similarities between the two rodent populations are striking, permitting us to identify developmental dates for GABAergic and subplate neural events in rats that were previously identified only in mice, as well as the timing in mouse development for events previously identified in rats. Primate comparative data are also compelling, although slight variability in statistical error measurement indicates differences in primate GABAergic and subplate events when compared to rodents. Although human extrapolations are challenging because fewer empirical data points are available, and because human data display more variability, we also produce estimates of dates for GABAergic and subplate neural events that have not yet been, or cannot be, determined empirically in humans.

Keywords: GABA, statistical analysis, persisting subplate neurons, layer VIb, white matter neurons, cross-species correlations, rodent development, human development

INTRODUCTION

Cortical GABAergic (γ-aminobutyric acidergic) neurons can be parceled into a number of subgroups based on variations in morphology, birthplaces, mature locations, colocalized peptides, and electrophysiological parameters (Hendry and Jones, 1991; Ascoli et al., 2008; Burkhalter, 2008). Despite such diversity, conventional models of cortical function include GABAergic neurons as participators only in local connectivity, such that the designation of "interneuron" is often used interchangeably with GABAergic to describe cortical neurons that play an inhibitory role. However, cortical GABAergic categories recently were extended to include a subset of phylogenetically conserved neurons that project axons across long distances – the newly identified long-range interneurons, perhaps more precisely called cortical GABAergic projection neurons (McDonald and Burkhalter, 1993; Gonchar et al., 1995; Fabri and Manzoni, 1996, 2004; Tomioka et al., 2005; Pinto et al., 2006; Higo et al., 2007; Tomioka and Rockland, 2007).

One intriguing aspect of the latest subgroup is that the majority of the long-range GABAergic projections extend from neurons located in cortical layer I, cortical white matter, and the subgriseal region of the cortex (subjacent to cortical layer VI) (Tomioka et al., 2005; Tomioka and Rockland, 2007). This prompted the suggestion that the GABAergic projection neurons might be a subset of the little-studied cells that persist in the adult brain from the developmental subplate (Tomioka et al., 2005; Tomioka and Rockland, 2007).

GABAergic AND SUBPLATE NEURONS

Early in development, the cells of the future layer I, as well as the future subplate/white matter neurons, are merged as the preplate before neurons of the developing cortical plate split the preplate into a superficial region (later called layer 1) and a subgriseal region (later called the subplate) (Marin-Padilla, 1978, 1988). The exact percentage of subplate cells that survive into adulthood is somewhat difficult to identify (Chun and Shatz, 1989a; Valverde et al., 1995; Price et al., 1997; Robertson et al., 2000), but it is well-documented that some survive an early wave of cell death to remain in the mature white matter in human and non-human primate cortex (Kostovic and Rakic, 1980; Somogyi et al., 1981; Schiffmann et al., 1988; Yan et al., 1996) and in carnivores (Chun and Shatz, 1989b). Moreover, they survive both in the white matter and as a well-defined structure subjacent to the cortex in rodents, species used for the majority of neural studies (Somogyi et al., 1984; Lauder et al., 1986; Huntley et al.,

1988; Luskin et al., 1988; Reep and Goodwin, 1988; Winer and Larue, 1989; Cobas et al., 1991; Woo et al., 1991; Woo and Finlay, 1996; Reep, 2000).

As depicted in **Figure 1**, even in rodents the persisting subplate cells that remain in a distinct layer are essentially positioned in the white matter. They sit above the conventionally-identified white matter, but below a stria of intracortical connections (Reep and Goodwin, 1988; Clancy and Cauller, 1999; Reep, 2000; Teague-Ross et al., 2008), making them more comparable to the persisting white matter (interstitial) population in primates and carnivores than is often acknowledged. GABAergic neurons account for 15–25% of all the neurons in the region depicted as the persisting subplate, similar to their percentage in the cortex overall (Hendry et al., 1983, 1987; Chun et al., 1987; Esclapez et al., 1987; Meinecke and Peters, 1987; Peduzzi, 1988; Del Rio et al., 2000; Tomioka et al., 2005; Burkhalter, 2008). The subplate may include a GABAergic population that survives from cells located at the intermediate zone/subventricular zone border early in development (Del Rio et al., 2000), as well as some lower intermediate zone GABAergic cells that may later merge with subplate cells (Van Eden et al., 1989).

CORRESPONDENCE BETWEEN GABAergic AND SUBPLATE NEURONS

The location of the long distance GABAergic neurons, and the possibility that some may be surviving subplate neurons, is interesting because GABAergic and subplate neurons (both developmental and those that persist across maturity, including neurons in the white matter) have a notable relationship, and share some comparable characteristics. Similar to the sometimes confusing GABAergic nomenclature recently addressed by the Pettilla committee (Ascoli et al., 2008), surviving subplate cells have been assigned a variety of different names in mature cortex, including border neurons (Hogan and Berman, 1992), white matter neurons (Kostovic and Rakic, 1980), subgriseal neurons (Clancy and Cauller, 1999), layer VII (Clancy and Cauller, 1999; Reep, 2000), layer VIb (Gomez-Pinilla and Cotman, 1992), deep layer VI (McDonald and Burkhalter, 1993), upper subplate neurons (Marin-Padilla and Marin-Padilla, 1982), and the deep cortical band (Kristt, 1979). In this study we will use the term "persisting subplate neurons" (Reep, 2000) for those resilient cells that remain from the developmental subplate in and above the white matter across maturation, where they continue to participate in cortical function (Clancy et al., 1997, 2001b; Bayer et al., 2004; Torres-Reveron and Friedlander, 2007; Pinon et al., 2009), and apparently include the GABAergic subset that sends projections for long distances (Tomioka et al., 2005; Tomioka and Rockland, 2007).

Subplate neurons can be activated by GABA, including intrinsic GABAergic activation from other subplate neurons (Princivalle et al., 2000; Hanganu et al., 2001). The subplate is equally important in GABAergic function as it is required for a developmental change that switches GABAergic input from producing a depolarizing response to its more familiar hyperpolarizing role (Lauder et al., 1986; Kanold and Shatz, 2006).

In fact, it is difficult to comprehensively characterize one population without including the other. Both GABAergic and subplate populations have been implicated in similar disorders associated with development, including epilepsy and schizophrenia (Akbarian et al., 1995, 1996; Kirkpatrick et al., 1999; Lein et al., 1999; Kanold, 2004; Woo et al., 2004; Levitt, 2005; Lewis et al., 2005; Eastwood and Harrison, 2006; Freund and Katona, 2007; Leviton and Gressens, 2007; Lazar et al., 2008; Metin et al., 2008). Both populations may be particularly vulnerable to developmental insults, including those associated with premature birth (Nie and Wong-Riley, 1996; Ulfig, 2002; McQuillen and Ferriero, 2005), and in the white matter damage that often follows intrauterine infection (Dammann et al., 2002; Jensen et al., 2003; Kostovic and Judas, 2006; Robinson et al., 2006; Leviton and Gressens, 2007). Both populations play roles that change across development and maturity (Owens and Kriegstein, 2002; Ben-Ari et al., 2004; Kanold and Shatz, 2006; Friedlander, 2008), and it has been proposed that both populations may establish mechanisms during early development that lie dormant until triggered at later ages (Kanold et al., 2003; Butt et al., 2007).

Both GABAergic and subplate populations include numerous and diverse morphological subsets that are different from the more prevalent cortical pyramidal neurons (although each population may include cells with pyramidal morphology), and both populations contain a subset whose projections may travel long distances,

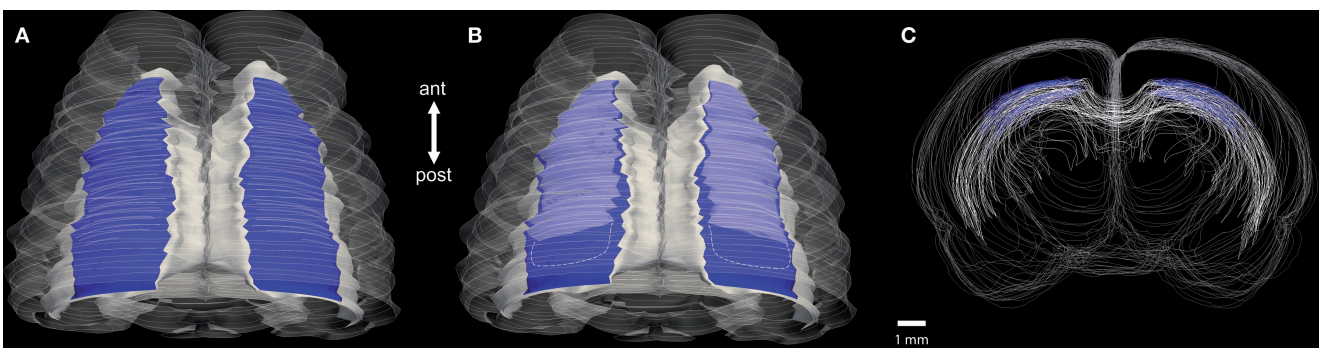

FIGURE 1 | (A) Schematic of a dorsal view of an adult rat brain produced from serial sections using Neurolucida (Version 8; MicroBrightField, Williston, VT, USA). Persisting subplate cells (blue) lie above the white matter (solid white). The brain outline is depicted in shadow. **(B)** The same brain with the infracortical stria, a fiber tract above the persisting subplate cells, shown in transparent white. The white, dashed line represents the continuity, but decreasing thickness, of the infracortical stria in posterior brain regions. **(C)** Overlapping coronal serial sections depicting the location of the persisting subplate cells (blue) with the conventional white matter outlined (white). Scale bar = approximately 1.0 mm. Abbreviations: ant (anterior); post (posterior).

sometimes crossing areal boundaries, as well as a subset that focuses projections on cortical layer I (Cauller et al., 1998; Clancy and Cauller, 1999; Tomioka et al., 2005; Silberberg and Markram, 2007; Tomioka and Rockland, 2007). Both populations are similarly heterogeneous in their electrophysiological properties (Friauf et al., 1990; Luhmann et al., 2000; Hanganu et al., 2001; Voigt et al., 2001; Miyoshi et al., 2004, 2007; Torres-Reveron and Friedlander, 2007), and in the numerous signaling chemicals they sequester (Chun and Shatz, 1989a,b; Bredt and Snyder, 1992; Gao et al., 1999; Tao et al., 1999; Robertson et al., 2000; Clancy et al., 2001b; Heuer et al., 2003; Bayer et al., 2004; Garbossa et al., 2004; Tomioka et al., 2005; Tomioka and Rockland, 2007; Burkhalter, 2008). Moreover, subsets of both populations may share a common non-cortical birthplace in the ganglionic eminences (Tamamaki et al., 1997; Lavdas et al., 1999; Anderson et al., 2001; McQuillen and Ferriero, 2005), raising the possibility that some may descend from similar sets of precursors. Supporting this notion, both populations use somewhat similar molecular modes of migration, different from the mechanism used by pyramidal cells (Gilmore and Herrup, 2001).

SPARSE CONNECTIVITY CAN HAVE POWERFUL EFFECTS

Although the contribution of the GABAergic interneurons to cortical function is undisputed, and the critical role of the subplate in cortical development is well-accepted (McConnell et al., 1989; Ghosh et al., 1990; De Carlos and O'Leary, 1992), conventional models of mature cognitive function do not yet incorporate contributions of either the projection GABAergic or the persisting subplate neurons. When numbers are reduced compared to other neural populations, there may be a tendency to simply dismiss those that persist as "sparse," "remnants," or "relics". Unfortunately, such terminology implies a fairly unessential function, and it seems important to avoid such categorization until additional information on their function is available. Persisting subplate cells in adult mammalian species have thus far eluded characterization as an easily observable and/or organized structure. Several studies, however, have suggested revising the whole idea of a "remnant" population, and provided evidence for the organization and participation of the persisting subplate cells in mature cortical function (Clancy et al., 2001b; Colombo and Bentham, 2006; Torres-Reveron and Friedlander, 2007; Chang et al., 2008; Friedlander, 2008; Friedlander and Torres-Reveron, 2009; Suarez-Sola et al., 2009).

With the recent indication that the long distance projection GABAergic neurons are associated with the persisting subplate population, including neurons that remain in isolated positions in both cortex and white matter, a role for the persisting subplate population is strengthened. Indeed, the mathematical principles underlying small-world networks suggest that sparse connectivity is a plausible design underlying important cognitive function. Long-range inhibition, even from relatively sparse connections, can be a potent network component (Sur and Rubenstein, 2005). In small-world networks (Watts and Strogatz, 1998) (inspired by the same mathematics behind "Six Degrees of Kevin Bacon"), clusters of cells link to their nearest neighbors, while some connect to distant clusters. This pattern can serve as the basis for a surprisingly strong communication network, especially when it is amplified by local input, as is likely the case for both the long-range GABAergic and the persisting subplate populations.

SPECIES SIMILARITIES AND DIFFERENCES

Most characteristics of GABAergic and subplate cells are conserved across species (Levitt, 2005; Wang and Kriegstein, 2009), and even some GABAergic features previously considered to be exclusive to primates, or wholly exclusive to humans, were later identified in other species (Meyer et al., 1998; Yuste, 2005; Petanjek et al., 2009). However, species differences have been reported in both populations, including in birthplace, migration, and final locations in mature cortices, suggesting that both populations may drive and/or be driven by evolutionary processes (Peduzzi, 1988; Letinic et al., 2002; Rakic, 2003, 2006; Watakabe et al., 2006; Petanjek et al., 2008; Suarez-Sola et al., 2009). In addition to the evolutionary aspects, the question of species differences has pragmatic impact because rodent models are necessary in studies for both normal (Rakic, 2006) and abnormal neural development (Goffinet and Rakic, 2000; Levitt, 2005; Robinson et al., 2005), and non-human primate and rodent studies are used routinely to fill in gaps in knowledge of human development e.g. (de Graaf-Peters and Hadders-Algra, 2006).

STATISTICAL MODELING OF SPECIES SIMILARITIES AND DIFFERENCES

In a series of previous studies, mathematical models have been used to successfully identify both similarities and relative differences in the timing of neural "events" when comparing primate and non-primate development (http://www.translatingtime.net) (Clancy et al., 2001a, 2007a,b; Nagarajan and Clancy, 2008). For the purpose of this review, "neural events" are defined as milestones pertaining to neural development such as the post conception (PC) date that neurons destined for the various cortical layers are generated. (Complete lists are included in **Table 3** at the end of this review.) Mathematical approaches are valid because despite species differences, including differences in the duration of development, the size of most brain regions scales similarly across species (Finlay and Darlington, 1995; Finlay et al., 1998, 2001). Central to this meta analysis, the timing of events that occur in most neural regions is remarkably conserved (Finlay and Darlington, 1995; Finlay et al., 1998, 2001).

Moreover, even neural regions that do display species-related duration differences can be modeled with appropriate mathematical adjustments (Clancy et al., 2000, 2001a). As a practical illustration, in a careful study of neurogenesis of the primate cortical plate (Smart et al., 2002), the authors urged caution when comparing histogenesis results between monkey and mouse, pointing out what seemed to be a disparity in that mouse PC days 11–12 compare to monkey PC days 46–65. In fact, our statistical modeling approach indicates the two time periods are remarkably comparable, predicting PC 11–12 in mice to correspond to PC 44–50 in macaques (these and other comparisons can be accessed at http://www.translatingtime.net).

STATISTICAL ANALYSES INCLUDING GABAergic AND SUBPLATE EMPIRICAL DATA

The most pragmatic application of statistical modeling is that neural events empirically derived in one species can be compared and successfully applied to another. Therefore, given the potentially important contributions of the long distance

GABAergic and persisting subplate populations, we reasoned any additional information about these two populations, including comparative cross-species data, is likely to be useful. At this time, no empirical developmental data are yet available specifically for the cortical GABAergic projection neurons. However developmental data are available for both general populations that include them, the GABAergic and subplate populations. We assembled a database of GABAergic and subplate developmental events (e.g. the PC day subplate neurogenesis begins, the day GABAergic cells are first found in the subplate). We then applied cross species statistical modeling, and tested if species similarities and differences might be indicated by statistical analysis of the developmental sequences.

For this review we generated three sets of results: (i) We compare and translate GABAergic and subplate developmental neural events between rats and mice, since a majority of the events have been documented in the literature in these two species. (ii) In order to understand the impact of translation from rodents to primates, we present the GABAergic and subplate predictions by pooling the events from mice, rats, and macaques, as sufficient data are available in macaques to allow such conversions. More importantly for this review, we discuss the prediction of common events across these three species with and without subplate and GABAergic events. (iii) Finally, we discuss the results obtained by pooling the data for mice, rats, macaques and humans. As would be expected, the number of empirically derived events in humans is considerably lower as compared to other three species. However, the results we present indicate that it might be possible to arrive at meaningful approximations to unknown human events by "translating" empirically derived events across other species.

STANDARD STATISTICAL TECHNIQUES

Our database was gleaned from the published literature, including the timing of 20 GABAergic and subplate neural events established in two or more mammalian species. We then incorporated these dates into our previously established database of 101 neural events freely available at http://www.translatingtime.net: (i) For translation of GABAergic and subplate developmental neural events between rats and mice, we considered a total of 135 events, comprised of dates from the empirical literature for mice, rats, and both mice and rats. (ii) For translation from rodents to primates, we considered the common events for mice, rats, and macaques, with GABAergic and subplate events (46 events) and without (38 events). We restricted the analysis to the common events in order to facilitate comparison of the regression results across these three species. (iii) Finally, we discuss the results obtained by pooling data on mice, rats, macaques and humans. This portion of the analysis consisted of events empirically derived in at least one of the species (119 events total). Tables that list the specific neural events, including empirically derived dates as well as the predictions generated by our analyses, are included at the end of this review (**Table 3**). In our analyses, data are standardized whenever possible such that the 24-h period following conception is designated PC 1, and the 24-h period immediately following birth is considered postnatal (PN) day 0. The "start" date is the day on which 5% of the neurons of a given structure are generated, and "end" is assigned similarly. If no clear "peak" is evident in the empirical neurogenesis data, a midpoint is used.

Because the timing of most developmental events in mammalian brains follows a similar pattern across species (Finlay and Darlington, 1995), standard regression techniques can be used to compare cross-species neural development (Clancy et al., 2001a, 2007a,b). The present study uses a general linear model to translate the events across the species. The response values (i.e. empirically derived event timings) were log-transformed and each of the predictors representing the species and the events were represented by dummy variables (Darlington, 1990). The model parameters were subsequently estimated using least squares regression. Confidence limits were determined in the log-scale for each of the estimated event timings (Statistical software "R" 2.8.1).

RESULTS OF STATISTICAL ANALYSES

Although we found empirical data for many developmental GABAergic and subplate data points in both rats and mice, there were a numbers of events for which we found empirical data points in only one of these important experimental species. Predictions for GABAergic and subplate events in the rodent species using the general linear model from the documented events are depicted in **Table 1**. The predicted values along with their confidence intervals are shown in the log-scale. The predicted values are also transformed back in the original scale (PC days) for clarity. Because so many developmental data points are available in these two species, cross-rodent conversions between these closely related species are especially compelling (Nagarajan and Clancy, 2008).

COMPARISONS OF COMMON EVENTS IN RATS, MICE, AND MACAQUES WITH AND WITHOUT SUBPLATE AND GABAergic EVENTS

There are reported species differences, most notably in rodent/primate comparisons, in the relative timing (heterochrony) and location of neurogenesis for both the GABAergic and subplate cell populations (Letinic et al., 2002; Smart et al., 2002; Rakic, 2003, 2006). Because a mathematical modeling approach has previously permitted identification of two neural systems whose temporal milestones "shift" in primates – the limbic system (shifted to occur earlier in primates) and cortical neurogenesis events (shifted to occur later when compared to non-primates) (Clancy et al., 2000), we anticipated our model would be able to establish if similar discrepancies occur in GABAergic and subplate neural development events when comparing the timing of rodent/primate events. In order to facilitate a direct comparison between rodents and macaques, we selected the common events across these species including the GABA/subplate events (46 events) and excluding them (38 events).

As depicted in the graphs in **Figure 2**, the sums of absolute error (e) between the empirical values and those predicted by the general linear model are similar for mice and rats (mice $e = 36.6$; rats $e = 39.7$), but much higher for macaques ($e = 235.8$). However, the correlation coefficient between the empirical and predicted values is significant for all three species (mice $r = 0.95$; rats $r = 0.96$; macaques $r = 0.93$; $p < 0.001$) indicating that a general linear model is a useful tool in cross species translations. Yet the results

Table 1 | GABAergic and subplate developmental neural events are statistically "translated" between rats and mice.

Translating mouse to rat/rat to mouse predicted by model in white type	Mouse PC day	Rat PC day	Lower CI log	Upper CI log
GABAergic cells first generated	**9.7**	11[7]	2.1	2.4
MGE begins generating burst spiking interneurons	9.5[1,5]	**10.8**	2.2	2.5
Tangential migration begins from MGE to subplate	11.5[11]	**13**	2.4	2.7
"Handshake" in ganglionic eminence (striate primordium)	13[8]	**14.7**	2.6	2.8
GAD positive cells in subplate and layer I	**13.2**	15[7]	2.5	2.7
CGE begins generating regular spiking interneurons (adapting)	13.5[5]	**15.2**	2.6	2.9
MGE begins generating fast spiking interneurons (PV)	13.5[6]	**15.3**	2.6	2.9
LGE generates GABA cells for proliferative zone – start	14[1]	**15.9**	2.6	2.9
GABAir cells in all cortical laminae formed at this point – start	**14.1**	16[12]	2.5	2.8
GABAir cells in all cortical laminae formed at this point – end	**15**	17[12]	2.6	2.8
GABA positive cells decrease in lower intermediate zone	**15**	17[7]	2.6	2.8
GABA positive cells conspicuous in white matter	**15.9**	18[7]	2.6	2.9
LGE generates cortical cells GABA – destined for proliferative zone – end	16[1]	**18.1**	2.8	3.0
Robust migration from MGE to cortex ends	16[1]	**18.1**	2.8	3.0
Subplate cells contain GABA in adult–like fashion (17%) – start	16[8]	**18.1**	2.8	3.0
Peak levels of GABA–induced migration	**16.3**	18.5[2,3]	2.7	2.9
GABAir cells no longer in lower intermediate zone	**16.8**	19[12]	2.7	2.9
GABA influences neurite outgrowth	**16.8**	19[10]	2.7	2.9
Subplate cells contain GABA in adult–like fashion (17%) – end	17[8]	**19.2**	2.8	3.1
Intra GABA subplate communication – start	**18.9**	21.5[9]	2.8	3.1
GABA switches from excitatory to inhibitory – start	**20.8**	23.5[4]	2.9	3.2
Intra GABA subplate communication – end	**21.6**	24.5[9]	2.9	3.2
GABA switches from excitatory to inhibitory – end*	**24.3**	28.5[4]		

**Base event; translation generated at www.translatingtime.net.*
References: [1] (Anderson et al., 2001), [2] (Behar et al., 1996), [3] (Behar et al., 1998), [4] (Ben-Ari et al., 1997), [5] (Butt et al., 2007), [6] (Butt et al., 2005), [7] (DeDiego et al., 1994), [8] (Del Rio et al., 2000), [9] (Hanganu et al., 2002), [10] (Maric et al., 2001), [11] (Marin and Rubenstein, 2001), [12] (Van Eden et al., 1989).
Abbreviations: MGE (medial ganglionic eminence); GABA (γ-aminobutyric acid); SOM (somatostatin); CR (calretinin); VIP (vasointestinal polypeptide); PV (parvalbumin); LGE (lateral ganglionic eminence); GAD (glutamic acid decarboxylase); GABAir (γ-aminobutyric acid immunoreactive).

of the sum of absolute error do indicate that translation of the events from rodent to primates can be more challenging. Some of the discrepancy in the sum of squared error may be attributed to additional variability in the macaque events as opposed to those of rats and mice.

We then ran the same analysis including GABAergic and subplate events because were especially interested if the development of these two populations might exhibit any differences when compared to the other neural events, based on the reports that these two populations might have particular species-specific differences (Kostovic and Rakic, 1990; Smart et al., 2002).

In each of the three species, the correlation coefficient values (r) do not change significantly with the inclusion of GABAergic and subplate events (**Figure 3**) when compared to the values generated without including these events (**Figure 2**). The r for mice increases from 0.95 to 0.97; for rats it increases from 0.96 to 0.97; for macaques it increases from 0.93 to 0.95 ($p = 0.001$ for all). When GABAergic and subplate events are considered, once again there is considerable similarity in the sum of absolute error between the empirical and predicted values in the rodents; mice ($e = 47.9$; $p = 0.001$) and rats ($e = 53.5$; $p = 001$), and again error for macaques is higher ($e = 312.1$; $p = 001$). However, the magnitude of the error (e) was higher across all three species when the data includes GABAergic and subplate events (**Figure 3**) as opposed to when they are excluded (**Figure 2**). For mice it increases from 36.6 to 47.9; for rats from 39.7 to 53.5; and for macaques it increases from 235.8 to 312.1 ($p = 0.001$ for all). This would appear to indicate something is indeed different in these GABAergic and subplate events when comparing rodents to primates, and it is more distinctive in primates.

These analyses are necessarily preliminary as they are based on a somewhat limited dataset, but they do support the neuroanatomical data noting slight species differences in the GABAergic and subplate populations (Peduzzi, 1988; Letinic et al., 2002; Rakic, 2003). Clearly a more detailed study with increased numbers of empirical events as they become available will be necessary in order to gain sufficient insight into the impact of GABAergic and subplate events on evolutionary modifications between rodent and primate species.

POOLING MAMMALIAN DATA TO PREDICT HUMAN EVENTS

The difficulty of establishing precise empirical dates for neural events in human development is extremely challenging, particularly due to individual variation and observational error. The

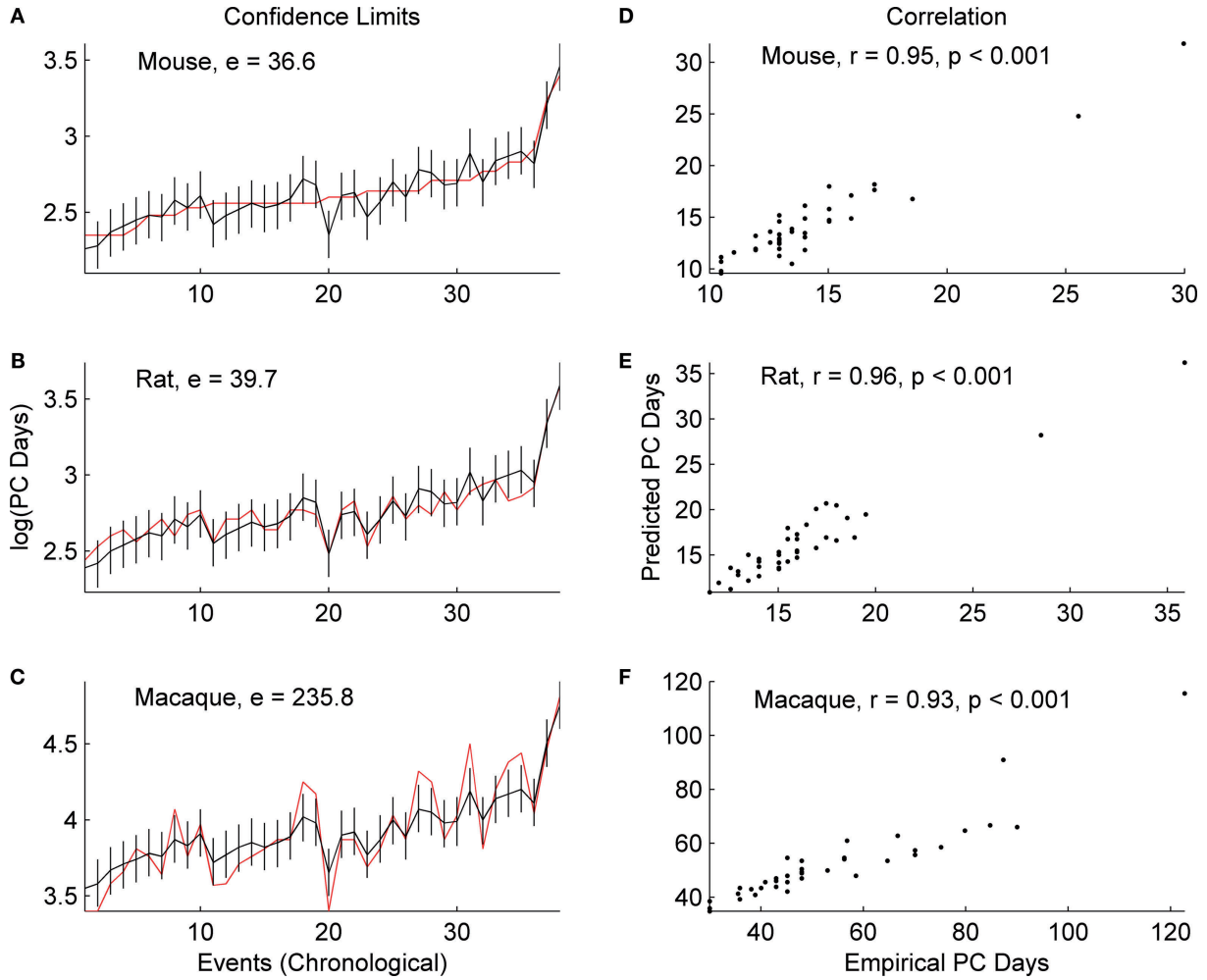

FIGURE 2 |The predicted (black) and empirical values (red) of the post-conceptional days in the log-scale, i.e. log(PC days), of the 38 common events excluding GABAergic and subplate events across mice, rats and macaques are shown in (A), (B) and (C) respectively. The rat and macaque events are in the same chronological order as that of mouse events. The confidence limits (log-scale) about the predicted values and the sum of absolute error (e) between the empirical and the predicted values are also shown in **(A)**, **(B)** and **(C)**. The correlation coefficient (*r*) between the predicted and the empirical values for mice, rats and macaques are shown in **(D)**, **(E)** and **(F)** respectively. The names of the events are included in Table 2 at the end of this review.

limitations are unavoidable: samples sizes are necessarily small, dates of conception are estimated, sampling intervals are essentially opportune, the most convincing techniques to establish dates of neurogenesis require invasive techniques, to name but a few. However, it is possible to arrive at meaningful approximation to unknown human events using statistical techniques to translating empirically derived dates from other mammalian species (Clancy et al., 2000).

Predictions for human GABAergic and subplate events translated using empirically derived statistical comparisons from our database (119 events) for at least one of the species (rat, mouse and macaque) are included in **Table 2**. For the predictions listed in **Table 2**, we used the statistical model to predict human events for data points that are not yet known, as well as for some events that are reported in the empirical literature. In most cases where empirical data are available, the model predictions were for dates earlier than those reported in the literature. This may be an indication of variability, but we have reason to propose that our data are accurate based on a principle, well known to statisticians, called the "bootstrap effect" (Cronbach and Meehl, 1955). Since the estimates generated by any mathematical model are based on all the empirical data used to build the model, errors can be "averaged out" such that the model's estimates may even be more accurate than empirical data. This seems especially compelling for human data, where techniques are extraordinarily challenging and sampling/observation intervals are often wide, such that an event might not be documented until well after it has occurred.

We have some direct evidence of the value of the bootstrap effect from a previous study (Clancy et al., 2000) that consistently predicted a much earlier date for human eye opening than any date then found in the empirical literature. When four-dimensional sonograms were perfected, it became clear that the statistical model had been accurate (Clancy et al., 2007a).

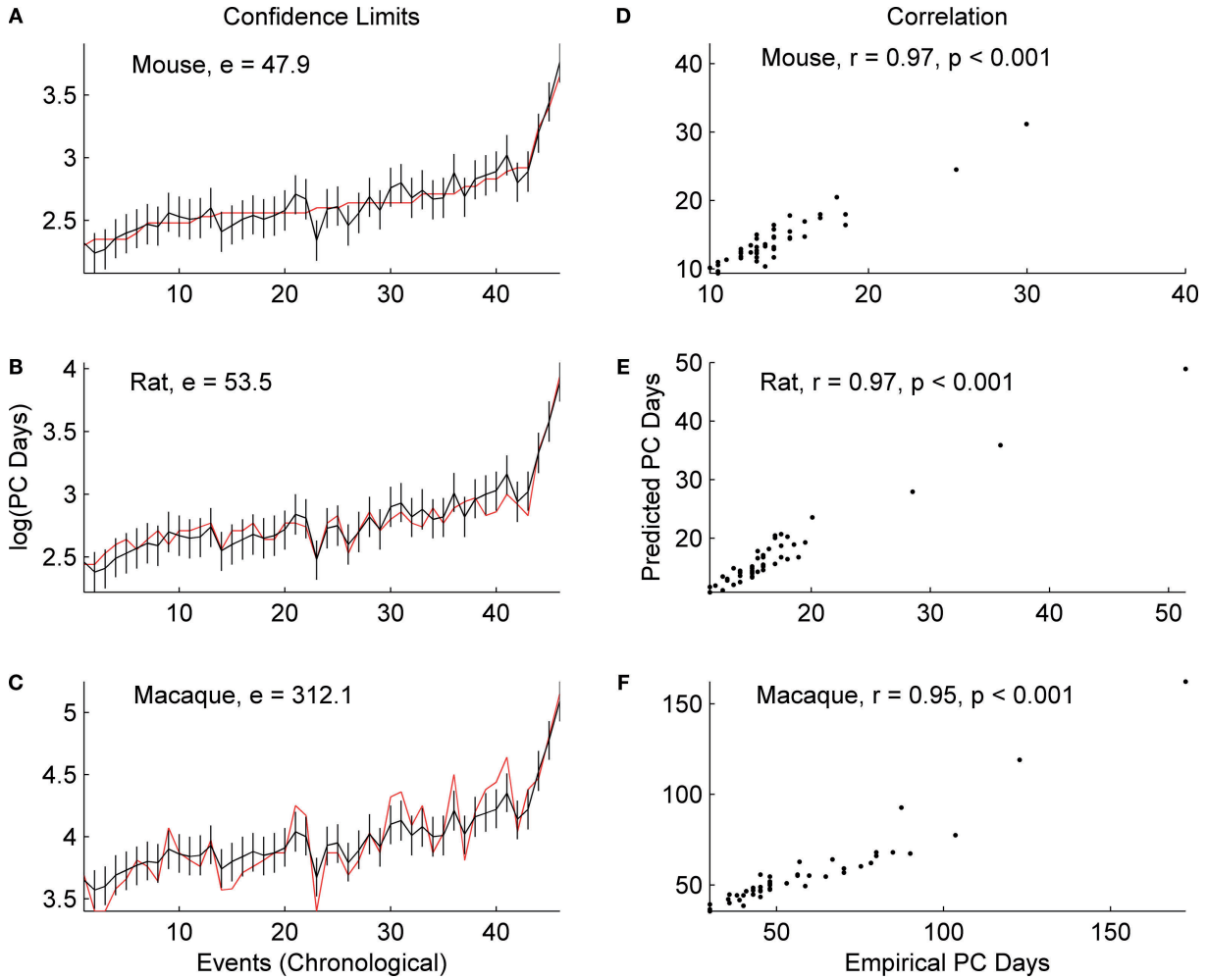

FIGURE 3 |The predicted (black) and empirical values (red) of the post-conceptional days in the log-scale, i.e. log(PC days), of the 46 common events including GABAergic and subplate events across mice, rats and macaques are shown in (A), (B) and (C) respectively. The rat and macaque events are in the same chronological order as the mouse events. The confidence limits (log-scale) for the predicted values and the sum of absolute error (*e*) between the empirical and the predicted values are also shown in **(A)**, **(B)** and **(C)**. The correlation coefficient (*r*) between the predicted and the empirical values for mice, rats and macaques are shown in **(D)**, **(E)** and **(F)** respectively. The specific events are included in Table 3 at the end of this review.

VALUE AND LIMITATIONS OF CROSS SPECIES TRANSLATIONS

The pragamatic value of the cross species translations in direct savings of time and resources when intervals for studies might be narrowed is a compelling reason to add this type of analysis to the growing array of modern neuroanatomical tools. In some case, it might eliminate the necessity of repeating a study already accomplished in one species (as our data suggest is possible when translating from mice to rats, or rats to mice), or at the least contribute to a narrowing of time intervals (as our data suggest is possible for humans). And although correlations between data available in the empirical literature and data produced by the model were already significant, we expect future predictions will become more accurate as additional data points are added to our database.

On the other hand, we certainly do not suggest that there are no differences in the brain development of diverse mammalian species. Mathematical modeling can successfully adjust for some rodent/primate differences (Clancy et al., 2001a), such that comparisons and predictions are not overly distorted by differences in brain sizes, or what might seem to be a relatively prolonged time window for neurogenesis in primates when compared to rats. Yet there are questions that analysis of our database does not yet permit us to address such as the possibility of an effect on differences in cell cycle mechanisms (Dehay and Kennedy, 2007), or the effect of variability in the location of GABAergic proliferation when comparing rodents to humans (Letinic et al., 2002; Molnar and Cheung, 2006; Molyneaux et al., 2007). Such differences may be exemplified by a recent statistical analysis of the pulvinar (Chalfin et al., 2007), which in humans also has a dual source of neurogenesis (Letinic and Rakic, 2001). However, it is clear that the timing of neurogenesis alone is an important factor in development, given the evidence that timing might predict such properties as laminar position, electrophysiology responses, and neuronal morphology, including projection patterns (Caviness, 1982; Rakic, 1988; Takahashi et al., 1999; Lai et al., 2008). As datapoints are added to our database,

Table 2 | The statistical model is used to produce estimates for postconception dates for GABAergic and subplate developmental neural events in humans.

Humans estimates GABAergic and subplate events	Empirical PC day	Model PC day	Prediction log	Lower CI log	Upper CI log
Layer 1 cells generated, first wave – start	53[8]	**49**	3.90	3.53	4.27
MGE produces Lhx6 in GABAergic cells – start	?	**61**	4.10	3.83	4.37
Subplate neurogenesis – start	35[4]	**66**	4.19	4.00	4.39
Subplate neurogenesis – end	42[4]	**70**	4.25	4.03	4.48
GABAir cells lower intermediate zone/subventricular border	77[7]	**71**	4.26	4.05	4.48
Lhx6 first in cortex in GABAergic cells	?	**75**	4.32	4.05	4.58
Thalamic axons in subplate	50[1]	**79**	4.37	4.17	4.56
Subplate expands rapidly – start	63[2]	**81**	4.40	4.14	4.65
GABA cells in subplate/cells from MGE reach cortex	140[3]	**82**	4.41	4.22	4.60
MGE produces Lhx6 in GABAergic cells – end	?	**92**	4.52	4.25	4.79
Waiting period – start	140[4]	**93**	4.53	4.34	4.73
Density of tangentially migrating cells increases	154[5]	**102**	4.62	4.36	4.88
Waiting period – end	182[4]	**116**	4.76	4.57	4.95
Subplate death/apoptosis – start	217[4]	**134**	4.90	4.71	5.09
GAD activity adult–like	280[6]	**197**	5.28	5.02	5.54
Subplate death/apoptosis – end	238[4]	**239**	5.48	5.29	5.67

References: [1] (Bourgeois, 1997), [2] (Bystron et al., 2008), [3] (Letinic et al., 2002), [4] (McQuillen and Ferriero, 2005), [5] (Petanjek et al., 2008), [6] (Romijn et al., 1991), [7] (Zecevic and Milosevic, 1997), [8] (Zecevic et al., 1999).
Abbreviations: MGE (medial ganglionic eminence); Lhx6 (LIM homeobox protein 6); GABA (γ-aminobutyric acid); GABAir (γ-aminobutyric acid immunoreactive); GAD (glutamic acid decarboxylase).

Tables 3 | The table below includes the neural events used in our statistical analysis, with GABAergic and subplate events indicated by an asterisk.
(A) Empirically derived and predicted mouse neural events in chronological order according to empirical dates, together with predictions and confidence limits on a log scale. Similar data are included for rats in **(B)**, and macaques in **(C)**. For comparative purposes these are listed in the same order as the mouse data.

	Empirical PC days	Prediction PC days	Prediction log	Lower CL log	Upper CL log
(A) MOUSE					
Subplate neurogenesis – start*	10.0	10.2	2.32	2.16	2.48
Retinal ganglion cell generation – start	10.5	9.4	2.24	2.08	2.40
Superficial SC laminae – start	10.5	9.7	2.27	2.11	2.43
dLGN – start	10.5	10.6	2.36	2.20	2.51
Purkinje cells – peak	10.5	11.0	2.40	2.24	2.55
Neurogenesis cortical layer VI – start (VC)	11.0	11.4	2.43	2.28	2.59
dLGN – peak	12.0	11.8	2.47	2.31	2.63
Amygdala – peak	12.0	11.6	2.45	2.30	2.61
Neurogenesis cortical layer V – start (VC)	12.0	13.0	2.56	2.41	2.72
Subplate neurogenesis – end*	12.0	12.6	2.53	2.37	2.69
GABA cells in subplate*	12.0	12.3	2.51	2.35	2.67
dLGN – end	12.5	12.4	2.52	2.36	2.68
Neurogenesis cortical layer VI – peak (VC)	12.5	13.5	2.60	2.44	2.76
Medial forebrain bundle appears	13.0	11.1	2.41	2.25	2.57
Optic axons at chiasm of optic tract	13.0	11.7	2.46	2.31	2.62
Superior colliculus – peak	13.0	12.3	2.51	2.35	2.66
Retinal ganglion cells – peak	13.0	12.7	2.54	2.39	2.70
Septal nuclei – peak	13.0	12.4	2.51	2.36	2.67
Entorhinal cortex – peak	13.0	12.6	2.54	2.38	2.69
Subiculum – peak	13.0	13.2	2.58	2.42	2.74
Neurogenesis cortical layer V – peak (VC)	13.0	15.0	2.71	2.55	2.86
Neurogenesis cortical lamina VI – end (VC)	13.0	14.4	2.67	2.51	2.83

(Continued)

Tables 3 | *(Continued)*

	Empirical PC days	Prediction PC days	Prediction log	Lower CL log	Upper CL log
Raphe complex – peak	13.5	10.4	2.34	2.18	2.50
Parasubiculum – peak	13.5	13.4	2.59	2.44	2.75
Presubiculum – peak	13.5	13.6	2.61	2.46	2.77
Fasciculus retroflexus appears	14.0	11.7	2.46	2.30	2.62
Caudoputamen – peak	14.0	13.0	2.56	2.40	2.72
Superficial SC laminae – end	14.0	14.7	2.69	2.53	2.84
Fornix appears	14.0	13.2	2.58	2.43	2.74
Neurogenesis cortical layer V – end (VC)	14.0	15.9	2.76	2.61	2.92
Thalamic axons in subplate*	14.0	16.4	2.80	2.64	2.95
Waiting period – start*	14.0	14.6	2.68	2.52	2.84
Neurogenesis cortical lamina IV – start (VC)	15.0	15.5	2.74	2.59	2.90
CA 1, CA 2 – peak	15.0	14.4	2.67	2.51	2.82
Retinal amacrine cells – peak	15.0	14.6	2.68	2.52	2.84
Neurogenesis cortical layer II/III – peak (VC)	15.0	17.8	2.88	2.72	3.03
Nucleus accumbens – peak	16.0	14.7	2.69	2.53	2.84
Cortical axons reach thalamus	16.0	16.9	2.83	2.67	2.98
Neurogenesis cortical layer IV – peak (VC)	17.0	17.5	2.86	2.70	3.02
Neurogenesis cortical layer IV – end (VC)	17.0	18.0	2.89	2.73	3.05
Subplate death/apoptosis – start*	18.0	20.5	3.02	2.86	3.18
Retinal ganglion cell generation – end	18.5	16.5	2.80	2.65	2.96
Waiting period – end*	18.5	18.0	2.89	2.73	3.05
ipsi/contra segregation in LGN and SC	25.5	24.4	3.20	3.04	3.35
Eye opening	30.0	31.3	3.44	3.29	3.60
Subplate death/apoptosis – end*	38.5	42.8	3.76	3.60	3.91
(B) RAT					
Subplate neurogenesis – start*	11.5	11.7	2.46	2.30	2.62
Retinal ganglion cell generation – start	11.5	10.8	2.38	2.22	2.54
Superficial SC laminae – start	12.5	11.1	2.41	2.25	2.56
dLGN – start	13.5	12.1	2.49	2.34	2.65
Purkinje cells – peak	14.0	12.6	2.53	2.37	2.69
Neurogenesis cortical layer VI – start (VC)	13.0	13.1	2.57	2.41	2.73
dLGN – peak	14.0	13.6	2.61	2.45	2.77
Amygdala – peak	15.0	13.3	2.59	2.43	2.75
Neurogenesis cortical layer V – start (VC)	13.5	14.9	2.70	2.54	2.86
Subplate neurogenesis – end*	15.0	14.4	2.67	2.51	2.83
GABA cells in subplate*	15.0	14.1	2.65	2.49	2.80
dLGN – end	15.5	14.2	2.66	2.50	2.81
Neurogenesis cortical layer VI – peak (VC)	16.0	15.4	2.74	2.58	2.89
Medial forebrain bundle appears	13.0	12.8	2.55	2.39	2.70
Optic axons at chiasm of optic tract	15.0	13.4	2.60	2.44	2.76
Superior colliculus – peak	15.0	14.0	2.64	2.48	2.80
Retinal ganglion cells – peak	16.0	14.6	2.68	2.52	2.84
Septal nuclei – peak	14.0	14.2	2.65	2.49	2.81
Entorhinal cortex – peak	14.0	14.5	2.67	2.51	2.83
Subiculum – peak	16.0	15.1	2.72	2.56	2.87
Neurogenesis cortical layer V – peak (VC)	16.0	17.1	2.84	2.68	3.00
Neurogenesis cortical lamina VI – end (VC)	15.5	16.6	2.81	2.65	2.96
Raphe complex – peak	12.0	11.9	2.48	2.32	2.63
Parasubiculum – peak	16.0	15.3	2.73	2.57	2.89
Presubiculum – peak	17.0	15.6	2.75	2.59	2.91

(Continued)

Tables 3 | ***(Continued)***

	Empirical PC days	Prediction PC days	Prediction log	Lower CL log	Upper CL log
Fasciculus retroflexus appears	12.5	13.4	2.60	2.44	2.75
Caudoputamen – peak	15.0	14.8	2.70	2.54	2.86
Superficial SC laminae – end	17.5	16.8	2.82	2.67	2.98
Fornix appears	15.0	15.2	2.72	2.56	2.88
Neurogenesis cortical layer V – end (VC)	16.5	18.2	2.90	2.74	3.06
Thalamic axons in subplate*	17.5	18.8	2.93	2.78	3.09
Waiting period – start*	16.0	16.7	2.82	2.66	2.97
Neurogenesis cortical lamina IV – start (VC)	15.5	17.8	2.88	2.72	3.04
CA 1, CA 2 – peak	18.0	16.5	2.80	2.65	2.96
Retinal amacrine cells – peak	16.0	16.7	2.82	2.66	2.97
Neurogenesis cortical layer II/III – peak (VC)	18.0	20.3	3.01	2.86	3.17
Nucleus accumbens – peak	19.0	16.8	2.82	2.66	2.98
Cortical axons reach thalamus	19.5	19.3	2.96	2.81	3.12
Neurogenesis cortical layer IV – peak (VC)	17.0	20.0	3.00	2.84	3.15
Neurogenesis cortical layer IV – end (VC)	17.5	20.6	3.03	2.87	3.18
Subplate death/apoptosis – end*	51.5	49.1	3.89	3.74	4.05
Retinal ganglion cell generation – end	18.5	18.9	2.94	2.78	3.10
Waiting period – end*	17.0	20.6	3.02	2.87	3.18
Ipsi/contra segregation in LGN and SC	28.5	28.0	3.33	3.17	3.49
Eye opening	36.0	35.8	3.58	3.42	3.74
Subplate death/apoptosis – end*	51.5	49.1	3.89	3.74	4.05
(C) MACAQUE					
Subplate neurogenesis – start*	40.0	38.6	3.65	3.50	3.81
Retinal ganglion cell generation – start	30.0	35.7	3.57	3.42	3.73
Superficial SC laminae – start	30.0	36.7	3.60	3.45	3.76
dLGN – start	36.0	40.0	3.69	3.53	3.85
Purkinje cells – peak	39.0	41.6	3.73	3.57	3.88
Neurogenesis cortical layer VI – start (VC)	45.0	43.2	3.77	3.61	3.92
dLGN – peak	43.0	44.9	3.80	3.65	3.96
Amygdala – peak	38.0	44.1	3.79	3.63	3.94
Neurogenesis cortical layer V – start (VC)	58.5	49.2	3.90	3.74	4.05
Subplate neurogenesis – end*	48.0	47.7	3.86	3.71	4.02
GABA cells in subplate*	45.0	46.7	3.84	3.69	4.00
dLGN – end	43.0	47.1	3.85	3.69	4.01
Neurogenesis cortical layer VI – peak (VC)	53.0	51.0	3.93	3.78	4.09
Medial forebrain bundle appears	35.5	42.2	3.74	3.59	3.90
Optic axons at chiasm of optic tract	36.0	44.5	3.80	3.64	3.95
Superior colliculus – peak	41.0	46.5	3.84	3.68	4.00
Retinal ganglion cells – peak	43.0	48.2	3.88	3.72	4.03
Septal nuclei – peak	45.0	46.8	3.85	3.69	4.00
Entorhinal cortex – peak	48.0	47.8	3.87	3.71	4.03
Subiculum – peak	48.0	50.0	3.91	3.76	4.07
Neurogenesis cortical layer V – peak (VC)	70.0	56.7	4.04	3.88	4.20
Neurogenesis cortical lamina VI – end (VC)	65.0	54.8	4.00	3.85	4.16
Raphe complex – peak	30.0	39.4	3.67	3.52	3.83
Parasubiculum – peak	48.0	50.7	3.93	3.77	4.08
Presubiculum – peak	48.0	51.7	3.95	3.79	4.10
Fasciculus retroflexus appears	40.0	44.4	3.79	3.64	3.95
Caudoputamen – peak	45.0	49.1	3.89	3.74	4.05
Superficial SC laminae – end	56.0	55.6	4.02	3.86	4.18

(Continued)

Tables 3 | *(Continued)*

	Empirical PC days	Prediction PC days	Prediction log	Lower CL log	Upper CL log
Fornix appears	48.0	50.2	3.92	3.76	4.07
Neurogenesis cortical layer V – end (VC)	75.0	60.1	4.10	3.94	4.25
Thalamic axons in subplate*	78.0	62.1	4.13	3.97	4.29
Waiting period – start*	60.0	55.2	4.01	3.85	4.17
Neurogenesis cortical lamina IV – start (VC)	70.0	58.9	4.08	3.92	4.23
CA 1, CA 2 – peak	48.0	54.6	4.00	3.84	4.16
Retinal amacrine cells – peak	56.0	55.2	4.01	3.85	4.17
Neurogenesis cortical layer II/III – peak (VC)	90.0	67.3	4.21	4.05	4.37
Nucleus accumbens – peak	45.0	55.6	4.02	3.86	4.17
Cortical axons reach thalamus	67.0	64.0	4.16	4.00	4.32
Neurogenesis cortical layer IV – peak (VC)	80.0	66.2	4.19	4.04	4.35
Neurogenesis cortical layer IV – end (VC)	85.0	68.2	4.22	4.07	4.38
Subplate death/apoptosis – start*	104.0	77.7	4.35	4.20	4.51
Retinal ganglion cell generation – end	57.0	62.5	4.14	3.98	4.29
Waiting period – end*	80.0	68.1	4.22	4.06	4.38
Ipsi/contra segregation in LGN and SC	87.0	92.5	4.53	4.37	4.69
Eye opening	123.0	118.5	4.78	4.62	4.93
Subplate death/apoptosis – end*	172.0	162.3	5.09	4.93	5.25

References for all data obtained from our database are available at www.translatingtime.net.
Abbreviations: SC (superior colliculus); dLGN (dorsal lateral geniculate nucleus); VC (visual cortex); CA (cornu ammonis).

we hope to test if a neuron's role is more closely related to the location of its birthplace, or the timing of its birth date.

One additional limitation we should point out arises because early PN days in rodent brain development correspond to *in utero* timing in humans. The result is that the effects of a perinatal wave of synaptogenesis (Zecevic and Rakic, 1991), the onslaught of experience surrounding birth, and the mother/offspring interaction are not included, as we have not yet identified data points associated with these events that fit into statistical models.

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REFERENCES

Akbarian, S., Huntsman, M. M., Kim, J. J., Tafazzoli, A., Potkin, S. G., Bunney, W. E., Jr., and Jones, E. G. (1995). GABAA receptor subunit gene expression in human prefrontal cortex: comparison of schizophrenics and controls. *Cereb. Cortex* 5, 550–560.

Akbarian, S., Kim, J. J., Potkin, S. G., Hetrick, W. P., Bunney, W. E., Jr., and Jones, E. G. (1996). Maldistribution of interstitial neurons in prefrontal white matter of the brains of schizophrenic patients. *Arch. Gen. Psychiatry* 53, 425–436.

Anderson, S. A., Marin, O., Horn, C., Jennings, K., and Rubenstein, J. L. (2001). Distinct cortical migrations from the medial and lateral ganglionic eminences. *Development* 128, 353–363.

Ascoli, G. A., Alonso-Nanclares, L., Anderson, S. A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., Buzsaki, G., Cauli, B., Defelipe, J., Fairen, A., Feldmeyer, D., Fishell, G., Fregnac, Y., Freund, T. F., Gardner, D., Gardner, E. P., Goldberg, J. H., Helmstaedter, M., Hestrin, S., Karube, F., Kisvarday, Z. F., Lambolez, B., Lewis, D. A., Marin, O., Markram, H., Munoz, A., Packer, A., Petersen, C. C., Rockland, K. S., Rossier, J., Rudy, B., Somogyi, P., Staiger, J. F., Tamas, G., Thomson, A. M., Toledo-Rodriguez, M., Wang, Y., West, D. C., and Yuste, R. (2008). Petilla terminology: nomenclature of features of, GABAergic interneurons of the cerebral cortex. *Nat. Rev.* 9, 557–568.

Bayer, L., Serafin, M., Eggermann, E., Saint-Mleux, B., Machard, D., Jones, B. E., and Muhlethaler, M. (2004). Exclusive postsynaptic action of hypocretin-orexin on sublayer 6b cortical neurons. *J. Neurosci.* 24, 6760–6764.

Behar, T. N., Li, Y. X., Tran, H. T., Ma, W., Dunlap, V., Scott, C., and Barker, J. L. (1996). GABA stimulates chemotaxis and chemokinesis of embryonic cortical neurons via calcum-dependent mechanisms. *J. Neurosci.* 16, 1808–1818.

Behar, T. N., Schaffner, A. E., Scott, C. A., O'Connell, C., and Barker, J. L. (1998). Differential response of cortical plate and ventricular zone cells to GABA as a migration stimulus. *J. Neurosci.* 18, 6378–6387.

Ben-Ari, Y., Khalilov, I., Represa, A., and Gozlan, H. (2004). Interneurons set the tune of developing networks. *Trends Neurosci.* 27, 422–427.

Ben-Ari, Y., Khazipov, R., Leinekugel, X., Caillard, O., and Gaiarsa, J. L. (1997). GABAA, NMDA and AMPA receptors: a developmentally regulated 'menage a trois'. *Trends Neurosci.* 20, 523–529.

Bourgeois, J. P. (1997). Synaptogenesis, heterochrony and epigenesis in the mammalian neocortex. *Acta Paediatr.* 422, 27–33.

Bredt, D. S., and Snyder, S. H. (1992). Nitric oxide, a novel neuronal messenger. *Neuron* 8, 3–11.

Burkhalter, A. (2008). Many specialists for suppressing cortical excitation. *Front. Neurosci.* 2, 155–167. doi: 10.3389/neuro.01.026.2008

Butt, S. J., Cobos, I., Golden, J., Kessaris, N., Pachnis, V., and Anderson, S. (2007). Transcriptional regulation of cortical interneuron development. *J. Neurosci.* 27, 11847–11850.

Butt, S. J., Fuccillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J. G., and Fishell, G. (2005). The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron* 48, 591–604.

Bystron, I., Blakemore, C., and Rakic, P. (2008). Development of the human cerebral cortex: Boulder Committee

revisited. *Nat. Rev. Neurosci.* 9, 110–122.

Cauller, L. J., Clancy, B., and Connors, B. W. (1998). Backward cortical projections to primary somatosensory cortex in rats extend long horizontal axons in layer I. *J. Comp. Neurol.* 390, 297–310.

Caviness, V. S., Jr. (1982). Early events of neocortical assembly: experimental studies and human pathology. *Int. J. Neurol.* 16–17, 102–109.

Chalfin, B. P., Cheung, D. T., Muniz, J. A., de Lima Silveira, L. C., and Finlay, B. L. (2007). Scaling of neuron number and volume of the pulvinar complex in New World primates: comparisons with humans, other primates, and mammals. *J. Comp. Neurol.* 504, 265–274.

Chang, A., Smith, M. C., Yin, X., Fox, R. J., Staugaitis, S. M., and Trapp, B. D. (2008). Neurogenesis in the chronic lesions of multiple sclerosis. *Brain* 131, 2366–2375.

Chun, J. J., Nakamura, M. J., and Shatz, C. J. (1987). Transient cells of the developing mammalian telencephalon are peptide-immunoreactive neurons. *Nature* 325, 617–620.

Chun, J. J., and Shatz, C. J. (1989a). The earliest-generated neurons of the cat cerebral cortex: characterization by MAP2 and neurotransmitter immunohistochemistry during fetal life. *J. Neurosci.* 9, 1648–1667.

Chun, J. J., and Shatz, C. J. (1989b). Interstitial cells of the adult neocortical white matter are the remnant of the early generated subplate neuron population. *J. Comp. Neurol.* 282, 555–569.

Clancy, B., and Cauller, L. J. (1999). Widespread projections from subgriseal neurons (layer VII) to layer I in adult rat cortex. *J. Comp. Neurol.* 407, 275–286.

Clancy, B., da Silva Filho, M., Hester, F., and Friedlander, M. J. (1997). Structure, function and connectivity of white matter neurons in mammalian visual cortex. *Abstr. - Soc. Neurosci.* 23, 1268.

Clancy, B., Darlington, R. B., and Finlay, B. L. (2000). The course of human events: predicting the timing of primate neural development. *Dev. Sci.* 3, 57–66.

Clancy, B., Darlington, R. B., and Finlay, B. L. (2001a). Translating developmental time across mammalian species. *Neuroscience* 105, 7–17.

Clancy, B., Silva-Filho, M., and Friedlander, M. J. (2001b). Structure and projections of white matter neurons in the postnatal rat visual cortex. *J. Comp. Neurol.* 434, 233–252.

Clancy, B., Finlay, B. L., Darlington, R. B., and Anand, K. J. (2007a). Extrapolating brain development from experimental species to humans. *Neurotoxicology* 28, 931–937.

Clancy, B., Kersh, B., Hyde, J., Darlington, R. B., Anand, K. J., and Finlay, B. L. (2007b). Web-based method for translating neurodevelopment from laboratory species to humans. *Neuroinformatics* 5, 79–94.

Cobas, A., Fairen, A., Alvarez-Bolado, G., and Sanchez, M. P. (1991). Prenatal development of the intrinsic neurons of the rat neocortex: a comparative study of the distribution of GABA-immunoreactive cells and the GABAA receptor. *Neuroscience* 40, 375–397.

Colombo, J. A., and Bentham, C. (2006). Immunohistochemical analysis of subcortical white matter astroglia of infant and adult primate brains, with a note on resident neurons. *Brain Res.* 1100, 93–103.

Cronbach, L. J., and Meehl, P. E. (1955). Construct validity in psychological tests. *Psychol. Bull.* 52, 281–302.

Dammann, O., Kuban, K. C., and Leviton, A. (2002). Perinatal infection, fetal inflammatory response, white matter damage, and cognitive limitations in children born preterm. *Ment. Retard. Dev. Disabil. Res. Rev.* 8, 46–50.

Darlington, R. B. (1990). Regression and Linear Models. New York, McGraw-Hill.

De Carlos, J. A., and O'Leary, D. D. (1992). Growth and targeting of subplate axons and establishment of major cortical pathways. *J. Neurosci.* 12, 1194–1211.

de Graaf-Peters, V. B., and Hadders-Algra, M. (2006). Ontogeny of the human central nervous system: what is happening when? *Early Hum. Dev.* 82, 257–266.

DeDiego, I., Smith-Fernandez, A., and Fairen, A. (1994). Cortical cells that migrate beyond area boundaries: characterization of an early neuronal population in the lower intermediate zone of prenatal rats. *Eur. J. Neurosci.* 6, 983–997.

Dehay, C., and Kennedy, H. (2007). Cell-cycle control and cortical development. *Nat. Rev.* 8, 438–450.

Del Rio, J. A., Martinez, A., Auladell, C., and Soriano, E. (2000). Developmental history of the subplate and developing white matter in the murine neocortex. Neuronal organization and relationship with the main afferent systems at embryonic and perinatal stages. *Cereb. Cortex* 10, 784–801.

Eastwood, S. L., and Harrison, P. J. (2006). Cellular basis of reduced cortical reelin expression in schizophrenia. *Am. J. Psychiatry* 163, 540–542.

Esclapez, M., Campistron, G., and Trottier, S. (1987). Immunocytochemical localization and morphology of GABA-containing neurons in the prefrontal and frontoparietal cortex of the rat. *Neurosci. Lett.* 77, 131–136.

Fabri, M., and Manzoni, T. (1996). Glutamate decarboxylase immunoreactivity in corticocortical projecting neurons of rat somatic sensory cortex. *Neuroscience* 72, 435–448.

Fabri, M., and Manzoni, T. (2004). Glutamic acid decarboxylase immunoreactivity in callosal projecting neurons of cat and rat somatic sensory areas. *Neuroscience* 123, 557–566.

Finlay, B. L., and Darlington, R. B. (1995). Linked regularities in the development and evolution of mammalian brains. *Science* 268, 1578–1584.

Finlay, B. L., Darlington, R. B., and Nicastro, N. (2001). Developmental structure in brain evolution. *Behav. Brain Sci.* 24, 263–278; discussion 278–308.

Finlay, B. L., Hersman, M. N., and Darlington, R. B. (1998). Patterns of vertebrate neurogenesis and the paths of vertebrate evolution. *Brain Behav. Evol.* 52, 232–242.

Freund, T. F., and Katona, I. (2007). Perisomatic inhibition. *Neuron* 56, 33–42.

Friauf, E., McConnell, S. K., and Shatz, C. J. (1990). Functional synaptic circuits in the subplate during fetal and early postnatal development of cat visual cortex. *J. Neurosci.* 10, 2601–2613.

Friedlander, M. J. (2008). Lifespan longitudinal multitasking by cortical neurons. *Future Neurol.* 3, 117–123.

Friedlander, M. J., and Torres-Reveron, J. (2009). The changing roles of neurons in the cortical subplate. *Front Neuroanat.* 3, 15. doi: 10.3389/neuro.05.015.2009

Gao, W. J., Newman, D. E., Wormington, A. B., and Pallas, S. L. (1999). Development of inhibitory circuitry in visual and auditory cortex of postnatal ferrets: immunocytochemical localization of GABAergic neurons. *J. Comp. Neurol.* 409, 261–273.

Garbossa, D., Fontanella, M., Tomasi, S., Ducati, A., and Vercelli, A. (2004). Differential distribution of NADPH-diaphorase histochemistry in human cerebral cortex. *Brain Res.* 1034, 1–10.

Ghosh, A., Antonini, A., McConnell, S. K., and Shatz, C. J. (1990). Requirement for subplate neurons in the formation of thalamocortical connections. *Nature* 347, 179–181.

Gilmore, E. C., and Herrup, K. (2001). Neocortical cell migration: GABAergic neurons and cells in layers I and VI move in a cyclin-dependent kinase 5-independent manner. *J. Neurosci.* 21, 9690–9700.

Goffinet, A., and Rakic, P. (2000). Mouse Brain Development. Berlin, NY, Springer.

Gomez-Pinilla, F., and Cotman, C. W. (1992). Transient lesion-induced increase of basic fibroblast growth factor and its receptor in layer VIb (subplate cells) of the adult rat cerebral cortex. *Neuroscience* 49, 771–780.

Gonchar, Y. A., Johnson, P. B., and Weinberg, R. J. (1995). GABA-immunopositive neurons in rat neocortex with contralateral projections to S-I. *Brain Res.* 697, 27–34.

Hanganu, I. L., Kilb, W., and Luhmann, H. J. (2001). Spontaneous synaptic activity of subplate neurons in neonatal rat somatosensory cortex. *Cereb. Cortex* 11, 400–410.

Hanganu, I. L., Kilb, W., and Luhmann, H. J. (2002). Functional synaptic projections onto subplate neurons in neonatal rat somatosensory cortex. *J. Neurosci.* 22, 7165–7176.

Hendry, S. H., Houser, C. R., Jones, E. G., and Vaughn, J. E. (1983). Synaptic organization of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. *J. Neurocytol.* 12, 639–660.

Hendry, S. H., and Jones, E. G. (1991). GABA neuronal subpopulations in cat primary auditory cortex: co-localization with calcium binding proteins. *Brain Res.* 543, 45–55.

Hendry, S. H., Schwark, H. D., Jones, E. G., and Yan, J. (1987). Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. *J. Neurosci.* 7, 1503–1519.

Heuer, H., Christ, S., Friedrichsen, S., Brauer, D., Winckler, M., Bauer, K., and Raivich, G. (2003). Connective tissue growth factor: a novel marker of layer VII neurons in the rat cerebral cortex. *Neuroscience* 119, 43–52.

Higo, S., Udaka, N., and Tamamaki, N. (2007). Long-range GABAergic projection neurons in the cat neocortex. *J. Comp. Neurol.* 503, 421–431.

Hogan, D., and Berman, N. E. (1992). The development of neuropeptide Y immunoreactive neurons in cat visual cortical areas. *Brain Res. Dev. Brain Res.* 67, 343–369.

Huntley, G. W., Hendry, S. H., Killackey, H. P., Chalupa, L. M., and Jones, E. G. (1988). Temporal sequence of neurotransmitter expression by developing neurons of fetal monkey visual cortex. *Brain Res.* 471, 69–96.

Jensen, A., Vaihinger, H. M., and Meier, C. (2003). [Perinatal brain damage--from neuroprotection to neuroregeneration using cord blood stem cells]. *Med. Klin. (Munich)* 98(Suppl. 2), 22–26.

Kanold, P. O. (2004). Transient microcircuits formed by subplate neurons and their role in functional development of thalamocortical connections. *Neuroreport* 15, 2149–2153.

Kanold, P. O., Kara, P., Reid, R. C., and Shatz, C. J. (2003). Role of subplate neurons in functional maturation of visual cortical columns. *Science* 301, 521–525.

Kanold, P. O., and Shatz, C. J. (2006). Subplate neurons regulate maturation of cortical inhibition and outcome of ocular dominance plasticity. *Neuron* 51, 627–638.

Kirkpatrick, B., Conley, R. C., Kakoyannis, A., Reep, R. L., and Roberts, R. C. (1999). Interstitial cells of the white matter in the inferior parietal cortex in schizophrenia: an unbiased cell-counting study. *Synapse* 34, 95–102.

Kostovic, I., and Judas, M. (2006). Prolonged coexistence of transient and permanent circuitry elements in the developing cerebral cortex of fetuses and preterm infants. *Dev. Med. Child Neurol.* 48, 388–393.

Kostovic, I., and Rakic, P. (1980). Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. *J. Neurocytol.* 9, 219–242.

Kostovic, I., and Rakic, P. (1990). Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J. Comp. Neurol.* 297, 441–470.

Kristt, D. A. (1979). Development of neocortical circuitry: histochemical localization of acetylcholinesterase in relation to the cell layers of rat somatosensory cortex. *J. Comp. Neurol.* 186, 1–15.

Lai, T., Jabaudon, D., Molyneaux, B. J., Azim, E., Arlotta, P., Menezes, J. R., and Macklis, J. D. (2008). SOX5 controls the sequential generation of distinct corticofugal neuron subtypes. *Neuron* 57, 232–247.

Lauder, J. M., Han, V. K., Henderson, P., Verdoorn, T., and Towle, A. C. (1986). Prenatal ontogeny of the GABAergic system in the rat brain: an immunocytochemical study. *Neuroscience* 19, 465–493.

Lavdas, A. A., Grigoriou, M., Pachnis, V., and Parnavelas, J. G. (1999). The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J. Neurosci.* 19, 7881–7888.

Lazar, N. L., Rajakumar, N., and Cain, D. P. (2008). Injections of NGF into neonatal frontal cortex decrease social interaction as adults: a rat model of schizophrenia. *Schizophr. Bull.* 34, 127–136.

Lein, E. S., Finney, E. M., McQuillen, P. S., and Shatz, C. J. (1999). Subplate neuron ablation alters neurotrophin expression and ocular dominance column formation. *Proc. Natl. Acad. Sci. U. S. A.* 96, 13491–13495.

Letinic, K., and Rakic, P. (2001). Telencephalic origin of human thalamic GABAergic neurons. *Nat. Neurosci.* 4, 931–936.

Letinic, K., Zoncu, R., and Rakic, P. (2002). Origin of GABAergic neurons in the human neocortex. *Nature* 417, 645–649.

Leviton, A., and Gressens, P. (2007). Neuronal damage accompanies perinatal white-matter damage. *Trends Neurosci.* 30, 473–478.

Levitt, P. (2005). Disruption of interneuron development. *Epilepsia* 46(Suppl. 7), 22–28.

Lewis, D. A., Hashimoto, T., and Volk, D. W. (2005). Cortical inhibitory neurons and schizophrenia. *Nat. Rev.* 6, 312–324.

Luhmann, H. J., Reiprich, R. A., Hanganu, I., and Kilb, W. (2000). Cellular physiology of the neonatal rat cerebral cortex: intrinsic membrane properties, sodium and calcium currents. *J. Neurosci. Res.* 62, 574–584.

Luskin, M. B., Pearlman, A. L., and Sanes, J. R. (1988). Cell lineage in the cerebral cortex of the mouse studied *in vivo* and *in vitro* with a recombinant retrovirus. *Neuron* 1, 635–647.

Maric, D., Liu, Q. Y., Maric, I., Chaudry, S., Chang, Y. H., Smith, S. V., Sieghart, W., Fritschy, J. M., and Barker, J. L. (2001). GABA expression dominates neuronal lineage progression in the embryonic rat neocortex and facilitates neurite outgrowth via GABA(A) autoreceptor/Cl– channels. *J. Neurosci.* 21, 2343–2360.

Marin, O., and Rubenstein, J. L. (2001). A long, remarkable journey: tangential migration in the telencephalon. *Nat. Rev.* 2, 780–790.

Marin-Padilla, M. (1978). Dual origin of the mammalian neocortex and evolution of the cortical plate. *Anat. Embryol.* 152, 109–126.

Marín-Padilla, M. (1988). Early ontogenesis of the human cerebral cortex. In Cerebral Cortex, Vol. VII, Development and Maturation of the Cerebral Cortex, A. Peters, and E. G. Jones, eds, (New York, Plenum), pp. 1–30.

Marin-Padilla, M., and Marin-Padilla, T. M. (1982). Origin, prenatal development and structural organization of layer I of the human cerebral (motor) cortex. A Golgi study. *Anat. Embryol.* 164, 161–206.

McConnell, S. K., Ghosh, A., and Shatz, C. J. (1989). Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science* 245, 978–982.

McDonald, C. T., and Burkhalter, A. (1993). Organization of long-range inhibitory connections with rat visual cortex. *J. Neurosci.* 13, 768–781.

McQuillen, P. S., and Ferriero, D. M. (2005). Perinatal subplate neuron injury: implications for cortical development and plasticity. *Brain Pathol.* 15, 250–260.

Meinecke, D. L., and Peters, A. (1987). GABA immunoreactive neurons in rat visual cortex. *J. Comp. Neurol.* 261, 388–404.

Metin, C., Vallee, R. B., Rakic, P., and Bhide, P. G. (2008). Modes and mishaps of neuronal migration in the mammalian brain. *J. Neurosci.* 28, 11746–11752.

Meyer, G., Soria, J. M., Martinez-Galan, J. R., Martin-Clemente, B., and Fairen, A. (1998). Different origins and developmental histories of transient neurons in the marginal zone of the fetal and neonatal rat cortex. *J. Comp. Neurol.* 397, 493–518.

Miyoshi, G., Bessho, Y., Yamada, S., and Kageyama, R. (2004). Identification of a novel basic helix-loop-helix gene, Heslike, and its role in GABAergic neurogenesis. *J. Neurosci.* 24, 3672–3682.

Miyoshi, G., Butt, S. J., Takebayashi, H., and Fishell, G. (2007). Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. *J. Neurosci.* 27, 7786–7798.

Molnar, Z., and Cheung, A. F. (2006). Towards the classification of subpopulations of layer V pyramidal projection neurons. *Neurosci. Res.* 55, 105–115.

Molyneaux, B. J., Arlotta, P., Menezes, J. R., and Macklis, J. D. (2007). Neuronal subtype specification in the cerebral cortex. *Nat. Rev.* 8, 427–437.

Nagarajan, R., and Clancy, B. (2008). Phylogenetic proximity revealed by neurodevelopmental event timings. *Neuroinformatics* 6, 71–79.

Nie, F., and Wong-Riley, M. T. (1996). Metabolic and neurochemical plasticity of gamma-aminobutyric acid-immunoreactive neurons in the adult macaque striate cortex following monocular impulse blockade: quantitative electron microscopic analysis. *J. Comp. Neurol.* 370, 350–366.

Owens, D. F., and Kriegstein, A. R. (2002). Is there more to GABA than synaptic inhibition? *Nat. Rev.* 3, 715–727.

Peduzzi, J. D. (1988). Genesis of GABA-immunoreactive neurons in the ferret visual cortex. *J. Neurosci.* 8, 920–931.

Petanjek, Z., Berger, B., and Esclapez, M. (2009). Origins of cortical GABAergic neurons in the cynomolgus monkey. *Cereb. Cortex* 19, 249–262.

Petanjek, Z., Dujmovic, A., Kostovic, I., and Esclapez, M. (2008). Distinct origin of GABA-ergic neurons in forebrain of man, nonhuman primates and lower mammals. *Coll. Antropol.* 32(Suppl. 1), 9–17.

Pinon, M. C., Jethwa, A., Jacobs, E., Campagnoni, A., and Molnar, Z. (2009). Dynamic integration of subplate neurons into the cortical barrel field circuitry during postnatal development in the Golli-tau-eGFP (GTE) mouse. *J. Physiol.* 587, 1903–1915.

Pinto, A., Fuentes, C., and Pare, D. (2006). Feedforward inhibition regulates perirhinal transmission of neocortical inputs to the entorhinal cortex: ultrastructural study in guinea pigs. *J. Comp. Neurol.* 495, 722–734.

Price, D. J., Aslam, S., Tasker, L., and Gillies, K. (1997). Fates of the earliest generated cells in the developing murine neocortex. *J. Comp. Neurol.* 377, 414–422.

Princivalle, A., Regondi, M. C., Frassoni, C., Bowery, N. G., and Spreafico, R. (2000). Distribution of GABA(B) receptor protein in somatosensory cortex and thalamus of adult rats and during postnatal development. *Brain Res. Bull.* 52, 397–405.

Rakic, P. (1988). Specification of cerebral cortical areas. *Science* 241, 170–176.

Rakic, P. (2003). Developmental and evolutionary adaptations of cortical radial glia. *Cereb. Cortex* 13, 541–549.

Rakic, P. (2006). A century of progress in corticoneurogenesis: from silver impregnation to genetic engineering. *Cereb. Cortex* 16(Suppl. 1), i3–i17.

Reep, R. L. (2000). Cortical layer VII and persistent subplate cells in mammalian brains. *Brain Behav. Evol.* 56, 212–234.

Reep, R. L., and Goodwin, G. S. (1988). Layer VII of rodent cerebral cortex. *Neurosci. Lett.* 90, 15–20.

Robertson, R. T., Annis, C. M., Baratta, J., Haraldson, S., Ingeman, J., Kageyama, G. H., Kimm, E., and Yu, J. (2000). Do subplate neurons comprise a transient population of cells in developing neocortex of rats? *J. Comp. Neurol.* 426, 632–650.

Robinson, S., Petelenz, K., Li, Q., Cohen, M. L., Dechant, A., Tabrizi, N., Bucek, M., Lust, D., and Miller, R. H. (2005). Developmental changes induced by graded prenatal systemic hypoxic-ischemic insults in rats. *Neurobiol. Dis.* 18, 568–581.

Robinson, S., Qing, L., DeChant, A., and Cohen, M. L. (2006). Neonatal loss of gamma-aminobutyric acid pathway expression after human perinatal brain injury. *J. Neurosurg.*104, 396–408.

Romijn, H. J., Hofman, M. A., and Gramsbergen, A. (1991). At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby? *Early Hum. Dev.* 26, 61–67.

Schiffmann, S., Campistron, G., Tugendhaft, P., Brotchi, J., Flament-Durand, J., Geffard, M., and Vanderhaeghen, J. J. (1988). Immunocytochemical detection of GABAergic nerve cells in the human temporal cortex using a direct gamma-aminobutyric acid antiserum. *Brain Res.* 442, 270–278.

Silberberg, G., and Markram, H. (2007). Disynaptic inhibition between neocortical pyramidal cells mediated by Martinotti cells. *Neuron* 53, 735–746.

Smart, I. H., Dehay, C., Giroud, P., Berland, M., and Kennedy, H. (2002). Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb. Cortex* 12, 37–53.

Somogyi, P., Cowey, A., Halasz, N., and Freund, T. F. (1981). Vertical organization of neurones accumulating 3H-GABA in visual cortex of rhesus monkey. *Nature* 294, 761–763.

Somogyi, P., Hodgson, A. J., Smith, A. D., Nunzi, M. G., Gorio, A., and Wu, J. Y. (1984). Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. *J. Neurosci.* 4, 2590–2603.

Suarez-Sola, M. L., Gonzalez-Delgado, F. J., Pueyo-Morlans, M., Medina-Bolivar, O. C., Hernandez-Acosta, N. C., Gonzalez-Gomez, M., and Meyer, G. (2009). Neurons in the white matter of the adult human neocortex. *Front. Neuroanat.* 3, 7. doi: 10.3389/neuro.05.007.2009

Sur, M., and Rubenstein, J. L. (2005). Patterning and plasticity of the cerebral cortex. *Science* 310, 805–810.

Takahashi, T., Goto, T., Miyama, S., Nowakowski, R. S., and Caviness, V. S., Jr. (1999). Sequence of neuron origin and neocortical laminar fate: relation to cell cycle of origin in the developing murine cerebral wall. *J. Neurosci.* 19, 10357–10371.

Tamamaki, N., Fujimori, K. E., and Takauji, R. (1997). Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. *J. Neurosci.* 17, 8313–8323.

Tao, Z., Van Gool, D., Lammens, M., and Dom, R. (1999). NADPH-diaphorase-containing neurons in cortex, subcortical white matter and neostriatum are selectively spared in Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 10, 460–468.

Teague-Ross, T. J., Lantz, S. M., Davis, C., James, A. L., Oonthonpan, L., Hyde, J., Bratton, G. N., and Clancy, B. (2008). Rat cortical subplate comparisons across development and aging. *Society for Neuroscience Conference Abstract.* 232, 14.

Tomioka, R., Okamoto, K., Furuta, T., Fujiyama, F., Iwasato, T., Yanagawa, Y., Obata, K., Kaneko, T., and Tamamaki, N. (2005). Demonstration of long-range GABAergic connections distributed throughout the mouse neocortex. *Eur. J. Neurosci.* 21, 1587–1600.

Tomioka, R., and Rockland, K. S. (2007). Long-distance corticocortical GABAergic neurons in the adult monkey white and gray matter. *J. Comp. Neurol.* 505, 526–538.

Torres-Reveron, J., and Friedlander, M. J. (2007). Properties of persistent postnatal cortical subplate neurons. *J. Neurosci.* 27, 9962–9974.

Ulfig, N. (2002). Ganglionic eminence of the human fetal brain – new vistas. *Anat. Rec.* 267, 191–195.

Valverde, F., Lopez-Mascaraque, L., Santacana, M., and De Carlos, J. A. (1995). Persistence of early-generated neurons in the rodent subplate: assessment of cell death in neocortex during the early postnatal period. *J. Neurosci.* 15, 5014–5024.

Van Eden, C. G., Mrzljak, L., Voorn, P., and Uylings, H. B. (1989). Prenatal development of GABA-ergic neurons in the neocortex of the rat. *J. Comp. Neurol.* 289, 213–227.

Voigt, T., Opitz, T., and de Lima, A. D. (2001). Synchronous oscillatory activity in immature cortical network is driven by GABAergic preplate neurons. *J. Neurosci.* 21, 8895–8905.

Wang, D. D., and Kriegstein, A. R. (2009). Defining the role of GABA in cortical development. *J. Physiol.* 587, 1873–1879.

Watakabe, A., Komatsu, Y., Nawa, H., and Yamamori, T. (2006). Gene expression profiling of primate neocortex: molecular neuroanatomy of cortical areas. *Genes Brain Behav.* 5(Suppl. 1), 38–43.

Watts, D. J., and Strogatz, S. H. (1998). Collective dynamics of 'small-world' networks. *Nature* 393, 440–442.

Winer, J. A., Larue, D. T. (1989). Populations of GABAergic neurons and axons in layer I of rat auditory cortex. *Neuroscience* 33, 499–515.

Woo, T. U., Beale, J. M., and Finlay, B. L. (1991). Dual fate of subplate neurons in a rodent. *Cereb. Cortex* 1, 433–443.

Woo, T. U., and Finlay, B. L. (1996). Cortical target depletion and ingrowth of geniculocortical axons: implications for cortical specification. *Cereb. Cortex* 6, 457–469.

Woo, T. U., Walsh, J. P., and Benes, F. M. (2004). Density of glutamic acid decarboxylase 67 messenger RNA-containing neurons that express the N-methyl-D-aspartate receptor subunit NR2A in the anterior cingulate cortex in schizophrenia and bipolar disorder. *Arch. Gen. Psychiatry* 61, 649–657.

Yan, X. X., Garey, L. J., and Jen, L. S. (1996). Prenatal development of NADPH-diaphorase-reactive neurons in human frontal cortex. *Cereb. Cortex* 6, 737–745.

Yuste, R. (2005). Origin and classification of neocortical interneurons. *Neuron* 48, 524–527.

Zecevic, N., and Milosevic, A. (1997). Initial development of gamma-aminobutyric acid immunoreactivity in the human cerebral cortex. *J. Comp. Neurol.* 380, 495–506.

Zecevic, N., Milosevic, A., Rakic, S., and Marin-Padilla, M. (1999). Early development and composition of the human primordial plexiform layer: an immunohistochemical study. *J. Comp. Neurol.* 412, 241–254.

Zecevic, N., and Rakic, P. (1991). Synaptogenesis in monkey somatosensory cortex. *Cereb. Cortex* 1, 510–523.

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Subtypes of GABAergic neurons project axons in the neocortex

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γ-aminobutyric acid (GABA)ergic neurons in the neocortex have been regarded as interneurons and speculated to modulate the activity of neurons locally. Recently, however, several experiments revealed that neuronal nitric oxide synthase (nNOS)-positive GABAergic neurons project cortico-cortically with long axons. In this study, we illustrate Golgi-like images of the nNOS-positive GABAergic neurons using a nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) reaction and follow the emanating axon branches in cat brain sections. These axon branches projected cortico-cortically with other non-labeled arcuate fibers, contra-laterally via the corpus callosum and anterior commissure. The labeled fibers were not limited to the neocortex but found also in the fimbria of the hippocampus. In order to have additional information on these GABAergic neuron projections, we investigated green fluorescent protein (GFP)-labeled GABAergic neurons in GAD67-Cre knock-in/GFP Cre-reporter mice. GFP-labeled axons emanate densely, especially in the fimbria, a small number in the anterior commissure, and very sparsely in the corpus callosum. These two different approaches confirm that not only nNOS-positive GABAergic neurons but also other subtypes of GABAergic neurons project long axons in the cerebral cortex and are in a position to be involved in information processing.

Keywords: GABA, cerebral cortex, nicotinamide adenine dinucleotide phosphate diaphorase, nitric oxide synthase

INTRODUCTION

GABAergic neurons regulate information processing and are involved in oscillations in the cerebral cortex (Freund and Buzsáki, 1996; Cardin et al., 2009; Sohal et al., 2009). Generally they were thought to be interneurons and act locally. Recently, however, a body of evidence has indicated that GABAergic neurons in the neocortex also project over longer distances. According to our previous report, long-range GABAergic projections originated in layers II, VI and the underlying white matter in mouse neocortex (Tomioka et al., 2005). Several reports have also described the existence of long-range GABAergic projections in the rat's neocortex (Matsubara and Boyd, 1992; McDonald and Burkhalter, 1993). Those projections were not limited to the rodent brain but seemed to exist in feline (Higo et al., 2007) and in primate brain (Barone and Kennedy, 2000; Tomioka and Rockland, 2007).

GABAergic neurons with axons projecting in the ipsilateral hemisphere seem to have similar chemical features and often contain somatostatin (SS)-immunoreactivity (IR) (91%), neuropeptide Y (NPY)-IR (82%), and neuronal nitric oxide synthase (nNOS)-IR (71%) (Tomioka et al., 2005; Higo et al., 2007). Considering these observations, the fact that most nNOS-positive neurons are a subpopulation of SS- and NPY-IR neurons, and nNOS-, NPY-, and SS-triple-positive cells are less than 0.5% of GABAergic neurons (Kubota et al., 1994; Gonchar and Burkhalter, 1997), it was speculated that the nNOS-positive GABAergic projection neurons compose a very small subpopulation in the neocortical GABAergic neurons. In addition, co-localization of GABA synthesizing enzyme, glutamic acid decarboxylase at 67 K-dalton (GAD67)-IR and a retrograde tracer injected into contralateral hemisphere revealed that another subtype of GABAergic neurons may be involved in the contralateral projection (Gonchar et al., 1995; Kimura and Baughman, 1997; Fabri and Manzoni, 2004). Parvalbumin (PV)-IR GABAergic neurons in the medial septum have been shown to terminate preferentially on hippocampal GABAergic neurons, and act to regulate the activity of pyramidal neurons in the hippocampus (Freund and Antal, 1988). SS-IR GABAergic neurons in the hippocampus project back to the medial septum. This circuit was interpreted as the mechanism to cause theta oscillation (Toth et al., 1997). GABAergic projection neurons in the neocortex may act similarly, by inhibiting local GABAergic interneurons and, in turn, allowing cortical principal cell firing.

Although GABAergic projection neurons have been recognized as a subset of neocortical neurons, it is very hard to reveal the projection axons technically. Simple immunohistochemistry of markers like calcium binding proteins or short peptides did not reveal the full axon trajectory from the soma to the terminals. Although nNOS-IR also does not reveal projection axons, NADPH-d reaction reveals nNOS-IR GABAergic neurons in Golgi-like images including their projection fibers (Yan et al., 1996; Barone and Kennedy, 2000; Smiley et al., 2000; Higo et al., 2007). Thus we utilized the NADPH-d reaction to reveal the projection axons of nNOS-IR neurons in the neocortex. In addition, we introduce a new tool, GAD67-Cre knock-in mouse, in which DNA encoding Cre DNA-recombinase in P1 phage was targeted to the locus encoding GAD67. Since the vast majority of GABAergic neurons are expected to be labeled by GFP in the offsprings obtained by mating GAD67-Cre knock-in mouse and GFP Cre-reporter mouse, we expect that all the subtypes of GABAergic projection neurons will be revealed at a glance.

MATERIALS AND METHODS

All procedures were carried out according to the guidelines for the care and use of animals approved by the Animal Care and Use Committee at Kumamoto University in accordance with the National Institutes of Health (NIH).

PRODUCTION OF GAD67-Cre KNOCK-IN MOUSE

The generation of GAD67-Cre knock-in mice will be described into detail elsewhere (Akashi et al. in preparation). To generate the GAD67-knock-in Cre mice, we designed a targeting vector in which Cre recombinase gene was inserted into immediately after the translational initiation site of the *GAD67* gene in frame. A knock-in vector pGAD67CreTV contained a 3 kb fragment at the 5′ side, a Cre gene placed behind the GAD67 translational start, a *Pgk*-neo-p(A) cassette flanked by two Flp recognition target (frt) site, a 7 kb fragment at the 3′ side, and a MC1 promoter-driven diphtheria toxin gene.

Culture of embryonic stem (ES) cells and generation of chimeric mice were performed as described previously (Kitayama et al., 2001). Briefly, linearized pGAD67CreTV was introduced into C57BL/6 mouse ES cells and then, G418-resistant clones were picked up. Homologous recombined ES clone was identified by Southern blotting. To produce germline chimera, the selected ES cells were microinjected into eight cell-stage embryos of CD-1 mouse strain. The germline chimera of GAD67-Cre mice were crossed with C57BL/6 mice to generate the GAD67-Cre mice line. Because the knock-out of both GAD67 alleles is lethal at birth (Asada et al., 1997), mice heterozygous for the altered GAD67 allele were used for all the observations in this study. Genotypes were identified by Southern blot hybridization or PCR. Tail genomic DNA was digested with Spe-I or Afl-II and hybridized with 5′ probe or 3′ probe, respectively. PCR was performed with specific three primers. The sequence of each primer and the approximate length of the amplified DNA fragments are described as follows: *Gad1*^{Cre}, g67-2 (5′-TTCCGGAGGTACCACACCTT-3′), g67-5 (5′-TAAGTCGACGCTAGCGAGCGCCTCCCCA-3′), and CreR1 (5′-TTGCCCCTGTTTCACTATCC-3′); wild type, 1.8 kbp; mutant: 1.4 kbp. The GAD67-Cre knock-in mice were mated with a GFP Cre-reporter mouse (Tanahira et al., 2009). Cre-positive GABAergic neurons are revealed by GFP-expression in the offsprings.

ANIMALS AND RECOVERY OF BRAINS

In this experiment, three adult cats (2.5- to 4-kg body weight) and five GAD67-Cre knock-in/GFP Cre-reporter mice were used. Cats were anesthetized with ketamine (40 mg/kg, i.m.) and Nembutal (50 mg/kg, i.p.). The animals were perfused with phosphate-buffered saline at pH 7.4 (PBS), followed by fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) through the left ventricle. Brains were removed, blocked, saturated with a cold solution of 30% phosphate-buffered sucrose overnight, cut serially on a freezing microtome into 50-μm thick coronal sections, and every fifteenth section was collected and used for NADPH-d reaction.

Mice were anesthetized with Nembutal (50 mg/kg i.p.), and perfused with PBS and the same fixative used for cats. Brains were removed, saturated with a cold solution of 30% phosphate-buffered sucrose overnight, and cut serially on a freezing microtome into 50 μm thick coronal sections, and every sixth section was collected and used for GFP-immunohistochemistry or NADPH-d reaction.

VISUALIZATION OF nNOS-POSITIVE NEURONS AND GFP-POSITIVE NEURONS

The procedure for NADPH-d staining was performed according to the method of Vincent and Kimura (1992). Briefly, floating sections were incubated in a solution of 0.1 M phosphate buffer, 0.3% Triton X-100, 0.1 mg/ml nitroblue tetrazolium, and 1.0 mg/ml β-NADPH at 37°C (pH 7.4) for 60 min. Sections were rinsed three times in 0.1 M phosphate buffer, 5 min per rinse, and mounted on gelatinized slides. Sections were then counterstained with 0.3% Neutral red.

Brain sections of GAD67-Cre knock-in mouse/GFP Cre-reporter mouse were incubated with a primary antibody against GFP, and the immunoreactive site was visualized by DAB reaction as reported previously (Tamamaki et al., 2000). This procedure allowed us to carry out the morphometry of GFP-positive structures in blight-field microscopy.

COUNTING NADPH-d POSITIVE FIBERS IN THE WHITE MATTER

To estimate the number of NADPH-d positive fibers in the white matter, we set 200-μm-site where labeled fibers could be traced as long as possible in the white matter. Then we counted labeled fibers in more than five windows. Since thickness of the sections was 50 μm, we could estimate the number of labeled fibers contained in 1×10^4 μm^2 of the cut end of the white matter.

RESULTS

NADPH-d POSITIVE CELLULAR STRUCTURES IN THE CAT NEOCORTEX

To reveal axons of nNOS-positive GABAergic neurons, we employed NADPH-d histochemistry in cat cerebral cortex. NADPH-d staining reveals large non-pyramidal neurons (type I) and small non-pyramidal neurons (type II) with dark-blue precipitate in Golgi-like images (Higo et al., 2007) (**Figure 1A**). nNOS-IR is co-localized with the precipitate in type I cell but not in the type II cells. These two types of NADPH-d-positive cells have been reported in other experimental species (Monkey: Yan and Garey, 1997; Smiley et al., 2000; Rat: Valtschanoff et al., 1993; Kubota et al., 1994) and also in human brain (Judas et al., 1999).

Somata of type I cells are found in the deep layers and white matter of the neocortex (**Figures 1A,B**). The number of type I cells is highest at the gray matter and white matter boundary. According to the depth in the white matter, the number of type I cells is reduced, and these are almost completely absent near the lateral ventricle (**Figure 1B**). On the other hand, somata of type II cells are found in the neocortical layers II–VI (Higo et al., 2007). NADPH-d positive cells are also found in the basal ganglia. Since the internal capsule is almost free of NADPH-d positive fibers, the NADPH-d positive fibers and their terminals in the cerebral cortex are assumed to belong to the type I and II NADPH-d positive cells in the cerebral cortex.

NADPH-d positive fibers branch and ramify in the gray matter of the neocortex (**Figure 1A**). On the other hand, NADPH-d positive thick fibers did not seldom branch in the white matter (**Figure 1C**). In parallel with the number of type I cells in the white

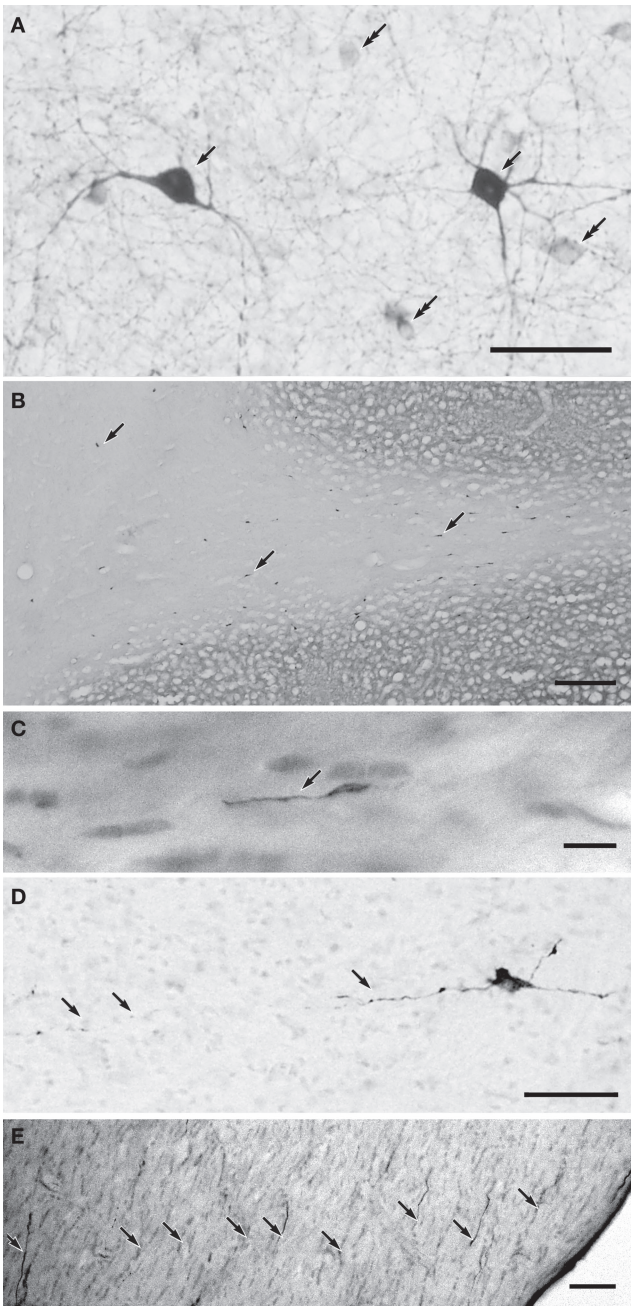

FIGURE 1 | (A) NADPH-d positive GABAergic neurons in the middle suprasylvian gyrus of the cat neocortex. **(B)** A low magnification photograph of a section with NADPH-d positive neurons in the middle suprasylvian gyrus. **(C)** A NADPH-d positive axon fiber fragments in the white matter of the neocortex. **(D)** An NADPH-d-positive type I cell extending an NADPH-d-positive axon fiber in the white matter of the neocortex. **(E)** NADPH-d positive axon fiber fragments in the fimbria. Arrows indicate NADPH-d positive type I cells and their axons. Double-arrows in **(A)** indicate NADPH-d type II cells. Calibration bars in **(A–E)** are 10 μm, 300 μm, 10 μm, 50 μm, and 50 μm, respectively.

matter, the number of NADPH-d positive thick axons also changes: they are highest at the boundary between the gray matter and the white matter and reduced in the deeper white matter. Moreover, large NADPH-d-positive cells originate NADPH-d positive thick axon in the white matter (**Figure 1D**). The labeled thick axons occur in fascicles and are extended with following the trajectory of other non-labeled axons at every depth of the white matter. We could not find the thick labeled axons giving rise large branches or turning to ascend to the gray matter. We found fragmented thick axons, many in the fimbria ($8.8 \pm 2.1/10^4$ μm^2; $n = 5$) (**Figure 1E**), a small number in the anterior commissure (1.2 ± 1.2 μm^2; $n = 5$), and only one in five windows in the corpus callosum (0.2 ± 0.4 μm^2; $n = 5$) (see Materials and Methods).

GABAergic PROJECTION AXONS IN THE MOUSE WHITE MATTER

Three brains were obtained from the mice at 10-weeks old and sectioned into coronal sections at 50-μm thickness. The GFP-positive cells in the mouse brain were distributed similarly to the GFP-positive cells in the GAD67-GFP knock-in mouse (Tamamaki et al., 2003). However, GFP-positive cells in the GAD67-Cre/GFP-reporter mouse are significantly less in number than that in GAD67-GFP mouse. GFP-IR is similar in every GFP-positive cells because the expression of GFP is driven by chick actin (CA)-promoter in all the GFP-positive cells.

Axon fibers with GFP-IR fill the neocortical grey matter, although individual fibers are hard to resolve. GFP-positive axon fibers also occur in the white matter. They can be traced in a few serial sections, but not sufficiently far to determine whether the axon fibers belong to GABAergic projection neurons or not. Therefore, we directed our attention to the fimbria, the anterior commissure, and the corpus callosum. We found a large number of labeled axon segments in the fimbria (**Figure 2A**). The density of the labeled axons in the fimbria is much higher than that of the NADPH-d positive fibers in the cat fimbria (**Figure 1D**). A small number of labeled axons traveled in the anterior commissure (**Figure 2B**), and a few sparsely in the corpus callosum.

The NADPH-d reaction in mouse brain sections does not reveal nNOS-positive neurons in Golgi-like fashion. Although NADPH-d reactions revealed somata and dendrites of type I-like non-pyramidal neurons in mouse neocortex, NADPH-d positive axons appear as dotted-lines and it was difficult to trace their trajectories. The number of labeled axon fragments is countable only in the fimbria. We found similar number of fragmented NADPH-d-labeled axons in the mouse fimbria ($3.7 \pm 1.5/10^4$ μm^2; $n = 6$), (**Figure 2C**) to the case in the cat fimbria.

DISCUSSION

The present study confirmed the presence of a subpopulation of GABAergic neurons with long projection axons in the cat and mouse neocortex. Moreover, we added the evidence that the GABAergic neurons with long projection axons include subpopulations larger than the nNOS-positive GABAergic neurons, especially in the archicortex.

NADPH-d-positive somata in the white matter originate thick NADPH-d-positive fibers in the white matter of the cat neocortex, which emanate with other non-labeled arcuate fibers, without originating any thick branches, and without bending toward the gray matter of the neocortex. These features may indicate that the total length of NADPH-d-positive fibers in the white matter is long enough to be called as projection fibers, while we regarded

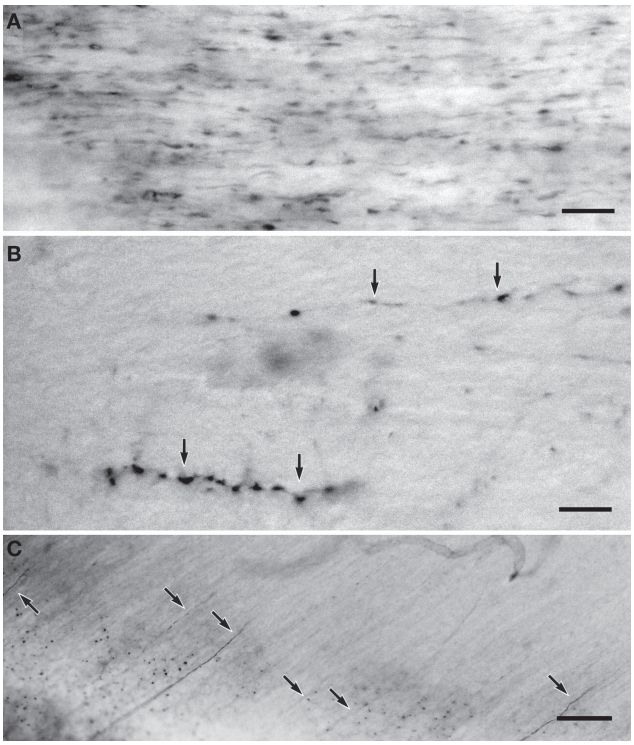

FIGURE 2 | (A) GFP-positive axon fibers in the fimbria of the GAD67-Cre/GFP Cre-reporter mouse brain. **(B)** GFP-positive axon fibers in the anterior commissure. **(C)** NADPH-d positive axon fiber fragments in the mouse fimbria. Calibration bar in **(A,B)** are 10 μm; calibration bar in **(C)** is 30 μm.

GABAergic neuron extending an axon longer than 1.5 mm from the soma as the GABAergic projection neuron in the mouse (Tomioka et al., 2005). The NADPH-d-positive fibers in the grey matter of the neocortex may include fibers originating from both type I and type II cells.

Formerly, we produced GAD67-GFP knock-in mouse in order to observe GAD67-positive cells and axon fibers traveling in the central nervous system (Tamamaki et al., 2003). The GFP-positive axon fibers were clearly revealed by GFP-fluorescence and could be traced long distances in the brain stem. However, projection axons belonging to the GABAergic neurons in the neocortex seemed to be thin and difficult to identify in the GAD67-GFP mouse. In addition, GAD67-promoter activity seemed to vary in each GABAergic neuron subtype, and the intensity of GFP-fluorescence differed from cell to cell (Tamamaki et al., 2003). With the approach reported here, however, Cre DNA-recombinase deletes floxed stop in the GFP Cre-reporter construct, and GFP was expressed depending on the CA-promoter in the construct (Tanahira et al., 2009). Since Cre-expression levels depend on the GAD67-promoter, some subtypes of GABAergic neurons with weak GAD67-promoter activity may fail to delete floxed stop and may not be revealed by GFP-expression. As the result, we may have underestimated the GABAergic neurons projecting contra-laterally via the corpus callosum (Gonchar et al., 1995; Kimura and Baughman, 1997; Fabri and Manzoni, 2004). We feel necessity of introducing additional techniques to estimate the number of GABAergic neurons projecting contra-laterally.

NADPH-d reaction labeled axon fibers of nNOS-positive GABAergic neurons in cats and mice, while GFP-immunohistochemistry in GAD67-Cre/GFP Cre-reporter mouse labeled both projection fibers originating in nNOS-positive GABAergic neurons and those in the other subtypes of GABAergic neurons. However, the distribution pattern of the labeled axon fragments was similar in both preparations. NADPH-d reactive axons and GFP-IR axons were found to be very sparse in the corpus callosum, sparsely in the anterior commissure, and many in the fimbria. Labeled fibers in the fimbria will include afferent- and efferent-fibers from and to the subcortical nuclei, in addition to the commissural fibers through the ventral hippocampal commissure. Excluding subcortical afferent- and efferent-fibers, we speculate that the GABAergic commissural fibers reciprocally interconnect archi- and paleo-cortex.

When GABAergic projection neurons terminate on GABAergic interneurons, they will inhibit the interneurons and thus disinhibit excitatory principle neurons in the hippocampus (Freund and Antal, 1988; Toth et al., 1997). Although NADPH-d positive fibers in cat neocortex ramified and seemed to terminate on many types of neurons (i.e. both principle neurons and inhibitory neurons), NADPH-d-positive fibers originating in the type I cells may innervate GABAergic neurons preferentially. To address this possibility, we plan to investigate the terminal of labeled nNOS-positive axon fibers in future. One of the important results elucidated in this study is that a large number of GABAergic neurons are also involved in the cortico-fugal, cortico-cortical, and callosal projections. Each subtype of GABAergic projection neuron may contribute to information processing in the neocortex in different ways.

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REFERENCES

Asada, H., Kawamura, Y., Maruyama, K., Kume, H., Ding, R. G., Kanbara, N., Kuzume, H., Sanbo, M., Yagi, T., and Obata, K. (1997). Cleft palate and decreased brain gamma-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. *Proc. Natl. Acad. Sci. U.S.A.* 94, 6496–6499.

Barone, P., and Kennedy, H. (2000). Non-uniformity of neocortex: areal heterogeneity of NADPH-diaphorase reactive neurons in adult macaque monkey. *Cereb. Cortex* 10, 160–174.

Cardin, J. A., Carlén, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., Tsai, L-H., and Moore, C. I. (2009). Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 459, 663–667.

Fabri, M., and Manzoni, T. (2004). Glutamic acid decarboxylase immunoreactivity in callosal projecting neurons of cat and rat somatic sensory areas. *Neuroscience* 123, 557–566.

Freund, T. F., and Antal, M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature* 336, 170–173.

Freund, T. F., and Buzsáki, G. (1996). Interneurons of the hippocampus. *Hippocampus* 6, 347–470.

Gonchar, Y., and Burkhalter, A. (1997). Three distinct families of GABAergic neurons in rat visual cortex. *Cereb. Cortex* 7, 347–358.

Gonchar, Y. A., Johnson, P. B., and Weinberg, R. J. (1995). GABA-immunopositive neurons in rat neocortex with contralateral projections to S-I. *Brain Res.* 697, 27–34.

Higo, S., Udaka, N., and Tamamaki, N. (2007). Long-range GABAergic

projection neurons in the cat neocortex. *J. Comp. Neurol.* 503, 421–431.

Judas, M., Sestan, N., and Kostović, I. (1999). Nitrinergic neurons in the developing and adult human telencephalon: Transient and permanent patterns of expression in comparison to other mammals. *Microsc. Res. Tech.* 45, 401–419.

Kimura, F., and Baughman, R. W. (1997). GABAergic transcallosal neurons in developing rat neocortex. *Eur. J. Neurosci.* 9, 1137–1143.

Kitayama, K., Abe, M., Kakizaki, T., Honma, ., Natsume, R., Fukaya, M., Watanabe, M., Miyazaki, J., Mishina, M., and Sakimura, K. (2001). Purkinje cell-specific and inducible gene recombination system generated from C57BL/6 mouse ES cells. *Biochem. Biophys. Res. Commun.* 281, 1134–1140.

Kubota, Y., Hattori, R., and Yui, Y. (1994). Three distinct subpopulation of GABAergic neurons in rat frontal agranular cortex. *Brain Res.* 649, 159–173.

Matsubara, J. A., and Boyd, J. D. (1992). Presence of GABA-immunoreactive neurons within intracortical patches in area 18 of the cat. *Brain Res.* 583, 161–170.

McDonald, C. T., and Burkhalter, A. (1993). Organization of long-range inhibitory connections within rat visual cortex. *J. Neruosci.* 13, 768–781.

Smiley, J. F., McGinnis, J. P., and Javitt, D. C. (2000). Nitric oxide synthase interneurons in the monkey cerebral cortex are subsets of the somatostatin, neuropeptide Y, and calbindin cells. *Brain Res.* 863, 205–212.

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

Tamamaki, N., Nakamura, K., Furuta, T., Asamoto, K., and Kaneko, T. (2000). Neurons in Golgi-stain-like images revealed by GFP-adenovirus infection *in vivo*. *Neurosci. Res.* 38, 231–236.

Tamamaki, N., Yanagawa, Y., Tomioka, R., Miyazaki, J., Obata, K., and Kaneko, T. (2003). Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. *J. Comp. Neurol.* 467, 60–79.

Tanahira, C., Higo, S., Watanabe, K., Tomioka, R., Ebihara, S., Kaneko, T., and Tamamaki, N. (2009). Parvalbumin neurons in the forebrain as revealed by parvalbumin-Cre transgenic mice. *Neurosci. Res.* 63, 213–223.

Tomioka, R., Okamoto, K., Furuta, T., Fujiyama, F., Iwasato, T., Yanagawa, Y., Obata, K., Kaneko, T., and Tamamaki, N. (2005). Demonstration of long-range GABAergic connections distributed throughout the mouse neocortex. *Eur. J. Neurosci.* 21, 1587–1600.

Tomioka, R., and Rockland K. S. (2007). Long-distance corticocortical GABAergic neurons in the adult monkey white and gray matter. *J. Comp. Neurol.* 505, 526–538.

Toth, K., Freund, T. F., and Miles, R. (1997). Disinhibition of rat hippocampal pyramidal cells by GABAergic afferents from the septum. *J. Physiol. (Lond.)* 500, 463–474.

Valtschanoff, J. G., Weinberg, R. J., Kharazia, V. N., Schmidt, H. H., Nakane, M., and Rustioni, A. (1993). Neurons in rat cerebral cortex that synthesize nitric oxide: NADPH-diaphorase histochemistry, NOS immunocytochemistry, and colocalization with GABA. *Neurosci. Lett.* 157, 157–161.

Vincent, S. R., and Kimura, H. (1992). Histochemical mapping of nitric oxide synthase in the rat brain. *Neuroscience* 46, 755–784.

Yan, X. X., and Garey, L. J. (1997). Morphological diversity of nitric oxide synthesizing neurons in mammalian cerebral cortex. *J. Hirnforsch.* 38, 165–172.

Yan, X. X., Jen, L. S., and Garey, L. J. (1996). NADPH-diaphorase-positive neurons in primate cerebral cortex colocalize with GABA and calcium-binding proteins. *Cereb. Cortex* 6, 524–529.

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Structural organization of long-range GABAergic projection system of the hippocampus

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GABA is a key mediator of neural activity in the mammalian central nervous system, and a diverse set of GABAergic neurons utilize GABA as a transmitter. It has been widely accepted that GABAergic neurons typically serve as interneurons while glutamatergic principal cells send excitatory signals to remote areas. In general, glutamatergic projection neurons monosynaptically innervate both principal cells and local GABAergic interneurons in each target area, and these GABAergic cells play a vital role in modulation of the activity of principal cells. The formation and recall of sensory, motor and cognitive representations require coordinated fast communication among multiple areas of the cerebral cortex, which are thought to be mostly mediated by glutamatergic neurons. However, there is an increasing body of evidence showing that specific subpopulations of cortical GABAergic neurons send long-range axonal projections to subcortical and other cortical areas. In particular, a variety of GABAergic neurons in the hippocampus project to neighboring and remote areas. Using anatomical, molecular and electrophysiological approaches, several types of GABAergic projection neurons have been shown to exist in the hippocampus. The target areas of these cells are the subiculum and other retrohippocampal areas, the medial septum and the contralateral dentate gyrus. The long-range GABAergic projection system of the hippocampus may serve to coordinate precisely the multiple activity patterns of widespread cortical cell assemblies in different brain states and among multiple functionally related areas.

Keywords: GABAergic neuron, long-range projection, hippocampus, medial septum, subiculum, retrosplenial cortex, multiregional coordination

INTRODUCTION

GABA is a key mediator of neural activity in the mammalian central nervous system, and a diverse set of GABAergic neurons utilize GABA as a transmitter. The majority of cortical GABAergic neurons are considered to work as local interneurons. Excitatory glutamatergic projection neurons monosynaptically innervate both principal cells and local GABAergic interneurons in each target area, where GABAergic synaptic inhibition strictly regulates the spatial and temporal extent of neuronal activity. As such, topographically organized GABAergic inputs are essential to regulate sleep, arousal, cognition, locomotion and mood. For instance, a specific subset of GABAergic neurons in the cerebral cortex can be part of the neurobiological substrate that underlies homeostatic sleep regulation (Gerashchenko et al., 2008). In addition, recent studies have emphasized that dysfunctions in the GABAergic system might be associated with various pathological conditions including epilepsy, Parkinson's disease, Alzheimer's disease and mental illness (Kalueff and Nutt, 2007). In particular, increasing evidence suggests the possible involvement of GABA in the neurobiology of mood disorder and the mechanisms of antidepressant action (Gos et al., 2009; Jinno and Kosaka, 2009; Sanacora and Saricicek, 2007).

It has been well established that the formation and recall of sensory, motor and cognitive representations require coordinated communication among multiple areas of the cerebral cortex, which are mostly mediated by long-range glutamatergic projections. Interestingly, a number of studies show that specific subpopulations of cortical GABAergic neurons also send long-range inhibitory projections to subcortical and other cortical areas. For instance, the medial septum is one of the extrahippocampal targets of a subset of GABAergic neurons in the hippocampus (Alonso and Köhler, 1982). The existence of additional remote targets of hippocampal GABAergic neurons, such as subiculum and retrosplenial cortex, has also been demonstrated (Jinno et al., 2007; Losonczy et al., 2002; Miyashita and Rockland, 2007; van Groen and Wyss, 2003). Long-range GABAergic projections have also been described in the isocortex (Fabri and Manzoni, 2004; Gonchar et al., 1995; Jinno and Kosaka, 2004; Tomioka and Rockland, 2007).

Starting with the seminal work of Ramón y Cajal (1911) revealing the morphological features of cortical neurons, many studies reported the diversity of GABAergic interneurons in the cortex based on anatomical, neurochemical and electrophysiological analyses (DeFelipe, 1993; Freund and Buzsáki, 1996; Maccaferri and Lacaille, 2003; Markram et al., 2004). Current evidence indicates that distinct classes of GABAergic neurons specifically coordinate the activity of cortical pyramidal neurons in a spatially and temporally different and brain-state-dependent manner (Klausberger and Somogyi, 2008). In this article, I aimed to summarize the recent findings and outline the structural organization of GABAergic projection system of the hippocampus.

EXTRA HIPPOCAMPAL TARGETS OF GABAergic PROJECTION SYSTEM OF THE HIPPOCAMPUS

The diagram of the long-range GABAergic projection system of the hippocampus is schematically summarized in **Figure 1**. In this section, I describe the diversity of GABAergic neurons projecting to the following major extrahippocampal targets: medial septum, subiculum, retrosplenial cortex and contralateral dentate gyrus (cDG). Using *in vivo* recording (Jinno et al., 2007), we have previously reported that some of the GABAergic neurons project to multiple areas, such as the medial septum and the subicular area (i.e., double projection cells). Here, I discuss the characteristics of GABAergic neurons innervating each target separately, and then summarize the significance of multiple targeting cells in the following section.

MEDIAL SEPTUM

The most studied remote target of the hippocampal GABAergic projection neurons is the medial septum (**Figure 1**). Using intraseptal injection of horseradish peroxidase as the retrograde tracer, Alonso and Köhler (1982) found that a subset of GABAergic neurons of

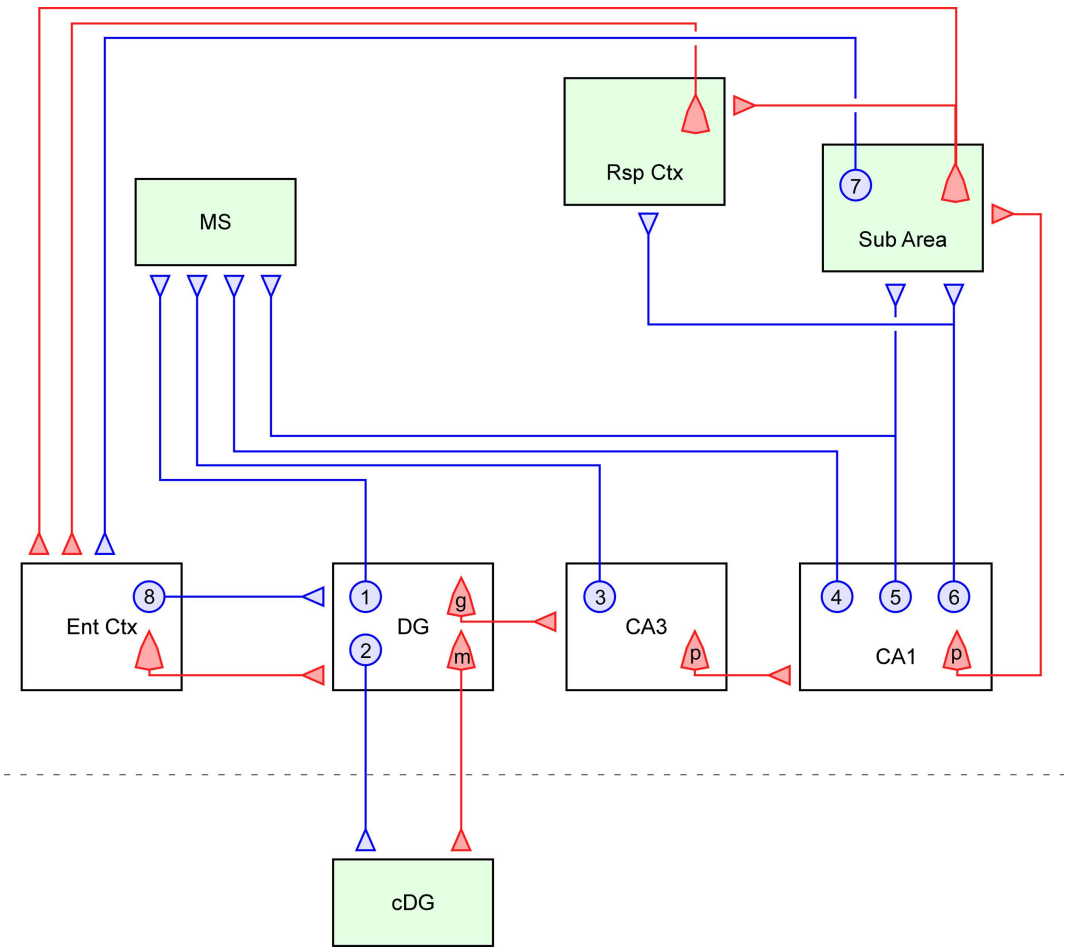

FIGURE 1 | Summary diagram of the GABAergic efferents of the hippocampus. The long-range GABAergic projection system (blue) and major glutamatergic circuit (red) of the hippocampus and related limbic structures are schematically described. Four extrahippocampal targets are colored in green: medial septum (MS), subiculum (Sub Area), retrosplenial cortex (Rsp Ctx) and contralateral dentate gyrus (cDG). This figure highlights the efferent GABAergic pathway of the hippocampus, and thus its local collaterals and related glutamatergic circuits are only partially described. In the dentate gyrus, some of the hilar GABAergic neurons project to the medial septum (1) and others project to the cDG via the commissural pathway (2). The existence of GABAergic cells projecting to both the medial septum and the cDG has not been proven. The granule cells (g) in the dentate gyrus (DG) send mossy fiber axons to the ipsilateral CA3 area, while hilar mossy cells (m) send commissural axon to the contralateral side of the dentate gyrus. In the CA3 region, GABAergic cells projecting to the medial septum are scattered throughout all the layers (3). Some of the CA3 pyramidal cells (p) project back to the dentate gyrus, but they were omitted from this diagram for simplicity. In the CA1 region, there are three types of GABAergic projection neurons, i.e., those projecting exclusively to the medial septum (4), projecting to both the medial septum and subicular/retrosplenial area (5), and projecting exclusively to the subicular/retrosplenial area (6). In the CA1 region, the pyramidal cells (p) project not only to the subicular area but also to the lateral septum (not shown). In addition to the above hippocampal GABAergic projection neurons, earlier studies have also shown the existence of other interareal GABAergic connections in parallel to the glutamatergic circuits. For instance, the projection from the presubiculum comprises a small inhibitory component from GABAergic neurons (7) and targets entorhinal interneurons (van Haeften et al., 1997). The perforant path that originates from the entorhinal cortex may also have a small GABAergic component (8) in addition to the main glutamatergic projection (Germroth et al., 1989).

the hippocampus innervate the medial septum (H-MS cells) in the rat brain. The H-MS cells were found in all regions of the hippocampus, but they were distributed in a layer specific manner: in the CA1 region, they were mainly located in the stratum oriens; in the CA3 region, they were scattered throughout all the layers; and in the dentate gyrus (DG), they were exclusively located in the hilar area (**Figure 2A**, Jinno and Kosaka, 2002; Totterdell and Hayes, 1987).

The morphological characteristics of H-MS cells were examined using fixed slice preparations of the rat hippocampus (Schwerdtfeger and Buhl, 1986), and the following cells were identified: stellate cells in the hilus, horizontal basket cells in the stratum oriens of CA1 and CA3, stellate cells in the stratum radiatum of CA3 and pyramid-like cells in the stratum radiatum of CA1. The main postsynaptic targets of H-MS cells in the medial septum were

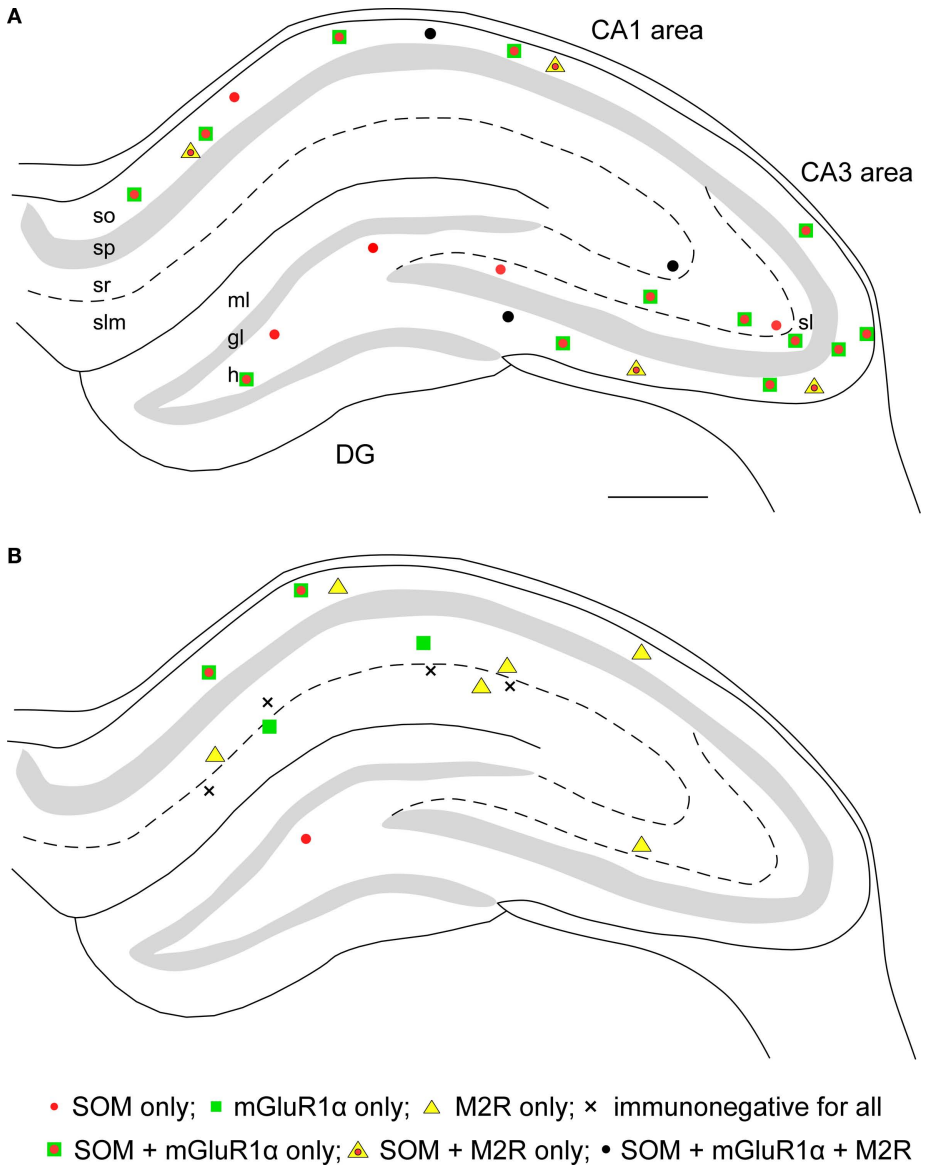

FIGURE 2 | Distributions of retrogradely labeled GABAergic projection neurons following Fluoro-gold injection into the medial septum (A) or the subiculum (B). Each panel represents a 70-μm thick triple immunofluorescence labeled section for somatostatin (SOM), metabotropic glutamate receptor 1α (mGluR1α) and muscarinic acetylcholine receptor type 2 (M2R). **(A)** The H-MS cells are distributed in all regions of the hippocampus. In the CA1 area, most of them are in the stratum oriens (so). In the CA3 area, they are in the all layers, whereas they are restricted to the hilus (h) in the dentate gyrus (DG). Virtually all H-MS cells are SOM-positive, and the majority of them are SOM-positive/mGluR1α-positive/M2R-negative. In addition, there are also some triple-positive cells and SOM-positive/mGluR1α-negative/M2R-positive cells. **(B)** The H-Sub cells are mainly present in the CA1 region, and rarely found in the CA3 region and in the dentate gyrus. In the CA1 area, SOM-positive/mGluR1α-positive/M2R-negative projection cells are mostly detected in the stratum oriens, whereas SOM-negative/mGluR1α-negative/M2R-positive cells are present throughout all layers. In addition, SOM-negative/mGluR1α-positive/M2R-negative cells are distributed in strata radiatum (sr) and lacunosum-moleculare (slm). There are a few H-Sub cells immunonegative for all three molecules. Scale bar in **(A)** = 500 μm [applies to **(A)** and **(B)**]. gl, granule cell layer; ml, molecular layer; sl, stratum lucidum; sp, stratum pyramidale. Panels **(A)** and **(B)** are modified and reproduced from Jinno et al. (2007), with permission of the publisher.

parvalbumin (PV)-expressing GABAergic neurons and, to a lesser extent, cholinergic neurons (Tóth et al., 1993). On the other hand, several studies, employing different methods, have demonstrated conflicting results with regard to the local targets of H-MS cells. In juvenile rats, the local axons of CA1 H-MS cells recorded *in vitro* were reported to innervate predominantly hippocampal GABAergic neurons (Gulyás et al., 2003). By contrast, the main local targets of *in vivo* recorded (Jinno et al., 2007) or retrogradely labeled (Takács et al., 2008) GABAergic cells projecting to the medial septum and subiculum were pyramidal neurons in the CA1 area of the adult rats. Although it is difficult to explain the discrepancy, differences in ages of animals and labeling methods might be related to the inconsistent results in local postsynaptic targets of H-MS cells.

The hippocampus also receives GABAergic inputs from the medial septum, and thus the medial septum and the hippocampus are connected reciprocally (Raisman, 1966). The two major components of the septo-hippocampal projection are cholinergic and GABAergic neurons (Köhler et al., 1984; Shute and Lewis, 1963). The cholinergic projection terminates on all types of hippocampal cells (Frotscher and Léránth, 1985), whereas septal GABAergic neurons specifically innervate hippocampal GABAergic neurons (Freund and Antal, 1988; Gulyás et al., 1991). Recently, Takács et al. (2008) demonstrated direct reciprocity by using combined retrograde and anterograde tracing; that is, H-MS cells are the postsynaptic targets of GABAergic septo-hippocampal axons. On the other hand, GABAergic terminals of H-MS cells in the medial septum were shown to innervate septo-hippocampal neurons retrogradely labeled from the ventral hippocampus (Tóth et al., 1993). This reciprocal loop between the hippocampus and the medial septum via GABAergic neurons is considered to play a critical role for generating the rhythmic activity and synchronization (Dragoi et al., 1999; Wang, 2002).

SUBICULUM

Recent studies have demonstrated the existence of hippocampal GABAergic cells projecting to the subiculum (H-Sub cells; **Figure 1**). The first hint of a possible projection of GABAergic neurons from the hippocampus to the subiculum was shown by using slice preparations (Losonczy et al., 2002). They found that the oriens-bistratified (O-Bi) cells located in the CA1 region innervated the CA3 region and the subiculum in juvenile mice. One trilaminar cell recorded *in vivo* was decorated by metabotropic glutamate receptor 8a (mGluR8a)-enriched boutons, and exhibited a large projection from the CA1 stratum oriens to the subiculum (Ferraguti et al., 2005).

Using retrograde labeling, the distributions of H-Sub cells were estimated in the rat brain (**Figure 2B**). Differently from the H-MS cells, the retrogradely labeled H-Sub cells were mainly found in the CA1 region, and only a few cells were detected in the CA3 region and the dentate hilus. In the CA1 region, H-Sub cells were scattered throughout all the layers. The majority of H-Sub cells in the stratum oriens were large-sized horizontal cells, while those in the strata radiatum and lacunosum-moleculare were small to medium-sized bipolar and multipolar cells. The postsynaptic targets of long-range axons in the subiculum were assumed to be pyramidal neurons, although the ratios were not determined due to technical limitations. The major local targets of seven H-Sub cells were the dendritic shafts of pyramidal neurons (Jinno et al., 2007). But, one H-Sub cell expressing enkephalin (ENK) innervated dendritic shafts of GABAergic and pyramidal neurons (Fuentealba et al., 2008). The targets of ENK-expressing H-Sub cells were associated with the location of axonal arbors, i.e., interneurons were the main targets in the alveus, both interneurons and pyramidal cell dendrites were innervated in the other layers, and interneurons were the exclusive targets in the subiculum.

It should be noted that four *in vivo* recorded cells projected from the CA1 stratum oriens to both the subiculum and the medial septum (Jinno et al., 2007). Thus, there might be three groups of projection neurons in the CA1 region: those sending axons exclusively to the medial septum (4 in **Figure 1**), those innervating both the medial septum and the subicular are (5 in **Figure 1**), and those exclusively sending axons to the subicular/retrosplenial cortex (6 in **Figure 1**). Although the cells exclusively projecting to the medial septum (4) have not yet been identified, their existence has been suggested by the numerical data showing that the numbers of retrogradely labeled cells after injection into the medial septum are much larger than those after injection into the subiculum (see **Figures 2A,B**).

RETROSPLENIAL CORTEX

A few GABAergic neurons in the CA1 region are identified by injections of retrograde tracers into the retrosplenial cortex (van Groen and Wyss, 2003; Wyss and van Groen, 1992). The majority (about 65%) of hippocampal GABAergic neurons projecting to the granular retrosplenial cortex (H-Rsp cells) were detected at the border between strata radiatum and lacunosum-moleculare of the CA1 region, and a smaller population was located in the stratum radiatum (Miyashita and Rockland, 2007). Many fewer cells (<10%) were found in the stratum oriens or stratum pyramidale of the CA1 region. In the CA3 and DG, virtually no cells were retrogradely labeled after the injection of tracer into the granular retrosplenial cortex. It should also be noted that two *in vivo* recorded cells projected from the CA1 region to the retrosplenial cortex through the subicular area (Jinno et al., 2007). The morphological characteristics of H-Rsp cells at the border of strata radiatum and lacunosum-moleculare are rather similar to those of H-Sub cells.

The retrosplenial cortex has been implicated in various functions including spatial navigation and memory (Cain et al., 2006; Keene and Bucci, 2009; Wolbers and Büchel, 2005). There is increasing evidence that the retrosplenial cortex has a close functional relationship with the hippocampus (Burwell and Amaral, 1998; van Groen and Wyss, 2003). Anatomical studies indicate that, in addition to the hippocampal glutamatergic output to the retrosplenial cortex relayed through the subiculum, direct GABAergic projections by H-Rsp cells might play a role in processing of spatial information, but the significance remains to be elucidated.

COMMISSURAL PROJECTION

A small number of GABAergic neurons in the hilus of the DG have axonal projections to the cDG through the hippocampal commissure (Ribak et al., 1986; Seress and Ribak, 1983). Although the targets of these neurons are not strictly extrahippocampal, hilar GABAergic neurons with commissural projection should logically be included as long-range GABAergic projection neurons.

The postsynaptic targets of GABAergic commissural projections are thought to be dendrites of granule cells (Deller, 1998). It has not been clearly proven whether the hilar cells innervating the medial septum (1 in **Figure 1**) simultaneously send commissural axons to the cDG (2 in **Figure 1**).

NEUROCHEMICAL CHARACTERIZATION OF GABAergic PROJECTION NEURONS IN THE HIPPOCAMPUS

A number of studies have reported the neurochemical characterization of hippocampal GABAergic projection neurons in combination with tracer labeling. Some of the previously identified molecular markers might be expressed in neurons innervating different target areas, because a given neurochemical marker is not always specifically related to the connectional characteristics (Freund and Buzsáki, 1996). But there is ample evidence for the importance of molecular markers in the classification of GABAergic neurons tested (Jinno and Kosaka, 2006). Thus in this section, I describe the eight major molecules identified so far in GABAergic neurons projecting to the extrahippocampal areas.

Before entering into a detailed discussion, it might be better to briefly argue on a possible species difference in chemical characteristics of GABAergic projection neurons. In the neocortex, there is a considerable difference between mice and rats with respect to the basic microcircuits (DeFelipe et al., 2002). Several species differences have also been reported in the functional organization of the hippocampus. Most notably, glutamatergic hilar mossy cells showed neurochemical discrepancies between mice and rats. The calcium-binding protein calretinin (CR) is expressed in mossy cells in the mouse ventral hilus, but not in the rat hilus (Blasco-Ibáñez and Freund, 1997; Fujise et al., 1998; Liu et al., 1996). On the contrary, calcitonin gene-related peptide is localized in the rat mossy cells, but not in the mouse mossy cells (Freund et al., 1997; Sakurai and Kosaka, 2007). However, interestingly enough, the morpho-functional similarities in hippocampal GABAergic neurons have been repeatedly reported in mice and rats. For example, Mátyás et al. (2004) showed that the neurochemical and morphological classifications of hippocampal GABAergic interneurons established in the rat were largely valid for mouse strains. The numerical densities of chemically defined subpopulations of GABAergic neurons in the mouse hippocampus were comparable to those in the rat hippocampus (Jinno and Kosaka, 2006; Nomura et al., 1997). Taken together, it is possible to hypothesize that the chemical characteristics of GABAergic projection neurons in the rat and mouse hippocampus are rather similar to each other. But I leave the matter open.

SOMATOSTATIN

Somatostatin (SOM) is a neuroactive peptide, and one of the key molecules of H-MS cells (Zappone and Sloviter, 2001). The vast majority (93.0%) of the H-MS cells express SOM in the mouse hippocampus (Jinno and Kosaka, 2002). The percentages were very high both in the Ammon's horn (95.5% in the CA1 region, 92.6% in the CA3 region) and the DG (92.1%) in the mouse hippocampus. In contrast, one half of SS-positive neuron in the CA3 region (49.6%) and DG (44.1%) projected to the medial septum, and 22.5% in the CA1 region projected to the medial septum. Similar results were obtained from the rat hippocampus.

Using slice preparations of juvenile mice, Losonczy et al. (2002) showed that a large population of O-Bi cells in the CA1 stratum oriens innervating both the CA3 and subiculum were SOM-positive (7 of 10 cells tested). The patterns of expression of SOM in the H-Sub cells in the rat hippocampus were systematically assessed by retrograde labeling (Jinno et al., 2007). In the CA1 region, 50% of H-Sub cells were SOM-positive, while no H-Sub cells were immunoreactive for SOM in the strata radiatum and lacunosum-moleculare. In the CA3 strata radiatum/lacunosum-moleculare and the dentate hilus, SOM was detected in 43 and 75% of H-Sub cells, respectively.

In the rat CA1 region, SOM was not detected in H-Rsp cells so far examined (Miyashita and Rockland, 2007). On the other hand, the vast majority (as high as 96%) of SOM-positive cells project to the contralateral hippocampus via the commissural pathway in the rat dentate hilus (Zappone and Sloviter, 2001). Some of the hilar commissurally projecting GABAergic cells receive cholinergic inputs (Léránth and Frotscher, 1987).

NEUROPEPTIDE Y

Neuropeptide Y (NPY) is a peptide belonging to the pancreatic polypeptide family (Tatemoto et al., 1982). The frequent co-localization of NPY and SOM has been reported in cortical and subcortical cells in the rodent and primate brain (Chan-Palay, 1987; Köhler et al., 1987). Using "mirror" technique, Tóth and Freund (1992) showed that 20% of H-MS cells were NPY-positive in the rat brain. Regional differences in the expression of NPY in H-MS cells were shown in the rat hippocampus (Jinno et al., 2007). In the Ammon's horn, less than half of H-MS cells (46% in the CA1 region, 25% in the CA3 region) were positive for NPY, whereas virtually all H-MS cells (92%) in the DG contained NPY. The expression ratios of NPY in H-Sub cells (20% in the CA1 region, 43% in the CA3 region, and 80% in the DG) were generally similar to those in H-MS cells.

The expression of NPY in H-Rsp cells has not yet been established. In the dentate hilar area, a small population (2%) of NPY-positive hilar neurons has been reported to project to the contralateral hippocampus via the commissural pathway (Deller and Léránth, 1990). But the percentage might be underestimated due to the limitation of detection sensitivity.

CALBINDIN D28K

Another key molecule of H-MS cells is calbindin D28K (CB), which belongs to the EF-hand calcium-binding protein family. It has been reported that the majority of H-MS cells are CB-positive in the rat hippocampus (Tóth and Freund, 1992). Although the authors did not demonstrate the GABAergic nature of cell bodies of retrogradely labeled CB-expressing H-MS cells, subsequently they showed that the anterogradely labeled terminals in the septum originating from the hippocampus were GABA like-immunoreactive (Tóth et al., 1993). Immunofluorescent multiple labeling showed that 38% of retrogradely identified H-MS cells were CB-positive in the mouse hippocampus (Jinno and Kosaka, 2002). Approximately half of H-MS cells in the Ammon's horn expressed CB (57% in the CA1 region, 41% in the CA3 region), while none of them were positive for CB in the DG. Similar results were shown in the rat hippocampus (Jinno et al., 2007).

Expression ratios of CB in retrogradely labeled H-Sub cells were generally lower than those in H-MS cells (Jinno et al., 2007). In the CA1 region, 27% of H-Sub cells in the stratum oriens expressed CB, and only a few cells (3–4%) in the strata radiatum and lacunosum-moleculare were CB-positive.

CB was not detected in H-Rsp cells so far examined (Miyashita and Rockland, 2007). Possible expression of CB in commissurally projecting GABAergic neurons has not yet been examined.

PARVALBUMIN

PV is a low-molecular-weight, high-affinity calcium-binding protein of the EF hand family. Although infrequently, PV is detected in 5–15% of H-MS cells in the mouse and rat brain (Jinno and Kosaka, 2000; Jinno et al., 2007). PV is also expressed in a small population (4%) of H-Sub cells in the rat hippocampus. On the other hand, no H-Rsp cells contained PV (Miyashita and Rockland, 2007). In the DG, the majority (as high as 84%) of PV-positive neurons commissurally project to the contralateral hippocampus (Goodman and Sloviter, 1992; Zappone and Sloviter, 2001).

CALRETININ

CR is a high affinity EF-hand calcium-binding protein. It is well known that CR is expressed in one of the populations of GABAergic neurons called interneuron-specific (IS) cells, i.e., those exclusively innervating other GABAergic neurons (Gulyás et al., 1999). In addition, CR is also expressed in 18% of H-MS cells of the rat hippocampus. But, neither H-Sub cells nor H-Rsp cells were positive for CR (Jinno et al., 2007; Miyashita and Rockland, 2007). In the DG, one third of CR-positive GABAergic neurons projected commissurally (Zappone and Sloviter, 2001).

MUSCARINIC ACETYLCHOLINE RECEPTOR TYPE 2

Acetylcholine (ACh) modulates learning and memory in many tasks through actions in the hippocampus and striatum (Bartus et al., 1982; Gold, 2003). Muscarinic ACh receptor type 2 (M2R) is commonly expressed in a considerable population of H-MS cells (38%, Hájos et al., 1998) and H-Sub cells (42%, Jinno et al., 2007) in the rat hippocampus. In contrast, only a smaller subset (14%) of H-Rsp cells was positive for M2R (Miyashita and Rockland, 2007). It has not been tested whether M2R is expressed in commissurally projecting GABAergic neurons in the dentate hilus.

mGluR1α

Synergic activation of M2R and mGluRs induces enhancement of burst firing, and is thought to be involved in intrinsic neuronal plasticity (Moore et al., 2009). Expression of mGluR1α in GABAergic projection neurons was tested in H-MS cells and H-Sub cells (Jinno et al., 2007). In the Ammon's horn, the majority of H-MS cells expressed mGluR1α (69% in the CA1, and 84% in the CA3 region), whereas only 15% of H-MS cells showed mGluR1α immunoreactivity in the DG. H-Sub cells also expressed mGluR1α, but the expression ratios were lower (40% in the CA1 region) than those of H-MS cells.

ENKEPHALIN

In the CA1 area, ENK has been detected in a population of IS cells (Blasco-Ibáñez et al., 1998). In addition, a recent retrograde labeling study showed that ENK was expressed in 11.3% of H-Sub cells using a highly sensitive viral vector for retrograde labeling (Fuentealba et al., 2008). The ENK-positive H-Sub cells ($n = 7$) were located in the middle and deep stratum radiatum, close to the stratum lacunosum-moleculare. All tested ENK-positive H-Sub cells were co-labeled for the orphan nuclear receptor chicken ovalbumin upstream promoter-transcription factor II, but none for CR.

MULTIREGIONAL COORDINATION BY LONG-RANGE GABAergic PROJECTION NEURONS

This final section considers the potential significance of long-range GABAergic projection systems both in basic and clinical research. It has been repeatedly described that GABAergic neurons exhibit substantial diversity in their anatomical, neurochemical and electrophysiological characteristics (Markram et al., 2004; Somogyi and Klausberger, 2005). Due to this phenotypic variety, the classification of cortical GABAergic neurons has been a big challenge. Although the situation appears difficult, by now, at least 21 distinct classes of cells have been identified in the rat CA1 region using multidisciplinary approaches (Klausberger and Somogyi, 2008). Interestingly, different types of cells innervating specific extrahippocampal targets fire with distinct spike timing during network oscillation (Jinno et al., 2007). The firing pattern of GABAergic neurons projecting to *both* the medial septum and the subiculum (double-projection cells) is an example of the spatiotemporal requirements of long-range coordination. During theta oscillations (4–10 Hz), which can co-occur in hippocampal and isocortical areas (Jones and Wilson, 2005), double-projection cells fire preferentially at or after the trough of theta cycles, recorded extracellularly in stratum pyramidale, with low discharge frequencies. In contrast, the firing of H-Sub cells in the stratum radiatum (radiatum-retrohippocampal cells) strongly increases during theta oscillation. In firing preferentially on the descending theta phase, radiatum-retrohippocampal cells are different from the local dendrite-innervating oriens-lacunosum-moleculare (O-LM), cholecystokinin-expressing and bistratified cells (Klausberger et al., 2003, 2004, 2005). During ripple oscillations (100–200 Hz), double-projection cells fire with a high discharge rate. This activity might lead to a suppression of the majority of cells in the medial septum and inhibit the generation of theta oscillations. Their local axons in the CA1 area innervate pyramidal cell dendrites, and cooperatively release GABA with the bistratified cells, which fire at high frequency during the ascending phase of the extracellular ripple cycle (Klausberger et al., 2004). Together, they temporally structure the excitation of the CA3 input in CA1 pyramidal dendrites at ripple frequency. The other long-range axonal arbors of double-projection cells in the subiculum also innervate dendrites of pyramidal cells and contribute to communicating the temporal structure of ripple oscillations to the subiculum, which oscillates coherently with the CA1 area (Chrobak and Buzsáki, 1996). Unlike double-projection cells, radiatum-retrohippocampal cells do not change their activity during ripples and are unlikely to contribute to coordinating this network state, indicating a different function for these neurons. The diversity of GABAergic projection cell classes in the hippocampus may result from the need to coordinate precisely the multiple activities of distributed neural circuits in different brain states and among multiple functionally related brain areas.

Alterations in GABA-mediated signaling have been found in various psychiatric disorders (Kalueff and Nutt, 2007). For instance, reduced levels of glutamic acid decarboxylase (GAD) 67 mRNA have been found in individuals with schizophrenia (Guidotti et al., 2000). Some postmortem studies showed that GAD activity was lower in patients with major depression compared to controls in several brain regions (Perry et al., 1977). A modern imaging study using proton magnetic resonance spectroscopy showed the reduced GABA levels in the prefrontal area of depression sufferers (Hasler et al., 2007). There is a growing body of evidence suggesting that the spatially and temporally organized synaptic inhibition mediated by specific subclasses of GABAergic neurons is impaired in mental illness (Gonzalez-Burgos and Lewis, 2008). Along these lines, it has also been postulated that cognitive and affective impairments in psychiatric disorders may be related to a failure to integrate the activity of widely spread neural circuits (Andreasen et al., 1999; Stephan et al., 2006). Interestingly, recent imaging data suggest that functional connectivity between remote regions is impaired in individuals with mental illness, such as schizophrenia (Zhou et al., 2007) and autism (Villalobos et al., 2005). Because GABAergic neurons are considered to play a critical role in the long-range fast synchronization of neural activities across brain regions (Buzsáki and Chrobak, 1995; Mann and Paulsen, 2007), these finding suggest that defects of long-range GABAergic projection system might be associated with the neurobiology of psychiatric disorders.

In summary, through fast oscillatory synchronization, distinct classes of hippocampal GABAergic neurons can contribute to the coordination of neural activity in multiple brain regions. I propose that such multiple long-range GABAergic projection systems acting in concert are of great importance in the maintenance of psychological normalcy. Understanding the characteristics of long-range GABAergic projection neurons might not only inform computational modeling of brain function, but will hopefully also make it possible to get a clue to novel treatment for psychiatric illness.

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REFERENCES

Alonso, A., and Köhler, C. (1982). Evidence for separate projections of hippocampal pyramidal and non-pyramidal neurons to different parts of the septum in the rat brain. *Neurosci. Lett.* 31, 209–214.

Andreasen, N. C., Nopoulos, P., O'Leary, D. S., Miller, D. D., Wassink, T., and Flaum, M. (1999). Defining the phenotype of schizophrenia: cognitive dysmetria and its neural mechanisms. *Biol. Psychiatry* 46, 908–920.

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.

Blasco-Ibáñez, J. M., and Freund, T. F. (1997). Distribution, ultrastructure, and connectivity of calretinin-immunoreactive mossy cells of the mouse dentate gyrus. *Hippocampus* 7, 307–320.

Blasco-Ibáñez, J. M., Martínez-Guijarro, F. J., and Freund, T. F. (1998). Enkephalin-containing interneurons are specialized to innervate other interneurons in the hippocampal CA1 region of the rat and guinea-pig. *Eur. J. Neurosci.* 10, 1784–1795.

Burwell, R. D., and Amaral, D. G. (1998). Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *J Comp. Neurol.* 398, 179–205.

Buzsáki, G., and Chrobak, J. J. (1995). Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. *Curr. Opin. Neurobiol.* 5, 504–510.

Cain, D. P., Humpartzoomian, R., and Boon, F. (2006). Retrosplenial cortex lesions impair water maze strategies learning or spatial place learning depending on prior experience of the rat. *Behav. Brain Res.* 170, 316–325.

Chan-Palay, V. (1987). Somatostatin immunoreactive neurons in the human hippocampus and cortex shown by immunogold/silver intensification on vibratome sections: coexistence with neuropeptide Y neurons, and effects in Alzheimer-type dementia. *J. Comp. Neurol.* 260, 201–223.

Chrobak, J. J., and Buzsáki, G. (1996). High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *J. Neurosci.* 16, 3056–3066.

DeFelipe, J. (1993). Neocortical neuronal diversity: chemical heterogeneity revealed by colocalization studies of classic neurotransmitters, neuropeptides, calcium-binding proteins, and cell surface molecules. *Cereb. Cortex* 3, 273–289.

DeFelipe, J., Alonso-Nanclares, L., and Arellano, J. I. (2002). Microstructure of the neocortex: comparative aspects. *J. Neurocytol.* 31, 299–316.

Deller, T. (1998). The anatomical organization of the rat fascia dentata: new aspects of laminar organization as revealed by anterograde tracing with *Phaseolus vulgaris*-Leucoagglutinin (PHAL). *Anat. Embryol. (Berl.)* 197, 89–103.

Deller, T., and Léránth, C. (1990). Synaptic connections of neuropeptide Y (NPY) immunoreactive neurons in the hilar area of the rat hippocampus. *J. Comp. Neurol.* 300, 433–447.

Dragoi, G., Carpi, D., Recce, M., Csicsvari, J., and Buzsáki, G. (1999). Interactions between hippocampus and medial septum during sharp waves and theta oscillation in the behaving rat. *J. Neurosci.* 19, 6191–6199.

Fabri, M., and Manzoni, T. (2004). Glutamic acid decarboxylase immunoreactivity in callosal projecting neurons of cat and rat somatic sensory areas. *Neuroscience* 123, 557–566.

Ferraguti, F., Klausberger, T., Cobden, P., Baude, A., Roberts, J. D., Szucs, P., Kinoshita, A., Shigemoto, R., Somogyi, P., and Dalezios, Y. (2005). Metabotropic glutamate receptor 8-expressing nerve terminals target subsets of GABAergic neurons in the hippocampus. *J. Neurosci.* 25, 10520–10536.

Freund, T. F., and Antal, M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature* 336, 170–173.

Freund, T. F., and Buzsáki, G. (1996). Interneurons of the hippocampus. *Hippocampus* 6, 347–470.

Freund, T. F., Hájos, N., Acsády, L., Görcs, T. J., and Katona, I. (1997). Mossy cells of the rat dentate gyrus are immunoreactive for calcitonin gene-related peptide (CGRP). *Eur. J. Neurosci.* 9, 1815–1830.

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.

Fuentealba, P., Tomioka, R., Dalezios, Y., Márton, L. F., Studer, M., Rockland, K., Klausberger, T., and Somogyi, P. (2008). Rhythmically active enkephalin-expressing GABAergic cells in the CA1 area of the hippocampus project to the subiculum and preferentially innervate interneurons. *J. Neurosci.* 28, 10017–10022.

Fujise, N., Liu, Y., Hori, N., and Kosaka, T. (1998). Distribution of calretinin immunoreactivity in the mouse dentate gyrus: II. Mossy cells, with special reference to their dorsoventral difference in calretinin immunoreactivity. *Neuroscience* 82, 181–200.

Gerashchenko, D., Wisor, J. P., Burns, D., Reh, R. K., Shiromani, P. J., Sakurai, T., de la Iglesia, H. O., and Kilduff, T. S. (2008). Identification of a population of sleep-active cerebral cortex neurons. *Proc. Natl. Acad. Sci. USA* 105, 10227–10232.

Germroth, P., Schwerdtfeger, W. K., and Buhl, E. H. (1989). GABAergic neurons in the entorhinal cortex project to the hippocampus. *Brain Res.* 494, 187–192.

Gold, P. E. (2003). Acetylcholine: cognitive and brain functions. *Neurobiol. Learn. Mem.* 80, 177.

Gonchar, Y. A., Johnson, P. B., and Weinberg, R. J. (1995). GABA-immunopositive neurons in rat neocortex

with contralateral projections to S-I. *Brain Res.* 697, 27–34.

Gonzalez-Burgos, G., and Lewis, D. A. (2008). GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. *Schizophr. Bull.* 34, 944–961.

Goodman, J. H., and Sloviter, R. S. (1992). Evidence for commissurally projecting parvalbumin-immunoreactive basket cells in the dentate gyrus of the rat. *Hippocampus* 2, 13–21.

Gos, T., Günther, K., Bielau, H., Dobrowolny, H., Mawrin, C., Trübner, K., Brisch, R., Steiner, J., Bernstein, H. G., Jankowski, Z., and Bogerts, B. (2009). Suicide and depression in the quantitative analysis of glutamic acid decarboxylase-immunoreactive neuropil. *J. Affect. Disord.* 113, 45–55.

Guidotti, A., Auta, J., Davis, J. M., Di-Giorgi-Gerevini, V., Dwivedi, Y., Grayson, D. R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., and Costa, E. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch. Gen. Psychiatry* 57, 1061–1069.

Gulyás, A. I., Hájos, N., Katona, I., and Freund, T. F. (2003). Interneurons are the local targets of hippocampal inhibitory cells which project to the medial septum. *Eur. J. Neurosci.* 17, 1861–1872.

Gulyás, A. I., Megías, M., Emri, Z., and Freund, T. F. (1999). Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. *J. Neurosci.* 19, 10082–10097.

Gulyás, A. I., Seress, L., Tóth, K., Acsády, L., Antal, M., and Freund, T. F. (1991). Septal GABAergic neurons innervate inhibitory interneurons in the hippocampus of the macaque monkey. *Neuroscience.* 41, 381–390.

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. *Neuroscience* 82, 355–376.

Hasler, G., van der Veen, J. W., Tumonis, T., Meyers, N., Shen, J., and Drevets, W. C. (2007). Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch. Gen. Psychiatry* 64, 193–200.

Jinno, S., Klausberger, T., Marton, L. F., Dalezios, Y., Roberts, J. D., Fuentealba, P., Bushong, E. A., Henze, D., Buzsáki, G., and Somogyi, P. (2007). Neuronal diversity in GABAergic long-range projections from the hippocampus. *J. Neurosci.* 27, 8790–8804.

Jinno, S., and Kosaka, T. (2000). Colocalization of parvalbumin and somatostatin-like immunoreactivity in the mouse hippocampus: quantitative analysis with optical dissector. *J. Comp. Neurol.* 428, 377–388.

Jinno, S., and Kosaka, T. (2002). Immunocytochemical characterization of hippocamposeptal projecting GABAergic nonprincipal neurons in the mouse brain: a retrograde labeling study. *Brain Res.* 945, 219–231.

Jinno, S., and Kosaka, T. (2004). Parvalbumin is expressed in glutamatergic and GABAergic corticostriatal pathway in mice. *J. Comp. Neurol.* 477, 188–201.

Jinno, S., and Kosaka, T. (2006). Cellular architecture of the mouse hippocampus: a quantitative aspect of chemically defined GABAergic neurons with stereology. *Neurosci. Res.* 56, 229–245.

Jinno, S., and Kosaka, T. (2009). Neuronal circuit-dependent alterations in expression of two isoforms of glutamic acid decarboxylase in the hippocampus following electroconvulsive shock: a stereology-based study. *Hippocampus* (in press).

Jones, M. W., and Wilson, M. A. (2005). Phase precession of medial prefrontal cortical activity relative to the hippocampal theta rhythm. *Hippocampus* 15, 867–873.

Kalueff, A. V., and Nutt, D. J. (2007). Role of GABA in anxiety and depression. *Depress. Anxiety* 24, 495–517.

Keene, C. S., and Bucci, D. J. (2009). Damage to the retrosplenial cortex produces specific impairments in spatial working memory. *Neurobiol. Learn. Mem.* 91, 408–414.

Klausberger, T., Magill, P. J., Marton, L., Roberts, J. D. B., Cobden, P. M., Buzsáki, G., and Somogyi, P. (2003). Brain state- and cell type-specific firing of hippocampal interneurons in vivo. *Nature* 421, 844–848.

Klausberger, T., Marton, L. F., Baude, A., Roberts, J. D. B., Magill, P., and Somogyi, P. (2004). Spike timing of dendrite-targeting bistratified cells during hippocampal network oscillations in vivo. *Nat. Neurosci.* 7, 41–47.

Klausberger, T., Marton, L. F., O'Neill, J., Huck, J. H. J., Dalezios, Y., Fuentealba, P., Suen, W. Y., Papp, E., Kaneko, T., Watanabe, M., Csicsvari, J., and Somogyi, P. (2005). Complementary roles of cholecystokinin- and parvalbumin-expressing GABAergic neurons in hippocampal network oscillations. *J. Neurosci.* 25, 9782–9793.

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

Köhler, C., Chan-Palay, V., and Wu, J. Y. (1984). Septal neurons containing glutamic acid decarboxylase immunoreactivity project to the hippocampal region in the rat brain. *Anat. Embryol. (Berl.)* 169, 41–44.

Köhler, C., Eriksson, L. G., Davies, S., and Chan-Palay, V. (1987). Co-localization of neuropeptide tyrosine and somatostatin immunoreactivity in neurons of individual subfields of the rat hippocampal region. *Neurosci. Lett.* 78, 1–6.

Léránth, C., and Frotscher, M. (1987). Cholinergic innervation of hippocampal GAD- and somatostatin-immunoreactive commissural neurons. *J. Comp. Neurol.* 261, 33–47.

Liu, Y., Fujise, N., and Kosaka, T. (1996). Distribution of calretinin immunoreactivity in the mouse dentate gyrus. I. General description. *Exp. Brain Res.* 108, 389–403.

Losonczy, A., Zhang, L., Shigemoto, R., Somogyi, P., and Nusser, Z. (2002). Cell type dependence and variability in the short-term plasticity of EPSCs in identified mouse hippocampal interneurones. *J. Physiol.* 542, 193–210.

Maccaferri, G., and Lacaille, J. C. (2003). Interneuron diversity series: hippocampal interneuron classifications – making things as simple as possible, not simpler. *Trends Neurosci.* 26, 564–571.

Mann, E. O., and Paulsen, O. (2007). Role of GABAergic inhibition in hippocampal network oscillations. *Trends Neurosci.* 30, 343–349.

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., and Wu, C. (2004). Interneurons of the neocortical inhibitory system. *Nat. Rev. Neurosci.* 5, 793–807.

Mátyás, F., Freund, T. F., and Gulyás, A. I. (2004). Immunocytochemically defined interneuron populations in the hippocampus of mouse strains used in transgenic technology. *Hippocampus* 14, 460–481.

Miyashita, T., and Rockland, K. S. (2007). GABAergic projections from the hippocampus to the retrosplenial cortex in the rat. *Eur. J. Neurosci.* 26, 1193–1204.

Moore, S. J., Cooper, D. C., and Spruston, N. (2009). Plasticity of burst firing induced by synergistic activation of metabotropic glutamate and acetylcholine receptors. *Neuron.* 61, 287–300.

Nomura, T., Fukuda, T., Aika, Y., Heizmann, C. W., Emson, P. C., Kobayashi, T., and Kosaka, T. (1997). Laminar distribution of non-principal neurons in the rat hippocampus, with special reference to their compositional difference among layers. *Brain Res.* 764, 197–204.

Perry, E. K., Gibson, P. H., Blessed, G., Perry, R. H., and Tomlinson, B. E. (1977). Neurotransmitter enzyme abnormalities in senile dementia. Choline acetyltransferase and glutamic acid decarboxylase activities in necropsy brain tissue. *J. Neurol. Sci.* 34, 247–265.

Raisman, G. (1966). The connexions of the septum. *Brain* 89, 317–348.

Ramóny y Cajal, S. (1911). Histologie du Systeme Nerveux de l'Homme et es Vertebres. Paris, Malonie.

Ribak, C. E., Seress, L., Peterson, G. M., Seroogy, K. B., Fallon, J. H., and Schmued, L. C. (1986). A GABAergic inhibitory component within the hippocampal commissural pathway. *J. Neurosci.* 6, 3492–3498.

Sakurai, O., and Kosaka, T. (2007). Nonprincipal neurons and CA2 pyramidal cells, but not mossy cells are immunoreactive for calcitonin gene-related peptide in the mouse hippocampus. *Brain Res.* 1186, 129–143.

Sanacora, G., and Saricicek, A. (2007). GABAergic contributions to the pathophysiology of depression and the mechanism of antidepressant action. *CNS Neurol. Disord. Drug Targets* 6, 127–140.

Schwerdtfeger, W. K., and Buhl, E. (1986). Various types of non-pyramidal hippocampal neurons project to the septum and contralateral hippocampus. *Brain Res.* 386, 146–154.

Seress, L., and Ribak, C. E. (1983). GABAergic cells in the dentate gyrus appear to be local circuit and projection neurons. *Exp. Brain Res.* 50, 173–182.

Shute, C. C., and Lewis, P. R. (1963). Cholinesterase-containing systems of the brain of the rat. *Nature* 199, 1160–1164.

Somogyi, P., and Klausberger, T. (2005). Defined types of cortical interneurone structure space and spike timing in the hippocampus. *J. Physiol.* 562, 9–26.

Stephan, K. E., Baldeweg, T., and Friston, K. J. (2006). Synaptic plasticity and dysconnection in schizophrenia. *Biol. Psychiatry* 59, 929–939.

Takács, V. T., Freund, T. F., and Gulyás, A. I. (2008). Types and synaptic connections of hippocampal inhibitory neurons reciprocally connected with the medial septum. *Eur. J. Neurosci.* 28, 148–164.

Tatemoto, K., Carlquist, M., and Mutt, V. (1982). Neuropeptide Y – a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* 296, 659–660.

Tomioka, R., and Rockland, K. S. (2007). Long-distance corticocortical

GABAergic neurons in the adult monkey white and gray matter. *J. Comp. Neurol.* 505, 526–538.

Tóth, K., Borhegyi, Z., and Freund, T. F. (1993). Postsynaptic targets of GABAergic hippocampal neurons in the medial septum-diagonal band of broca complex. *J. Neurosci.* 13, 3712–3724.

Tóth, K., and Freund, T. F. (1992). Calbindin D28k-containing nonpyramidal cells in the rat hippocampus: their immunoreactivity for GABA and projection to the medial septum. *Neuroscience* 49, 793–805.

Totterdell, S., and Hayes, L. (1987). Non-pyramidal hippocampal projection neurons: a light and electron microscopic study. *J. Neurocytol.* 16, 477–485.

van Groen, T., and Wyss, J. M. (2003). Connections of the retrosplenial granular b cortex in the rat. *J. Comp. Neurol.* 463, 249–263.

van Haeften, T., Wouterlood, F. G., Jorritsma-Byham, B., and Witter, M. P. (1997). GABAergic presubicular projections to the medial entorhinal cortex of the rat. *J. Neurosci.* 17, 862–874.

Villalobos, M. E., Mizuno, A., Dahl, B. C., Kemmotsu, N., and Müller, R. A. (2005). Reduced functional connectivity between V1 and inferior frontal cortex associated with visuomotor performance in autism. *Neuroimage* 25, 916–925.

Wang, X. J. (2002). Pacemaker neurons for the theta rhythm and their synchronization in the septohippocampal reciprocal loop. *J. Neurophysiol.* 87, 889–900.

Wolbers, T., and Büchel, C. (2005). Dissociable retrosplenial and hippocampal contributions to successful formation of survey representations. *J. Neurosci.* 25, 3333–3340.

Wyss, J. M., and van Groen, T. (1992). Connections between the retrosplenial cortex and the hippocampal formation in the rat: a review. *Hippocampus* 2, 1–11.

Zappone, C. A., and Sloviter, R. S. (2001). Commissurally projecting inhibitory interneurons of the rat hippocampal dentate gyrus: a colocalization study of neuronal markers and the retrograde tracer Fluoro-gold. *J. Comp. Neurol.* 441, 324–344.

Zhou, Y., Liang, M., Tian, L., Wang, K., Hao, Y., Liu, H., Liu, Z., and Jiang, T. (2007). Functional disintegration in paranoid schizophrenia using resting-state fMRI. *Schizophr. Res.* 97, 194–205.

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Subplate neurons: crucial regulators of cortical development and plasticity

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The developing cerebral cortex contains a distinct class of cells, subplate neurons, which form one of the first functional cortical circuits. Subplate neurons reside in the cortical white matter, receive thalamic inputs and project into the developing cortical plate, mostly to layer 4. Subplate neurons are present at key time points during development. Removal of subplate neurons profoundly affects cortical development. Subplate removal in visual cortex prevents the maturation of thalamocortical synapse, the maturation of inhibition in layer 4, the development of orientation selective responses in individual cortical neurons, and the formation of ocular dominance columns. In addition, monocular deprivation during development reveals that ocular dominance plasticity is paradoxical in the absence of subplate neurons. Because subplate neurons projecting to layer 4 are glutamatergic, these diverse deficits following subplate removal were hypothesized to be due to lack of feed-forward thalamic driven cortical excitation. A computational model of the developing thalamocortical pathway incorporating feed-forward excitatory subplate projections replicates both normal development and plasticity of ocular dominance as well as the effects of subplate removal. Therefore, we postulate that feed-forward excitatory projections from subplate neurons into the developing cortical plate enhance correlated activity between thalamus and layer 4 and, in concert with Hebbian learning rules in layer 4, allow maturational and plastic processes in layer 4 to commence. Thus subplate neurons are a crucial regulator of cortical development and plasticity, and damage to these neurons might play a role in the pathology of many neurodevelopmental disorders.

Keywords: subplate, cortical maturation, GABA, ocular dominance plasticity, KCC2

INTRODUCTION

Subplate neurons are among the earliest generated neurons in the cerebral cortex of mammals and are located in the developing white matter of all cortical regions (Luskin and Shatz, 1985; Valverde and Facal-Valverde, 1987, 1988; Mrzljak et al., 1988; Kostovic and Rakic, 1990; Allendoerfer and Shatz, 1994; Reep, 2000; Kostovic et al., 2002; Kostovic and Judas, 2006; Perkins et al., 2008). In humans subplate neurons comprise up to 50% of the cortical neurons in the second trimester and are present in the first few years of life (depending on cortical area) (Luskin and Shatz, 1985; Valverde and Facal-Valverde, 1987, 1988; Mrzljak et al., 1988; Kostovic and Rakic, 1990; Allendoerfer and Shatz, 1994; Reep, 2000; Kostovic et al., 2002; Kostovic and Judas, 2006; Perkins et al., 2008). In rodents some subplate neurons can remain into adulthood forming layer 6b (Woo et al., 1991; Wood et al., 1992; Price et al., 1997; Arias et al., 2002; Torres-Reveron and Friedlander, 2007). Subplate neurons thus comprise additional cortical circuits that are only present during cortical development, and these circuits appear to play a major role in development and early cortical function, but are only beginning to be characterized.

CONNECTIVITY OF SUBPLATE NEURON AND RELATIONSHIP WITH CORTICAL CELLS

The cell bodies of subplate neurons are located in the cerebral white matter (Mrzljak et al., 1988; Kostovic and Rakic, 1990). A diagram of the early cortical circuitry that the subplate participates in has been established from physiological and anatomical studies (**Figure 1**). Subplate neurons are a diverse neuropil encompassing glutamatergic and GABAergic neurons and receiving glutamatergic, GABAergic, cholinergic and glycinergic inputs (Wahle et al., 1987, 1994; Chun and Shatz, 1989a,b; Cobas et al., 1991; Meinecke and Rakic, 1992; Matute et al., 1993; Zecevic and Milosevic, 1997; Hanganu et al., 2002; Hanganu and Luhmann, 2004; Hirsch and Luhmann, 2008; Kilb et al., 2008).

Subplate neurons receive glutamatergic input from the thalamus before these thalamic axons grow to their targets in layer 4 (Friauf et al., 1990; Allendoerfer and Shatz, 1994; Hanganu et al., 2002; Higashi et al., 2002; Molnar et al., 2003; Torres-Reveron and Friedlander, 2007). Subplate axons mainly project to cortical layer 4 (Friauf and Shatz, 1991; Allendoerfer and Shatz, 1994; Pinon et al., 2009), thus there is a time period when subplate neurons are in a key position to relay thalamic input to layer 4 (**Figure 1** left) (Valverde and Facal-Valverde, 1987, 1988; Robinson and Dreher, 1990; Catalano et al., 1991; Friauf and Shatz, 1991; Allendoerfer and Shatz, 1994; Molnar and Blakemore, 1995; Clancy et al., 2001; Pinon et al., 2009). After thalamic axons grow into layer 4, thalamocortical synapses and GABAergic circuits in layer 4 undergo refinement and maturation and over this time are particularly influenced by sensory experience (defining the "critical period") (Friauf and Shatz, 1991; Allendoerfer and Shatz, 1994; Clancy et al., 2001; Chen et al., 2001a; Kanold et al., 2003; Kanold and Shatz, 2006). During this time subplate neurons are still present, receive

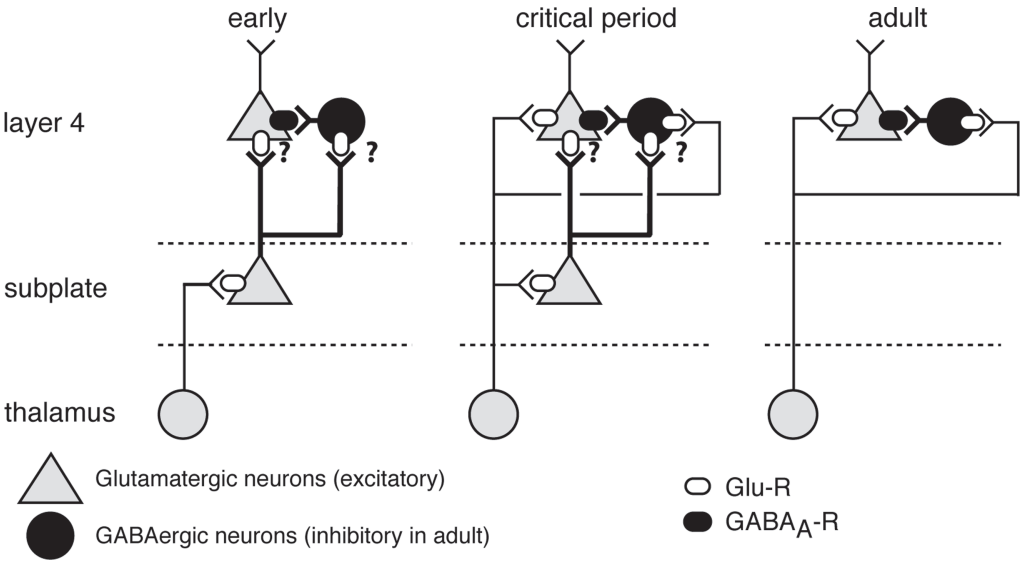

FIGURE 1 | Subplate neurons and their connectivity during development. Early: subplate neurons (green) receive inputs from thalamus and subplate neuron axons project to layer 4, but precise targets are unknown ('?'). **Onset of critical period** (coincides with time of subplate ablation in lesion studies): Both subplate neurons and thalamus project to layer 4. **Adult**: SPNs have been eliminated by programmed cell death and layer 4 neurons receive inputs from thalamus. Subplate neurons can depolarize layer 4 neurons via two pathways: directly via excitatory inputs and indirectly via exciting GABAergic neurons (red) and driving GABAergic depolarization.

direct thalamic input, and project to layer 4 (**Figure 1** middle). The majority of subplate neurons are gradually eliminated postnatally by programmed cell death (**Figure 1** right) and remaining neurons are retained as interstitial neurons (Allendoerfer and Shatz, 1994; Arias et al., 2002; Kanold et al., 2003; Torres-Reveron and Friedlander, 2007).

Functional evidence for these changing circuits was provided by current source density analysis in developing cat visual cortex (V1) (Friauf and Shatz, 1991). White matter stimulations in V1 at early ages (P0) show short latency sinks in the subplate and long latency sinks in layer 4. The difference in latencies suggests that subplate neurons make excitatory connections to layer 4 neurons and drive their activity (Friauf and Shatz, 1991). The presence of a disynaptic sink in layer 4 also implies that subplate to layer 4 connections are relatively strong. At later ages short latency sinks emerge in layer 4 after white matter stimulation. Thus now thalamic activity could directly activate layer 4 neurons indicating that thalamocortical circuits had matured. Similar results were obtained from recordings in rodent somatosensory system (Higashi et al., 2002; Molnar et al., 2003). These recordings show that thalamic stimulation activates subplate neurons by E18-19 while cortical plate activation is seen at E21. The difference in timing (prenatal in rodent vs. postnatal in cat) might reflect the early maturation of the somatosensory system relative to the visual systems or a difference between rodent and cat. Retrograde labeling studies show that most of the subplate neurons projecting to layer 4 are glutamatergic (Finney et al., 1998). Thus, subplate neurons are thought to provide excitatory input to layer 4. Since subplate neurons receive GABAergic, cholinergic and glycinergic inputs (Wahle et al., 1987, 1994; Chun and Shatz, 1989a,b; Cobas et al., 1991; Meinecke and Rakic, 1992; Matute et al., 1993; Zecevic and Milosevic, 1997; Hanganu et al., 2002; Hanganu and Luhmann, 2004; Hirsch and Luhmann, 2008; Kilb et al., 2008) this feed-forward excitation to layer 4 can be modified by processing within the subplate.

SUBPLATE NEURONS ENABLE THALAMOCORTICAL TARGET FINDING

Subplate neurons can be selectively ablated in early development by excitotoxic kainic acid injections (Ghosh et al., 1990; Ghosh and Shatz, 1992, 1993, 1994). Ablation of subplate neurons before thalamic axons invade layer 4 (**Figure 1** left) causes these axons to bypass the ablated area and grow into layer 4 at areas that contain subplate neurons (Ghosh et al., 1990). Thus subplate neurons seem to provide a guidance role in targeting thalamic axons to layer 4. Since subplate neurons project radially to layer 4 they might provide a scaffold that enables thalamic axons, which travel tangentially below their eventual target layer, to find their targets.

SUBPLATE NEURONS ENABLE THALAMOCORTICAL MATURATION

After thalamic axons grow into layer 4 they make synaptic connections with layer 4 neurons and build up these connections over time to adult strength. The strengthening of the thalamocortical synapses from an initially weak state occurs while there is already strong input from subplate neurons (Friauf and Shatz, 1991) (**Figure 1**) and possibly intracortical connections.

Recent experiments indicate that subplate neurons play a major role in the developmental strengthening of thalamocortical projections. Subplate ablation after thalamic afferents have grown into layer 4 but before these afferents have made a strong synapse with layer 4 neurons prevents the strengthening of thalamocortical connections (Kanold et al., 2003). In addition, the frequency – but not

amplitude – of spontaneous excitatory synaptic events in layer 4 cells is increased (Kanold et al., 2003), which is consistent with a lack of functional refinement of cortical connections. Together these data indicated that without subplate neurons, there is a failure of appropriate synapses to strengthen and others to weaken, and the visual cortex becomes functionally decoupled from its thalamic inputs.

SUBPLATE NEURONS CONTROL INHIBITORY MATURATION

The maturation of intracortical inhibition is central to normal cortical function. In addition GABAergic activity is thought to be involved in the maturation of glutamatergic circuits (Ben-Ari, 2002; Ben-Ari et al., 2004). Despite this importance of inhibition, the cells and circuits that control inhibitory development are unknown.

Key processes of inhibitory maturation occur postsynaptically by changes in the subunit composition of the $GABA_A$ receptor (**Figure 2A**) and the intracellular Cl^--concentration (which affects the ion flow through the $GABA_A$ receptor). The Cl^--reversal potential (E_{Cl}) controls if $GABA_A$-ergic activity is depolarizing or hyperpolarizing. E_{Cl} is mediated by Cl^- transporters such as KCC2 and NKCC1 that control Cl^- levels in the cytosol (Shimizu-Okabe et al., 2002, 2007; Yamada et al., 2004; Blaesse et al., 2009). KCC2 levels are low (E_{Cl} high) in early development, thus GABA can be depolarizing (Rivera et al., 1999; Ganguly et al., 2001; Owens and Kriegstein, 2002; Kanold and Shatz, 2006; Blaesse et al., 2009). Depending on the amount of depolarization, depolarizing GABA can be excitatory or have a shunting inhibitory influence (Blaesse et al., 2009). Over development, KCC2 levels increase (decreasing E_{Cl}), rendering GABA inhibitory (Rivera et al., 1999; Ganguly et al., 2001; Owens and Kriegstein, 2002; Shimizu-Okabe et al., 2002; Yamada et al., 2004; Kanold and Shatz, 2006; Blaesse et al., 2009). The strengthening of both excitatory and inhibitory circuits while maintaining "appropriate" activity levels might be achieved by wiring up GABAergic circuits first (Ben-Ari et al., 2004) and then utilizing depolarizing GABA to aid in maturing glutamatergic connections (Ben-Ari, 2002; Ben-Ari et al., 2004).

The maturation of inhibition depends on normal sensory experience. Sensory deprivations (i.e. dark rearing, deafness, whisker trimming) prevent inhibitory maturation and high expression levels of BNDF, which is involved in inhibitory maturation (Fuchs and Salazar, 1998; Huang et al., 1999; Lein and Shatz, 2000; Chen et al., 2001b; Morales et al., 2002; Gianfranceschi et al., 2003; Vale et al., 2003; Jiang et al., 2005; Kotak et al., 2005; Jiao et al., 2006; Huang, 2009). Because subplate neurons form a crucial relay of sensory information, and because subplate neurons provide excitation to developing circuits, subplate neurons are in a key position to regulate the maturation of cortical GABAergic inhibition. In particular since subplate neurons are driven by thalamic afferents, strong synaptic inputs between subplate neurons and cortical neurons might amplify the action of sensory inputs.

Removal of subplate neurons at early ages, when inhibition is immature, prevents both the developmental increase in KCC2 expression and the expression of a mature complement of $GABA_A$ receptor subunits (Kanold and Shatz, 2006) (**Figure 2B**). Consistent with these molecular abnormalities, electrophysiological recordings showed that GABAergic circuits remain depolarizing (Kanold and Shatz, 2006). How then is KCC2 regulated, and how are subplate neurons involved? Recent experiments have led to the hypothesis that KCC2 expression can be regulated by GABAergic depolarization (Ganguly et al., 2001; Leitch et al., 2005), while others report no influence of GABAergic signaling on KCC2 expression (Ludwig et al., 2003; Titz et al., 2003; Sipila et al., 2009). However, there are other sources for depolarization. Blocking glutamatergic signaling during early ages *in vivo* is sufficient to prevent the developmental increase in KCC2 (Kanold and Shatz, 2006). Thus early glutamatergic activity might be required for GABAergic maturation in layer 4 (Kanold and Shatz, 2006). There are three sources of glutamatergic inputs to cortical layer 4: intracortical, thalamus and subplate neurons. Subplate removal by itself prevents inhibitory development despite the presence of intracortical and thalamic inputs (Kanold and Shatz, 2006). Thus, together these data suggest that glutamatergic excitation from subplate neurons is needed for inhibitory maturation (**Figure 1**). Such a role of subplate neurons in inhibitory maturation would require that subplate neurons depolarize layer 4 neurons, which can be achieved either by exciting GABAergic neurons and increasing early depolarizing GABAergic activity or by exciting the targets of GABAergic neurons directly. Thus by

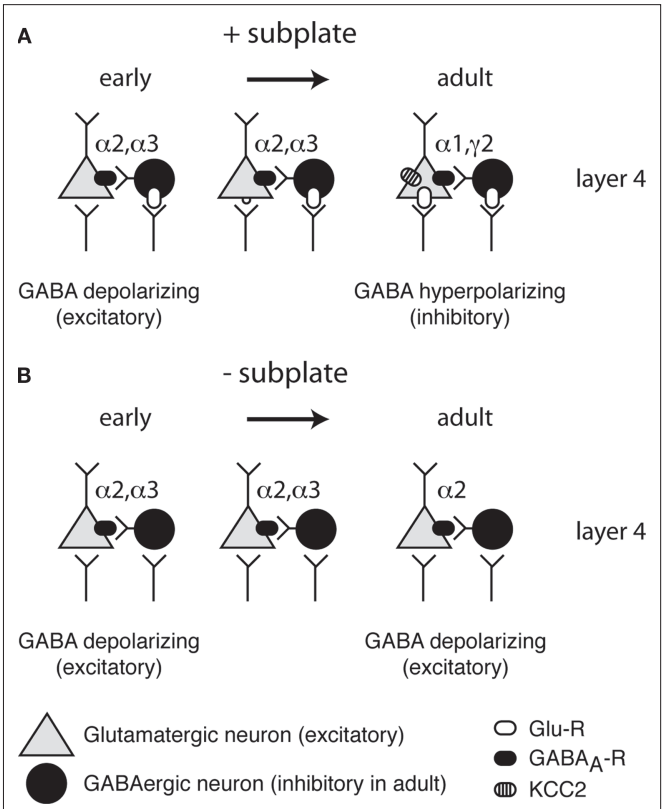

FIGURE 2 | Maturation of GABA(A)ergic circuitry. (A) Normal development with subplate present. Early in development (left) GABAergic inputs to neurons are depolarizing due to the lack of KCC2. As development progresses (middle) glutamatergic synapses appear and further depolarize neurons. Glutamatergic synapses strengthen and at a certain point in development a critical threshold of depolarization is reached (right) and KCC2 levels are increased, rendering GABA hyperpolarizing (inhibitory). In addition, mature GABAergic receptor subunits are expressed. **(B)** Abnormal development with subplate absent. KCC2 levels remain low and immature GABA receptor subunits remain expressed, while levels of mature subunits remain low.

providing feed-forward excitation to the developing cortical circuits subplate neurons can regulate both the maturation of glutamatergic thalamocortical and GABAergic intracortical synapses.

By controlling cortical inhibition, subplate neurons might also play a role in regulating cortical activity levels after GABAergic circuits have matured. Since subplate ablation prevents inhibitory maturation, maybe by directly activating GABAergic circuits, subplate activation is likely able to dampen cortical activity levels. Thus even temporary depression of subplate activity could lead to cortical hyperexcitability, which might underlie pathophysiological conditions (see below).

SUBPLATE NEURONS ENABLE THE FUNCTIONAL MATURATION OF CORTICAL RESPONSES AND SENSORY MAPS

Lesioning subplate at a time when thalamocortical axons are present in layer 4 (**Figure 1** middle), but before these projections have refined into a mature pattern, revealed a role for subplate neurons in thalamocortical patterning. The organizational pattern observed in the visual cortex is that of the ocular dominance columns (ODCs). These columns are formed by the segregation of thalamic afferents innervated by either eye into alternating bands of left eye or right eye dominance. In cat ODCs form during the postnatal period from an initially non-segregated state (see **Figure 3A** left). Analysis of the ODCs following ablation of subplate neurons in V1 with either kainic acid injections (Ghosh et al., 1990; Ghosh and Shatz, 1992, 1993, 1994) or immunoablation (Kanold et al., 2003) shows that subplate ablation prevents the formation of ODCs (Ghosh and Shatz, 1992, 1993, 1994; Kanold et al., 2003). This deficit in thalamocortical patterning is present even though both thalamic axons and their target neurons are present in layer 4 (Ghosh and Shatz, 1992, 1993, 1994; Kanold et al., 2003). Thus subplate neurons are necessary for the patterned organization of the cerebral cortex.

Activity dependent mechanisms have been known to be required for normal ODC formation (Shatz and Stryker, 1978; Chapman et al., 1986; Reiter et al., 1986; Stryker and Harris, 1986; Reiter and Stryker, 1988; Cang et al., 2005; Huberman et al., 2006). Indeed failure of thalamocortical strengthening after subplate removal is paralleled by decreased visual responsiveness and the lack of functional refinement of visual responses (Kanold et al., 2003). Thus, the fidelity of visual evoked responses in visual cortex is severely impaired and it is likely that these functional deficits in cortical processing subsequent to subplate ablation underlie the lack of ODCs consequent to subplate ablation (Ghosh and Shatz, 1992, 1993, 1994; Kanold et al., 2003).

In addition to thalamocortical and intracortical inhibitory circuits, long-range excitatory intracortical circuits also refine with sensory experience (Innocenti and Frost, 1980; Frost et al., 1990; Callaway and Katz, 1991; Sur et al., 1999). Thus, while the status of intracortical excitatory connections following subplate lesions has not been investigated, it is likely that subplate lesions, which abolish normal responses to patterned stimuli, will also affect the development of long-range intracortical circuits. Cognitive defects present after subplate damage, as discussed below, might be due from both deficits in thalamocortical and intracortical connections. A lack of refinement in intracortical excitatory circuits might functionally be evident as a larger number of smaller synaptic contacts which would be consistent with the observation that rates of spontaneous EPSC's but not their amplitude were increased in ablated areas (Kanold et al., 2003). However, these recordings were performed in layer 4, thus the source of the spontaneous EPSCs could also be from non-refined thalamocortical projections.

SUBPLATE NEURONS CONTROL PLASTICITY IN THE CRITICAL PERIOD

The function of inhibitory circuits is crucial for critical period plasticity in the visual cortex (Hensch, 2004; Kanold and Shatz, 2006). Sensory manipulations that alter inhibition also result in impaired synaptic plasticity mechanisms that underlie critical period plasticity. Thus, there is a co-regulation of inhibition and critical period plasticity (Kirkwood et al., 1995, 1996; Kotak et al., 2007; Kanold et al., 2009). Because subplate neurons play a crucial role in maturation of inhibition, it is likely that they also mediate cortical plasticity during the critical period.

In normal animals, if visual experience is altered, for example by monocular eye closure (monocular deprivation, MD) during the critical period then the pattern of ODCs is perturbed (Wiesel and Hubel, 1963; Hubel and Wiesel, 1970; Shatz and Stryker, 1978). MD causes a rearrangement of thalamocortical projections such that projections representing the open eye occupy a larger territory in layer 4, while projections representing the deprived eye occupy a smaller area (**Figure 3B** left). There is also a matching shift in physiological ocular dominance in cortical neurons towards the open eye.

As described above, under normal visual experience, removal of subplate neurons prevents the formation of ODCs from initially non-segregated inputs (Ghosh and Shatz, 1992, 1993, 1994; Kanold et al., 2003) (**Figure 3A** right). These results could be interpreted as thalamocortical projections remaining in their early non-segregated projection pattern. However, after subplate ablation, following MD, there is a "paradoxical" form of OD plasticity (Kanold and Shatz, 2006), meaning that the sign of the OD change is reversed. In these experiments subplate neurons were ablated before OD columns formed, meaning that projections from both eyes were present in all areas of the visual cortex. Eye closure is performed at the time of ablation, before the normal opening of the eyes. Transneuronal labeling at later ages shows that, in subplate ablated cortex, the deprived eye projections occupy a *larger* area than in cortical areas where the subplate was intact while projections representing the open eye occupy a *smaller* area in ablated compared to non-ablated areas (Kanold and Shatz, 2006) (**Figure 3B** right). Thus, paradoxically projections representing the more active eye have been removed while those representing the less active closed eye have been spared removal. A similar paradoxical removal of the more active inputs has been observed when cortical activity was pharmacologically silenced (Hata and Stryker, 1994; Hata et al., 1999).

Genetic manipulations that decrease inhibitory efficacy prevent OD plasticity entirely and it is thought that the maturation of inhibition is a key process enabling critical period plasticity (Hensch, 2004; Kanold and Shatz, 2006). Computational modeling studies (Kanold and Shatz, 2006) suggest that the outcome of OD plasticity following MD might exist on a continuum between "normal" and "paradoxical" and that the levels of inhibition might control where on this continuum the circuit operates. By regulating both

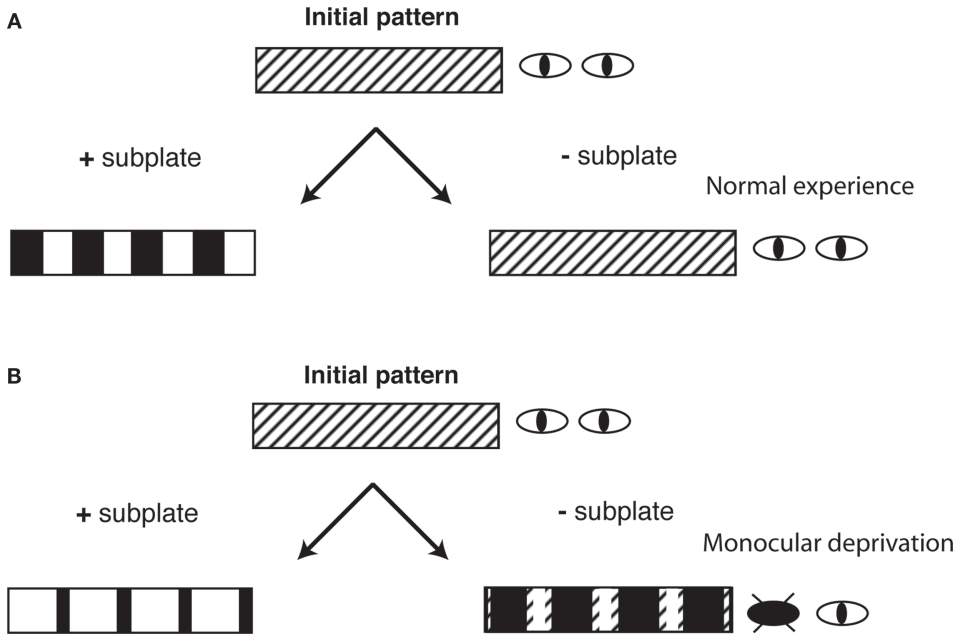

FIGURE 3 | Subplate neurons control the sign of OD plasticity. Shown are schematic diagrams of the ocular dominance columns in cat visual cortex under 4 conditions. ODCs form from an initial non-segregated pattern at early stages of development. **(A)** With normal experience and with subplate present (+subplate), each eye is represented in equally large regions of visual cortex. If subplate is removed (–subplate), ODCs do not form and thalamic afferents representing both eyes are intermingled. **(B)** If one eye is closed (monocular deprivation) and if subplate is present then the open eye representation expands. If subplate is absent there is a paradoxical removal of open eye projections.

the maturation of excitation and the maturation of inhibition, subplate neurons seem to control processes in layer 4 that are required for normal plasticity.

The disappearance of subplate neurons might ensure that certain processes, such as critical period plasticity, occur only once. While critical period plasticity might be distinct from adult plasticity by its extent and transience, the underlying mechanisms might be similar to processes underlying adult learning via attention based mechanisms (Hensch, 2004; Keuroghlian and Knudsen, 2007). This attentional modulation of cortical circuits develops postnatally, thus subplate neurons could provide a circuit that enables large-scale cortical plasticity mechanisms before attention is functioning.

A MODEL FOR THE ROLE OF SUBPLATE NEURONS IN DEVELOPMENT

The three developmental deficits observed after subplate ablation might have a common explanation when considering that each of these processes can be driven by depolarization. Synaptic strengthening in the brain is governed by synaptic plasticity rules such as long-term potentiation (LTP) and long-term depression (LTD) (Bear and Malenka, 1994; Malenka and Bear, 2004). While LTP at a particular synapse can be evoked by the activity of that synapse (homosynaptic LTP), this strengthening requires that this synapse is already of sufficient strength to modulate activity levels in the postsynaptic neuron. An alternative way of inducing LTP is associative LTP. Associative LTP of a weak synapse can be induced by simultaneous activation of another synapse, which is sufficiently strong to induce activity increases in the postsynaptic neuron.

Subplate activity can influence layer 4 synapses and enable thalamocortical strengthening to occur. In particular, subplate input to layer 4 can strongly depolarize layer 4 cells. Since subplate neurons are driven by thalamic activity, this subplate mediated depolarization of layer 4 cells occurs at the same time as direct thalamocortical input to layer 4 and may lead to a strengthening of thalamocortical synapses by associative LTP. The strength of the subplate to layer 4 connection is evidenced by the evoked disynaptic sinks in layer 4 during white matter stimulation (Friauf and Shatz, 1991). Therefore, subplate neurons can act somewhat like a "teacher" entraining layer 4 neurons to respond to appropriate thalamic inputs.

Formal implementation of this intuitive model using a computational simulation can replicate a large body of experimental data in normal development and after subplate removal (Kanold and Shatz, 2006). The topology of the computational model is based on the circuits present in development (**Figure 1**). A layer 4 neuron receives input from two LGN cells representing inputs from each eye. In addition the layer 4 neuron receives input from one subplate neuron. The subplate neuron receives input from the two LGN cells. All synapses on the layer 4 neuron can be modified according to spike-time dependent plasticity (STDP) rules (Abbott and Nelson, 2000; Bi and Poo, 2001) that are found at many synapses in the brain. In STDP synapses between neurons that are active within a certain time window prior to postsynaptic firing are strengthened while synapses that are active at other times are weakened.

At the start of the simulations LGN inputs to layer 4 are too weak to drive spiking in layer 4 and are not biased towards either eye. In contrast LGN inputs to subplate and subplate inputs to layer

4 are strong. The simulations show that subplate input to layer 4 induces correlations between thalamic firing and layer 4 activity. Since subplate neurons fire action potentials after thalamocortical synapses are active there is a positive time delay between the thalamocortical EPSCs and layer 4 firing. This time delay falls within the LTP window of the STDP rule and over time weak thalamocortical synapses are strengthened to adult strength (Kanold and Shatz, 2006).

Simulations of the effects of subplate removal show that correlations between the thalamic and layer 4 activity are not present. Thus, thalamic input to layer 4 does not strengthen, and sensory driven activity is absent in layer 4 (Kanold and Shatz, 2006), which is also observed in physiological experiments (Kanold et al., 2003). This is because the STDP window for LTD is longer than that for LTP (Abbott and Nelson, 2000; Feldman, 2000; Bi and Poo, 2001) and therefore if pre- and postsynaptic activity are uncorrelated, synaptic weakening occurs. Since in these simulations the activity levels in both eyes are equal, no refinement of ocular dominance is observed. In fact, these simulation results suggest that thalamic inputs will be weakened from their initial values over long periods of time.

Activity manipulations, such as MD reveal the explanatory power of this simple model. In MD the activity between the two eyes is unequal: the pathway driven by the open eye is more active while the pathway from the closed eye is less active. Simulations show that thalamic fibers driven by the open eye have a larger amount of uncorrelated activity with layer 4 neurons than fibers driven by the closed eye (Kanold and Shatz, 2006). Thus, in models with an asymmetric STDP rule, the more active inputs to layer 4 are weakened at a much faster rate than the less active inputs. Thus over time the projections representing the open eye disappear while projections representing the closed eye will be retained (Kanold and Shatz, 2006). This parallels experimental observations (Kanold and Shatz, 2006). Additionally, homeostatic mechanisms known to be present in the cortex (Turrigiano, 1999), can influence inputs from both eyes and thereby amplify this difference.

These simulation results support the view that subplate neurons can promote strengthening of thalamocortical connections by enabling correlations between thalamus and cortex that lead to synaptic strengthening. By this feed-forward mechanism, subplate neurons could also impart their stimulus selectivity to layer 4 neurons. Hence, cortical maps might be partially set up in the subplate and then transferred to and refined in layer 4 as has been proposed previously (Grossberg and Seitz, 2003).

SUBPLATE NEURONS IN DISEASE

Given the central role of subplate neurons in the maturation of cortical circuits, damage to subplate neurons at any point during development could lead to neurological diseases. Subplate neurons are particularly prone to injury (especially hypoxic-ischemic injuries) during development and are especially vulnerable at time points when injuries are associated with many neurodevelopmental disorders (second trimester) (Volpe, 1996, 2000; du Plessis and Volpe, 2002; McQuillen et al., 2003; McQuillen and Ferriero, 2005). The enhanced vulnerability of subplate neurons may be due to their early maturation and therefore higher metabolic requirements. This vulnerability of subplate neurons might be more pronounced in infants born prematurely that are at a higher risk for neurodevelopmental disorders disorders as a large period of development occur *ex utero*. In animals, neonatal hypoxia damages subplate neurons and prevents normal critical period plasticity (McQuillen et al., 2003; McQuillen and Ferriero, 2004; Failor et al., 2006), supporting the idea that such injuries damage circuits needed for the development of normal tuning and plasticity. In humans, such hypoxic-ischemic injuries, especially in the second trimester are associated with various neurodevelopmental disorders such as cerebral palsy and epilepsy (Volpe, 1996, 2000; Cioni et al., 1997; Schatz et al., 1997; Lanzi et al., 1998; Krageloh-Mann et al., 1999; Jacobson and Dutton, 2000; Deukmedjian et al., 2004; McQuillen and Ferriero, 2004; Meberg and Broch, 2004; Ozduman et al., 2004; Robinson, 2005; Robinson et al., 2006).

Subplate ablation in animals is followed by a period of seizures (Lein et al., 1999) indicating hyperactivity. These seizures develop a couple days after the time of ablation and thus are likely reflecting adjustments of the cortical network (Lein et al., 1999). The origin of these seizures is unclear. Seizures could be generating by depolarizing GABAergic activity (Kanold and Shatz, 2006) or alternatively be generated by glutamatergic activity that is not balanced appropriately by inhibitory GABAergic circuits. The different possible origins of seizures after subplate lesion are of clinical relevance. $GABA_A$ agonists can be used to treat seizures if they decrease firing probability. However, a GABAergic origin of seizures would also indicate that $GABA_A$ agonists would increase seizures instead of preventing them.

Many neurodevelopmental disorders are characterized by abnormal neuronal activity, hyperexcitability, and learning impairments due to impaired inhibition (Mathern et al., 2000; Lewis and Levitt, 2002; Cepeda et al., 2003; Christ et al., 2003; Lewis et al., 2004; Robinson et al., 2006), suggesting that altered inhibitory development underlies these disorders. Thus, a common outcome for early injuries and deprivations is that both alter inhibitory development, which in turn might alter critical period plasticity and normal development.

In addition to subplate lesions, the activity of subplate neurons can be altered by neuromodulators such as GABA, acetylcholine and glycine (see above). The subplate is also innervated by serotonergic fibers (Nakazawa et al., 1992) and subplate neurons selectively express progestin receptor (Lopez and Wagner, 2009). Thus, maternal or neonatal exposure to drugs (ranging from nicotine to sedatives and antidepressants) or hormones might alter subplate activity and thereby potentially disrupt cortical development. Therefore monitoring the status of subplate neurons in human infants is of high clinical relevance. Animal studies have shown that sensory activity can trigger cortical spindle bursts (Hanganu et al., 2006). While, the involvement of subplate neurons in the generation of spindle bursts is unclear, subplate neurons are thought to play a role in driving oscillatory activity in cortex (Dupont et al., 2006; Hanganu et al., 2009). *In vitro* MRI has been used to identify the subplate in humans (Rados et al., 2006), but functional MRI studies of subplate neurons have not been performed. However, such oscillatory activity can be detected using EEG, which has been used to monitor spontaneous and sensory evoked activity in human preterm and full-term infants (Vanhatalo et al., 2005; Vanhatalo and Kaila, 2006; Colonnese et al., 2008). These EEG studies identify the emergence of spontaneous activity transients (SATs) when thalamocortical fibers start to innervate the cortical plate (~30 GW)

(Vanhatalo and Kaila, 2006) and also show bipolar responses to early sensory stimuli (Colonnese et al., 2008). Thus by quantifying the emergence and characteristics of EEG features and studying the alteration of such features in subplate lesioned cortex one might be able to clinically assess the function of the early thalamocortical network of which the subplate is a key component.

WHY ARE THERE SUBPLATE NEURONS?

Subplate neurons are present in the cerebral cortex of all mammals (Luskin and Shatz, 1985; Valverde and Facal-Valverde, 1987, 1988; Allendoerfer and Shatz, 1994; Reep, 2000). Subplate neurons are more prominent in species with increased radial and tangential cortical connectivity such as cat, monkey and human (Mrzljak et al., 1988; Kostovic and Rakic, 1990; Kostovic et al., 2002; Kostovic and Judas, 2006), suggesting that subplate neurons might be needed for the establishment of more complex processing capabilities. The disappearance of subplate neurons over development suggests that their role is purely developmental. As discussed above, subplate neurons enable the functional maturation of cortical circuits. However, other areas in the brain (such as subcortical areas) seem mature without neurons equivalent to subplate neurons. Thus one can speculate that the role of subplate neurons might have to do with unique properties of the cerebral cortex. One hallmark of the cerebral cortex is complex interconnectivity and its ability to adjust its connectivity during early development in response to altered patterns of spontaneous and sensory inputs. This capability for rewiring of the cerebral cortex is greatly diminished after the critical period. Thus removing these enabling (or "teacher") circuits is one way to ensure that plasticity occurs only once and only during early development and might allow the development of higher cognitive processes at later stages of cortical processing at later ages.

CONCLUSION

Subplate neurons are an integral part of the developing cerebral cortex but their role in cortical development has been enigmatic. Recent progress has shown that they are required for the functional maturation of cortical circuits and for cortical plasticity. Because of their vulnerabilities, subplate neurons provide a key link between early brain injury and altered cortical function in the adult.

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REFERENCES

Abbott, L. F., and Nelson, S. B. (2000). Synaptic plasticity: taming the beast. *Nat. Neurosci.* 3(Suppl.), 1178–1183.

Allendoerfer, K. L., and Shatz, C. J. (1994). The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. *Annu. Rev. Neurosci.* 17, 185–218.

Arias, M. S., Baratta, J., Yu, J., and Robertson, R. T. (2002). Absence of selectivity in the loss of neurons from the developing cortical subplate of the rat. *Brain Res. Dev. Brain Res.* 139, 331–335.

Bear, M. F., and Malenka, R. C. (1994). Synaptic plasticity: LTP and LTD. *Curr. Opin. Neurobiol.* 4, 389–399.

Ben-Ari, Y. (2002). Excitatory actions of gaba during development: the nature of the nurture. *Nat. Rev. Neurosci.* 3, 728–739.

Ben-Ari, Y., Khalilov, I., Represa, A., and Gozlan, H. (2004). Interneurons set the tune of developing networks. *Trends Neurosci.* 27, 422–427.

Bi, G., and Poo, M. (2001). Synaptic modification by correlated activity: Hebb's postulate revisited. *Annu. Rev. Neurosci.* 24, 139–166.

Blaesse, P., Airaksinen, M. S., Rivera, C., and Kaila, K. (2009). Cation-chloride cotransporters and neuronal function. *Neuron* 61, 820–838.

Callaway, E. M., and Katz, L. C. (1991). Effects of binocular deprivation on the development of clustered horizontal connections in cat striate cortex. *Proc. Natl. Acad. Sci. U.S.A.* 88, 745–749.

Cang, J., Renteria, R. C., Kaneko, M., Liu, X., Copenhagen, D. R., and Stryker, M. P. (2005). Development of precise maps in visual cortex requires patterned spontaneous activity in the retina. *Neuron* 48, 797–809.

Catalano, S. M., Robertson, R. T., and Killackey, H. P. (1991). Early ingrowth of thalamocortical afferents to the neocortex of the prenatal rat. *Proc. Natl. Acad. Sci. U.S.A.* 88, 2999–3003.

Cepeda, C., Hurst, R. S., Flores-Hernandez, J., Hernandez-Echeagaray, E., Klapstein, G. J., Boylan, M. K., Calvert, C. R., Jocoy, E. L., Nguyen, O. K., Andre, V. M., Vinters, H. V., Ariano, M. A., Levine, M. S., and Mathern, G. W. (2003). Morphological and electrophysiological characterization of abnormal cell types in pediatric cortical dysplasia. *J. Neurosci. Res.* 72, 472–486.

Chapman, B., Jacobson, M. D., Reiter, H. O., and Stryker, M. P. (1986). Ocular dominance shift in kitten visual cortex caused by imbalance in retinal electrical activity. *Nature* 324, 154–156.

Chen, L., Yang, C., and Mower, G. D. (2001a). Developmental changes in the expression of GABA(A) receptor subunits (alpha(1), alpha(2), alpha(3)) in the cat visual cortex and the effects of dark rearing. *Brain Res. Mol. Brain Res.* 88, 135–143.

Chen, L., Yang, C., and Mower, G. D. (2001b). Developmental changes in the expression of GABA(A) receptor subunits (alpha(1), alpha(2), alpha(3)) in the cat visual cortex and the effects of dark rearing. *Brain Res. Mol. Brain Res.* 88, 135–143.

Christ, S. E., White, D. A., Brunstrom, J. E., and Abrams, R. A. (2003). Inhibitory control following perinatal brain injury. *Neuropsychology* 17, 171–178.

Chun, J. J., and Shatz, C. J. (1989a). The earliest-generated neurons of the cat cerebral cortex: characterization by MAP2 and neurotransmitter immunohistochemistry during fetal life. *J. Neurosci.* 9, 1648–1667.

Chun, J. J., and Shatz, C. J. (1989b). Interstitial cells of the adult neocortical white matter are the remnant of the early generated subplate neuron population. *J. Comp. Neurol.* 282, 555–569.

Cioni, G., Fazzi, B., Coluccini, M., Bartalena, L., Boldrini, A., and van Hof-van Duin, J. (1997). Cerebral visual impairment in preterm infants with periventricular leukomalacia. *Pediatr. Neurol.* 17, 331–338.

Clancy, B., Darlington, R. B., and Finlay, B. L. (2001). Translating developmental time across mammalian species. *Neuroscience* 105, 7–17.

Cobas, A., Fairen, A., Alvarez-Bolado, G., and Sanchez, M. P. (1991). Prenatal development of the intrinsic neurons of the rat neocortex: a comparative study of the distribution of GABA-immunoreactive cells and the GABAA receptor. *Neuroscience* 40, 375–397.

Colonnese, M. T., Kaminska, A., and Khazipov, R. (2008). Developmental Switch in Sensory Signal Processing in Visual Cortex Prior to the Onset of Vision. Washington, DC, Abstracts Society for Neuroscience Annual Meeting.

Deukmedjian, A. J., King, M. A., Cuda, C., and Roper, S. N. (2004). The GABAergic system of the developing neocortex has a reduced capacity to recover from in utero injury in experimental cortical dysplasia. *J. Neuropathol. Exp. Neurol.* 63, 1265–1273.

du Plessis, A. J., and Volpe, J. J. (2002). Perinatal brain injury in the preterm and term newborn. *Curr. Opin. Neurol.* 15, 151–157.

Dupont, E., Hanganu, I. L., Kilb, W., Hirsch, S., and Luhmann, H. J. (2006). Rapid developmental switch in the mechanisms driving early cortical columnar networks. *Nature* 439, 79–83.

Failor, S. W., Evans, M. M., Cang, J., Stryker, M. P., and McQuillen, P. S. (2006). Impaired Cortical Plasticity After Early Hypoxia-ischemia. Atlanta, GA, Abstracts Society for Neuroscience Annual Meeting.

Feldman, D. E. (2000). Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. *Neuron* 27, 45–56.

Finney, E. M., Stone, J. R., and Shatz, C. J. (1998). Major glutamatergic projection from subplate into visual cortex during development. *J. Comp. Neurol.* 398, 105–118.

Friauf, E., McConnell, S. K., and Shatz, C. J. (1990). Functional synaptic circuits in the subplate during fetal and early

postnatal development of cat visual cortex. *J. Neurosci.* 10, 2601–2613.

Friauf, E., and Shatz, C. J. (1991). Changing patterns of synaptic input to subplate and cortical plate during development of visual cortex. *J. Neurophysiol.* 66, 2059–2071.

Frost, D. O., Moy, Y. P., and Smith, D. C. (1990). Effects of alternating monocular occlusion on the development of visual callosal connections. *Exp. Brain Res.* 83, 200–209.

Fuchs, J. L., and Salazar, E. (1998). Effects of whisker trimming on GABA(A) receptor binding in the barrel cortex of developing and adult rats. *J. Comp. Neurol.* 395, 209–216.

Ganguly, K., Schinder, A. F., Wong, S. T., and Poo, M. (2001). GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell* 105, 521–532.

Ghosh, A., Antonini, A., McConnell, S. K., and Shatz, C. J. (1990). Requirement for subplate neurons in the formation of thalamocortical connections. *Nature* 347, 179–181.

Ghosh, A., and Shatz, C. J. (1992). Involvement of subplate neurons in the formation of ocular dominance columns. *Science* 255, 1441–1443.

Ghosh, A., and Shatz, C. J. (1993). A role for subplate neurons in the patterning of connections from thalamus to neocortex. *Development* 117, 1031–1047.

Ghosh, A., and Shatz, C. J. (1994). Segregation of geniculocortical afferents during the critical period: a role for subplate neurons. *J. Neurosci.* 14, 3862–3880.

Gianfranceschi, L., Siciliano, R., Walls, J., Morales, B., Kirkwood, A., Huang, Z. J., Tonegawa, S., and Maffei, L. (2003). Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF. *Proc. Natl. Acad. Sci. U.S.A.* 100, 12486–12491.

Grossberg, S., and Seitz, A. (2003). Laminar development of receptive fields, maps and columns in visual cortex: the coordinating role of the subplate. *Cereb. Cortex* 13, 852–863.

Hanganu, I. L., Ben-Ari, Y., and Khazipov, R. (2006). Retinal waves trigger spindle bursts in the neonatal rat visual cortex. *J. Neurosci.* 26, 6728–6736.

Hanganu, I. L., Kilb, W., and Luhmann, H. J. (2002). Functional synaptic projections onto subplate neurons in neonatal rat somatosensory cortex. *J. Neurosci.* 22, 7165–7176.

Hanganu, I. L., and Luhmann, H. J. (2004). Functional nicotinic acetylcholine receptors on subplate neurons in neonatal rat somatosensory cortex. *J. Neurophysiol.* 92, 189–198.

Hanganu, I. L., Okabe, A., Lessmann, V., and Luhmann, H. J. (2009). Cellular mechanisms of subplate-driven and cholinergic input-dependent network activity in the neonatal rat somatosensory cortex. *Cereb. Cortex* 19, 89–105.

Hata, Y., and Stryker, M. P. (1994). Control of thalamocortical afferent rearrangement by postsynaptic activity in developing visual cortex. *Science* 265, 1732–1735.

Hata, Y., Tsumoto, T., and Stryker, M. P. (1999). Selective pruning of more active afferents when cat visual cortex is pharmacologically inhibited. *Neuron* 22, 375–381.

Hensch, T. K. (2004). Critical period regulation. *Annu. Rev. Neurosci.* 27, 549–579.

Higashi, S., Molnar, Z., Kurotani, T., and Toyama, K. (2002). Prenatal development of neural excitation in rat thalamocortical projections studied by optical recording. *Neuroscience* 115, 1231–1246.

Hirsch, S., and Luhmann, H. J. (2008). Pathway-specificity in *N*-methyl-D-aspartate receptor-mediated synaptic inputs onto subplate neurons. *Neuroscience* 153, 1092–1102.

Huang, Z. J. (2009). Activity-dependent development of inhibitory synapses and innervation pattern: role of GABA signalling and beyond. *J. Physiol.* 587, 1881–1888.

Huang, Z. J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M. F., Maffei, L., and Tonegawa, S. (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell* 98, 739–755.

Hubel, D. H., and Wiesel, T. N. (1970). The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J. Physiol.* 206, 419–436.

Huberman, A. D., Speer, C. M., and Chapman, B. (2006). Spontaneous retinal activity mediates development of ocular dominance columns and binocular receptive fields in v1. *Neuron* 52, 247–254.

Innocenti, G. M., and Frost, D. O. (1980). The postnatal development of visual callosal connections in the absence of visual experience or of the eyes. *Exp. Brain Res.* 39, 365–375.

Jacobson, L. K., and Dutton, G. N. (2000). Periventricular leukomalacia: an important cause of visual and ocular motility dysfunction in children. *Surv. Ophthalmol.* 45, 1–13.

Jiang, B., Huang, Z. J., Morales, B., and Kirkwood, A. (2005). Maturation of GABAergic transmission and the timing of plasticity in visual cortex. *Brain Res. Brain Res. Rev.* 50, 126–133.

Jiao, Y., Zhang, C., Yanagawa, Y., and Sun, Q. Q. (2006). Major effects of sensory experiences on the neocortical inhibitory circuits. *J. Neurosci.* 26, 8691–8701.

Kanold, P. O., Kara, P., Reid, R. C., and Shatz, C. J. (2003). Role of subplate neurons in functional maturation of visual cortical columns. *Science* 301, 521–525.

Kanold, P. O., Kim, Y. A., Grandpre, T., and Shatz, C. J. (2009). Co-regulation of ocular dominance plasticity and NMDA receptor subunit expression in glutamic acid decarboxylase-65 knock-out mice. *J. Physiol.* 587 (Pt 12), 2857–2867.

Kanold, P. O., and Shatz, C. J. (2006). Subplate neurons regulate maturation of cortical inhibition and outcome of ocular dominance plasticity. *Neuron* 51, 627–638.

Keuroghlian, A. S., and Knudsen, E. I. (2007). Adaptive auditory plasticity in developing and adult animals. *Prog. Neurobiol.* 82, 109–121.

Kilb, W., Hanganu, I. L., Okabe, A., Sava, B. A., Shimizu-Okabe, C., Fukuda, A., and Luhmann, H. J. (2008). Glycine receptors mediate excitation of subplate neurons in neonatal rat cerebral cortex. *J. Neurophysiol.* 100, 698–707.

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.

Kirkwood, A., Rioult, M. C., and Bear, M. F. (1996). Experience-dependent modification of synaptic plasticity in visual cortex. *Nature* 381, 526–528.

Kostovic, I., and Judas, M. (2006). Prolonged coexistence of transient and permanent circuitry elements in the developing cerebral cortex of fetuses and preterm infants. *Dev. Med. Child Neurol.* 48, 388–393.

Kostovic, I., Judas, M., Rados, M., and Hrabac, P. (2002). Laminar organization of the human fetal cerebrum revealed by histochemical markers and magnetic resonance imaging. *Cereb. Cortex* 12, 536–544.

Kostovic, I., and Rakic, P. (1990). Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J. Comp. Neurol.* 297, 441–470.

Kotak, V. C., Breithaupt, A. D., and Sanes, D. H. (2007). Developmental hearing loss eliminates long-term potentiation in the auditory cortex. *Proc. Natl. Acad. Sci. U.S.A.* 104, 3550–3555.

Kotak, V. C., Fujisawa, S., Lee, F. A., Karthikeyan, O., Aoki, C., and Sanes, D. H. (2005). Hearing loss raises excitability in the auditory cortex. *J. Neurosci.* 25, 3908–3918.

Krageloh-Mann, I., Toft, P., Lunding, J., Andresen, J., Pryds, O., and Lou, H. C. (1999). Brain lesions in preterms: origin, consequences and compensation. *Acta Paediatr.* 88, 897–908.

Lanzi, G., Fazzi, E., Uggetti, C., Cavallini, A., Danova, S., Egitto, M. G., Ginevra, O. F., Salati, R., and Bianchi, P. E. (1998). Cerebral visual impairment in periventricular leukomalacia. *Neuropediatrics* 29, 145–150.

Lein, E. S., Finney, E. M., McQuillen, P. S., and Shatz, C. J. (1999). Subplate neuron ablation alters neurotrophin expression and ocular dominance column formation. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13491–13495.

Lein, E. S., and Shatz, C. J. (2000). Rapid regulation of brain-derived neurotrophic factor mRNA within eye-specific circuits during ocular dominance column formation. *J. Neurosci.* 20, 1470–1483.

Leitch, E., Coaker, J., Young, C., Mehta, V., and Sernagor, E. (2005). GABA type-A activity controls its own developmental polarity switch in the maturing retina. *J. Neurosci.* 25, 4801–4805.

Lewis, D. A., and Levitt, P. (2002). Schizophrenia as a disorder of neurodevelopment. *Annu. Rev. Neurosci.* 25, 409–432.

Lewis, D. A., Volk, D. W., and Hashimoto, T. (2004). Selective alterations in prefrontal cortical GABA neurotransmission in schizophrenia: a novel target for the treatment of working memory dysfunction. *Psychopharmacology (Berl).* 174, 143–150.

Lopez, V., and Wagner, C. K. (2009). Progestin receptor is transiently expressed perinatally in neurons of the rat isocortex. *J. Comp. Neurol.* 512, 124–139.

Ludwig, A., Li, H., Saarma, M., Kaila, K., and Rivera, C. (2003). Developmental up-regulation of KCC2 in the absence of GABAergic and glutamatergic transmission. *Eur. J. Neurosci.* 18, 3199–3206.

Luskin, M. B., and Shatz, C. J. (1985). Studies of the earliest generated cells of the cat's visual cortex: cogeneration of subplate and marginal zones. *J. Neurosci.* 5, 1062–1075.

Malenka, R. C., and Bear, M. F. (2004). LTP and LTD: an embarrassment of riches. *Neuron* 44, 5–21.

Mathern, G. W., Cepeda, C., Hurst, R. S., Flores-Hernandez, J., Mendoza, D., and Levine, M. S. (2000). Neurons recorded from pediatric epilepsy surgery patients with cortical dysplasia. *Epilepsia* 41(Suppl. 6), S162–S167.

Matute, C., Wahle, P., Gutierrez-Igarza, K., and Albus, K. (1993). Distribution of neurons expressing substance P receptor messenger RNA in immature and adult cat visual cortex. *Exp. Brain Res.* 97, 295–300.

McQuillen, P. S., and Ferriero, D. M. (2004). Selective vulnerability in the developing central nervous system. *Pediatr. Neurol.* 30, 227–235.

McQuillen, P. S., and Ferriero, D. M. (2005). Perinatal subplate neuron injury: implications for cortical development and plasticity. *Brain Pathol.* 15, 250–260.

McQuillen, P. S., Sheldon, R. A., Shatz, C. J., and Ferriero, D. M. (2003). Selective vulnerability of subplate neurons after early neonatal hypoxia-ischemia. *J. Neurosci.* 23, 3308–3315.

Meberg, A., and Broch, H. (2004). Etiology of cerebral palsy. *J. Perinat. Med.* 32, 434–439.

Meinecke, D. L., and Rakic, P. (1992). Expression of GABA and GABAA receptors by neurons of the subplate zone in developing primate occipital cortex: evidence for transient local circuits. *J. Comp. Neurol.* 317, 91–101.

Molnar, Z., and Blakemore, C. (1995). Guidance of thalamocortical innervation. *Ciba Found. Symp.* 193, 127–149; discussion 192–199.

Molnar, Z., Kurotani, T., Higashi, S., Yamamoto, N., and Toyama, K. (2003). Development of functional thalamocortical synapses studied with current source-density analysis in whole forebrain slices in the rat. *Brain Res. Bull.* 60, 355–371.

Morales, B., Choi, S. Y., and Kirkwood, A. (2002). Dark rearing alters the development of GABAergic transmission in visual cortex. *J. Neurosci.* 22, 8084–8090.

Mrzljak, L., Uylings, H. B., Kostovic, I., and Van Eden, C. G. (1988). Prenatal development of neurons in the human prefrontal cortex: I. A qualitative Golgi study. *J. Comp. Neurol.* 271, 355–386.

Nakazawa, M., Koh, T., Kani, K., and Maeda, T. (1992). Transient patterns of serotonergic innervation in the rat visual cortex: normal development and effects of neonatal enucleation. *Brain Res. Dev. Brain Res.* 66, 77–90.

Owens, D. F., and Kriegstein, A. R. (2002). Is there more to GABA than synaptic inhibition? *Nat. Rev. Neurosci.* 3, 715–727.

Ozduman, K., Pober, B. R., Barnes, P., Copel, J. A., Ogle, E. A., Duncan, C. C., and Ment, L. R. (2004). Fetal stroke. *Pediatr. Neurol.* 30, 151–162.

Perkins, L., Hughes, E., Srinivasan, L., Allsop, J., Glover, A., Kumar, S., Fisk, N., and Rutherford, M. (2008). Exploring cortical subplate evolution using magnetic resonance imaging of the fetal brain. *Dev. Neurosci.* 30, 211–220.

Pinon, M. C., Jethwa, A., Jacobs, E., Campagnoni, A., and Molnar, Z. (2009). Dynamic integration of subplate neurons into the cortical barrel field circuitry during postnatal development in the Golli-tau-eGFP (GTE) mouse. *J. Physiol.* 587, 1903–1915.

Price, D. J., Aslam, S., Tasker, L., and Gillies, K. (1997). Fates of the earliest generated cells in the developing murine neocortex. *J. Comp. Neurol.* 377, 414–422.

Rados, M., Judas, M., and Kostovic, I. (2006). In vitro MRI of brain development. *Eur. J. Radiol.* 57, 187–198.

Reep, R. L. (2000). Cortical layer VII and persistent subplate cells in mammalian brains. *Brain Behav. Evol.* 56, 212–234.

Reiter, H. O., and Stryker, M. P. (1988). Neural plasticity without postsynaptic action potentials: less-active inputs become dominant when kitten visual cortical cells are pharmacologically inhibited. *Proc. Natl. Acad. Sci. U.S.A.* 85, 3623–3627.

Reiter, H. O., Waitzman, D. M., and Stryker, M. P. (1986). Cortical activity blockade prevents ocular dominance plasticity in the kitten visual cortex. *Exp. Brain Res.* 65, 182–188.

Rivera, C., Voipio, J., Payne, J. A., Ruusuvuori, E., Lahtinen, H., Lamsa, K., Pirvola, U., Saarma, M., and Kaila, K. (1999). The K+/Cl− co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397, 251–255.

Robinson, S. (2005). Systemic prenatal insults disrupt telencephalon development: implications for potential interventions. *Epilepsy Behav.* 7, 345–363.

Robinson, S., Li, Q., Dechant, A., and Cohen, M. L. (2006). Neonatal loss of gamma-aminobutyric acid pathway expression after human perinatal brain injury. *J. Neurosurg.* 104, 396–408.

Robinson, S. R., and Dreher, B. (1990). The visual pathways of eutherian mammals and marsupials develop according to a common timetable. *Brain Behav. Evol.* 36, 177–195.

Schatz, J., Craft, S., Koby, M., and Park, T. S. (1997). Associative learning in children with perinatal brain injury. *J. Int. Neuropsychol. Soc.* 3, 521–527.

Shatz, C. J., and Stryker, M. P. (1978). Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. *J. Physiol.* 281, 267–283.

Shimizu-Okabe, C., Okabe, A., Kilb, W., Sato, K., Luhmann, H. J., and Fukuda, A. (2007). Changes in the expression of cation-Cl− cotransporters, NKCC1 and KCC2, during cortical malformation induced by neonatal freeze-lesion. *Neurosci. Res.* 59, 288–295.

Shimizu-Okabe, C., Yokokura, M., Okabe, A., Ikeda, M., Sato, K., Kilb, W., Luhmann, H. J., and Fukuda, A. (2002). Layer-specific expression of Cl− transporters and differential [Cl−]i in newborn rat cortex. *Neuroreport* 13, 2433–2437.

Sipila, S. T., Huttu, K., Yamada, J., Afzalov, R., Voipio, J., Blaesse, P., and Kaila, K. (2009). Compensatory enhancement of intrinsic spiking upon NKCC1 disruption in neonatal hippocampus. *J. Neurosci.* 29, 6982–6988.

Stryker, M. P., and Harris, W. A. (1986). Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. *J. Neurosci.* 6, 2117–2133.

Sur, M., Angelucci, A., and Sharma, J. (1999). Rewiring cortex: the role of patterned activity in development and plasticity of neocortical circuits. *J. Neurobiol.* 41, 33–43.

Titz, S., Hans, M., Kelsch, W., Lewen, A., Swandulla, D., and Misgeld, U. (2003). Hyperpolarizing inhibition develops without trophic support by GABA in cultured rat midbrain neurons. *J. Physiol.* 550, 719–730.

Torres-Reveron, J., and Friedlander, M. J. (2007). Properties of persistent postnatal cortical subplate neurons. *J. Neurosci.* 27, 9962–9974.

Turrigiano, G. G. (1999). Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci.* 22, 221–227.

Vale, C., Schoorlemmer, J., and Sanes, D. H. (2003). Deafness disrupts chloride transporter function and inhibitory synaptic transmission. *J. Neurosci.* 23, 7516–7524.

Valverde, F., and Facal-Valverde, M. V. (1987). Transitory population of cells in the temporal cortex of kittens. *Brain Res.* 429, 283–288.

Valverde, F., and Facal-Valverde, M. V. (1988). Postnatal development of interstitial (subplate) cells in the white matter of the temporal cortex of kittens: a correlated Golgi and electron microscopic study. *J. Comp. Neurol.* 269, 168–192.

Vanhatalo, S., and Kaila, K. (2006). Development of neonatal EEG activity: from phenomenology to physiology. *Semin. Fetal Neonatal Med.* 11, 471–478.

Vanhatalo, S., Palva, J. M., Andersson, S., Rivera, C., Voipio, J., and Kaila, K. (2005). Slow endogenous activity transients and developmental expression of K+–Cl− cotransporter 2 in the immature human cortex. *Eur. J. Neurosci.* 22, 2799–2804.

Volpe, J. J. (1996). Subplate neurons – missing link in brain injury of the premature infant? *Pediatrics* 97, 112–113.

Volpe, J. J. (2000). Overview: normal and abnormal human brain development. *Ment. Retard. Dev. Disabil. Res. Rev.* 6, 1–5.

Wahle, P., Lubke, J., and Naegele, J. R. (1994). Inverted pyramidal neurons and interneurons in cat cortical subplate zone are labelled by monoclonal antibody SP1. *Eur. J. Neurosci.* 6, 1167–1178.

Wahle, P., Meyer, G., Wu, J. Y., and Albus, K. (1987). Morphology and axon terminal pattern of glutamate decarboxylase-immunoreactive cell types in the white matter of the cat occipital cortex during early postnatal development. *Brain Res.* 433, 53–61.

Wiesel, T. N., and Hubel, D. H. (1963). Single-cell responses in striate cortex of kittens deprived of vision in one eye. *J. Neurophysiol.* 26, 1003–1017.

Woo, T. U., Beale, J. M., and Finlay, B. L. (1991). Dual fate of subplate neurons in a rodent. *Cereb. Cortex* 1, 433–443.

Wood, J. G., Martin, S., and Price, D. J. (1992). Evidence that the earliest generated cells of the murine cerebral cortex form a transient population in the subplate and marginal zone. *Brain Res. Dev. Brain Res.* 66, 137–140.

Yamada, J., Okabe, A., Toyoda, H., Kilb, W., Luhmann, H. J., and Fukuda, A. (2004). Cl− uptake promoting depolarizing GABA actions in immature rat neocortical neurones is mediated by NKCC1. *J. Physiol.* 557, 829–841.

Zecevic, N., and Milosevic, A. (1997). Initial development of gamma-aminobutyric acid immunoreactivity in the human cerebral cortex. *J. Comp. Neurol.* 380, 495–506.

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Subplate cells: amplifiers of neuronal activity in the developing cerebral cortex

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Due to their unique structural and functional properties, subplate cells are ideally suited to function as important amplifying units within the developing neocortical circuit. Subplate neurons have extensive dendritic and axonal ramifications and relatively mature functional properties, i.e. their action potential firing can exceed frequencies of 40 Hz. At earliest stages of corticogenesis subplate cells receive functional synaptic inputs from the thalamus and from other cortical and non-cortical sources. Glutamatergic and depolarizing GABAergic inputs arise from cortical neurons and neuromodulatory inputs arise from the basal forebrain and other sources. Activation of postsynaptic metabotropic receptors, i.e. muscarinic receptors, elicits in subplate neurons oscillatory burst discharges which are transmitted via electrical and chemical synapses to neighbouring subplate cells and to immature neurons in the cortical plate. The tonic non-synaptic release of GABA from GABAergic subplate cells facilitates the generation of burst discharges. These cellular bursts are amplified by prominent gap junction coupling in the subplate and cortical plate, thereby eliciting 10–20 Hz oscillations in a local columnar network. Thus, we propose that neuronal networks are organized at earliest stages in a gap junction coupled columnar syncytium. We postulate that the subplate does not only serve as a transient relay station for afferent inputs, but rather as an active element amplifying the afferent and intracortical activity.

Keywords: neocortex, development, subplate, electrophysiology, microciruitry, GABA, glutamate, NMDA

INTRODUCTION

The subplate forms a transient layer in the developing cerebral cortex and consists of migratory and postmigratory neurons, dendrites, axons, growth cones, synapses and glial cells. The subplate is located between the intermediate zone and the cortical plate, which during further development differentiates into the neocortical layers II to VI (**Figure 1A**). Kostovic and Molliver were the first who identified the subplate as a distinct layer in the embryonic human cerebral cortex (Kostovic and Molliver, 1974). Subsequently Rakic (1977) described this layer in the monkey neocortex. A subplate can be identified in all mammals although its relative thickness, developmental profile and persistence in adulthood vary among species. Anatomical data indicate an evolutionary difference in the ontogenetic fate of the subplate. In the rat and other rodents many subplate cells survive into adulthood forming layer VIb or VII (Aboitiz and Montiel, 2007). Prominent species differences also exist in the relative thickness of the subplate, which increased during evolution. In humans the subplate develops to approximately six times the thickness of the cortical plate around 29 weeks of gestation (Mrzljak et al., 1990), whereas in rodents it remains a relatively thin layer during development (Uylings et al., 1990). The majority of the subplate cells are born early before the first cortical plate neurons (Luskin and Shatz, 1985; Valverde et al., 1989). In rodents subplate neurons may be also generated later in development (Hevner et al., 2004). A substantial proportion of subplate cells are not born in the ventricular neuroepithelium, but instead originate in the medial ganglionic eminence and follow a tangential migratory route to their positions in the developing cortex (Lavdas et al., 1999). Subplate cells represent a rather heterogeneous neuronal population according to their morphology, neurotransmitter identity and connectivity (for review Allendoerfer and Shatz, 1994).

Subplate cells play important roles in the structural and functional organization of the cerebral cortex and in early necortical plasticity. Axons arising from subplate neurons pioneer the corticofugal pathway and have been proposed to form a cellular scaffold for guiding thalamocortical axons (McConnell et al., 1989; Ghosh et al., 1990) (for review Allendoerfer and Shatz, 1994; Molnár, 1998). Subplate neurons receive a transient synaptic input from "waiting" thalamic axons (Lund and Mustari, 1977; Rakic, 1977, 1983) and early deletion of subplate neurons in kitten visual cortex prevents the segregation of thalamocortical axons within layer IV and the formation of ocular dominance columns (Ghosh and Shatz, 1992; Kanold et al., 2003). Furthermore, subplate cells regulate the maturation of GABAergic synaptic transmission and establish the balance between excitation and inhibition in the developing neocortical network (Kanold and Shatz, 2006).

STRUCTURAL PROPERTIES OF SUBPLATE NEURONS

Subplate neurons reveal a large variety of morphologies (**Figure 1B**) (Hanganu et al., 2002). Inverted pyramidal-like and horizontal cells as well as polymorphic neurons with different shapes and spiny or smooth dendrites have been classified as subplate neurons (Kostovic and Rakic, 1980; Wahle et al., 1987; Valverde et al., 1989; Kostovic and Rakic, 1990). Due to their earlier generation and more mature developmental stage, subplate neurons show a relatively extensive dendritic tree when compared to the more immature pyramidal

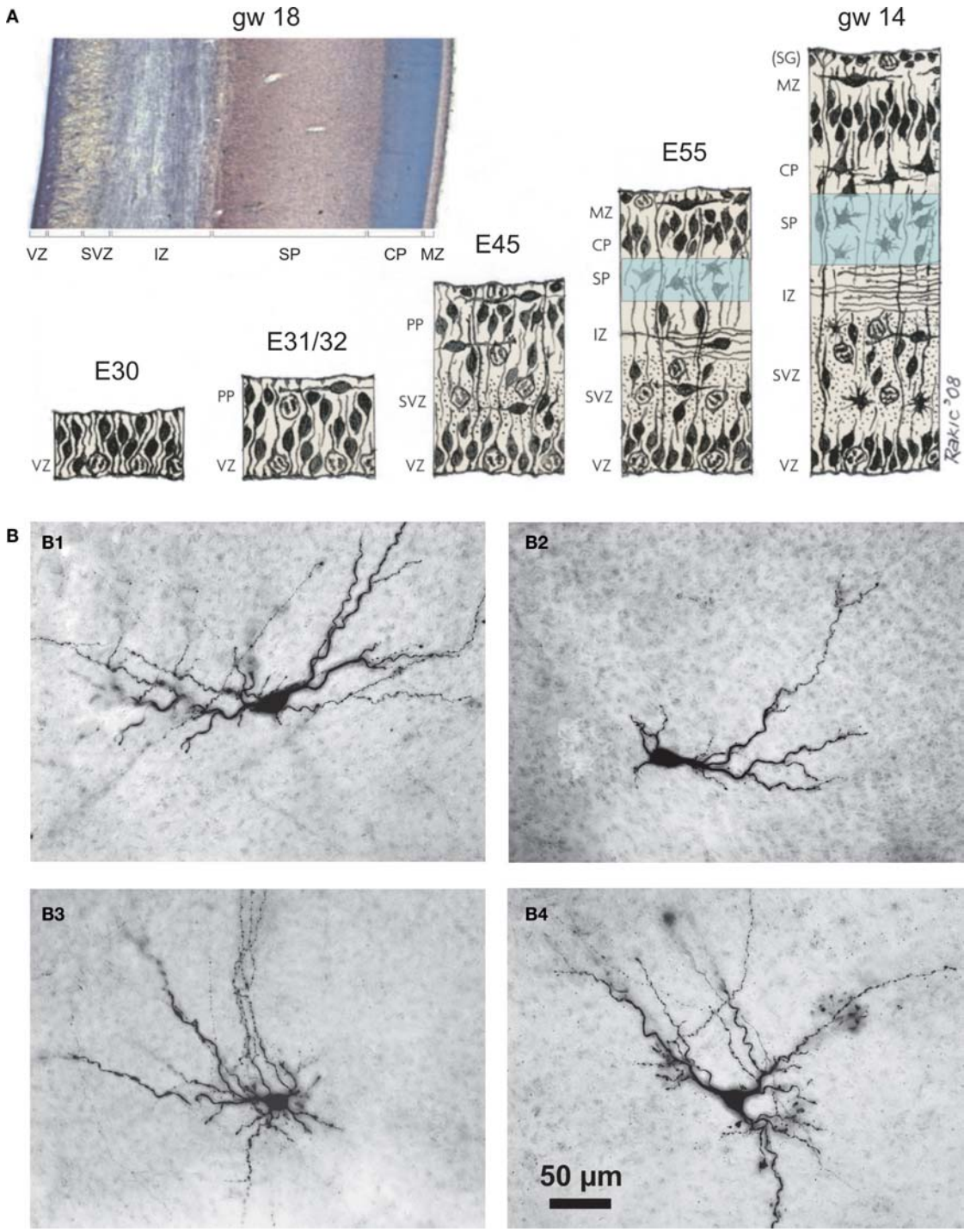

FIGURE 1 | Emergence of the subplate during neocortical development and structural diversity of subplate cells. (A) Prenatal development of the human cerebral cortex as suggested by Bystron et al. (2008). Drawing from Pasko Rakic. Photograph shows coronal brain section stained with cresyl violet of a gestational week 18 human neocortex. Note large expansion of subplate as compared to other neocortical layers. Approximate embryonic day (E) and gestational week (gw) are given for each developmental stage. CP, cortical plate; IZ, intermediate zone; MZ, marginal zone; PP, preplate; SP, subplate; SVZ, subventricular zone; (SG), subpial granular layer (part of MZ); VZ, ventricular zone. **(B)** Morphological properties of biocytin-stained subplate neurons in newborn rat cerebral cortex. **(B1)** Postnatal day (P) three horizontal bitufted cell with large fusiform soma and primary dendrites oriented parallel to the pial surface. **(B2)** P2 horizontal monotufted SPn. **(B3)** P3 multipolar cell with extensive dendritic arborization within SP and layers V/VI. **(B4)** P2 inverted pyramidal neuron with triangular soma and "apical" dendrite oriented towards white matter. Scale bar in **(B4)** corresponds to **(B1–B4)** and pial surface is located toward the top in all photomicrographs of **(B)**. Reproduced and modified with permission from (Bystron et al., 2008) **(A)**, (Kostovic et al., 2002) [photograph in **(A)**] and from (Hanganu et al., 2002) **(B)**.

neurons of the cortical plate (Mrzljak et al., 1992). Descending dendrites from subplate neurons may invade the underlying intermediate zone and ascending dendrites may extend into the cortical plate (Del Río et al., 2000). This morphological heterogeneity is accompanied by a large variation in immunoreactivity. Subplate neurons reveal markers for GABA or glutamate and may co-express various peptides (Chun et al., 1987; Finney et al., 1998). The morphological and neurochemical heterogeneity also explains, why various attempts failed to identify a specific marker for subplate neurons (Wahle et al., 1994). Recently Hoerder-Suabedissen et al. (2009) succeeded in identifying a number of novel markers for murine subplate cells, which may soon allow the definition of different subpopulations of subplate neurons.

Subplate cells participate in local and long-distance axonal connections indicating that these neurons may function as local circuit as well as projection neurons. They show a dense axonal arborization within the subplate, but also project to the marginal zone/layer I (Clancy and Cauller, 1999) and to the cortical plate, where they form axonal collaterals within layer IV (Friauf et al., 1990). In ferrets and cats, the majority of the subplate neurons projecting into the cortical plate reside in the upper half of the subplate and provide a glutamatergic synaptic input to the developing cortical plate, including the layer IV neurons (Friauf et al., 1990; Finney et al., 1998). Long-distance axons from subplate neurons invade the thalamus during early stages of corticogenesis and form an axonal scaffold for the establishment of cortical efferent and afferent projections (McConnell et al., 1989, 1994; Kim et al., 1991; De Carlos and O'Leary, 1992). Beside these local and long-distance projections arising from glutamatergic subplate neurons, GABAergic subplate cells also project to both neighbouring and more distant neocortical regions and form a corticocortical synaptic network (Tomioka et al., 2005; Higo et al., 2007). Since GABA may act as an excitatory neurotransmitter during early cortical development (for review Ben-Ari et al., 2007), the postsynaptic action of GABAergic subplate neurons may be also depolarizing (Figure 6 in Hanganu et al., 2002).

Ultrastructural studies of subplate cells in various species have demonstrated symmetrical as well as asymmetrical synapses with relatively mature properties (Kostovic and Rakic, 1980, 1990; Chun and Shatz, 1988; Herrmann et al., 1994), indicating that subplate neurons receive GABAergic as well as glutamatergic synaptic inputs. As suggested by Kostovic and Rakic (1980), glutamatergic inputs onto subplate neurons may arise from the thalamus and other neocortical areas, whereas GABAergic synaptic inputs may originate from GABAergic interneurons in the subplate. Thalamocortical synaptic contacts with spines and shafts of subplate neuron dendrites have been demonstrated in the neonatal ferret (Herrmann et al., 1994) and a dense network of corticocortical fibers have been reported in the subplate of the embryonic mouse (Crandall and Caviness, 1984).

N-Methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) and kainate receptors and the essential subunits for their receptor function have been demonstrated in the subplate of various species, suggesting the presence of functional glutamatergic synapses in subplate neurons (Herrmann, 1996; Aoki, 1997; Catalano et al., 1997; Furuta and Martin, 1999). The expression of benzodiazepine binding sites (Schlumpf et al., 1983), $GABA_A$ receptors (Huntley et al., 1990) and $GABA_A$ receptor subunits (Meinecke and Rakic, 1992) in the subplate indicate that functional GABAergic synaptic inputs should also be present in subplate neurons.

These morphological, ultrastructural and immunohistochemical data are complemented by functional studies on the properties of the subplate and single subplate cells in different mammalian species.

FUNCTIONAL PROPERTIES OF SUBPLATE NEURONS

In contrast to the heterogeneity in morphological and chemical appearance, electrophysiological recordings from single subplate neurons in rodents demonstrate rather homogeneous functional properties. Subplate neurons exhibit relatively uniform passive membrane properties. Whole-cell patch-clamp recordings from subplate cells in newborn rodent neocortical slices revealed resting membrane potentials and input resistances in the range of −55 mV and 1–1.2 GΩ, respectively (Luhmann et al., 2000; Hanganu et al., 2001; Hirsch and Luhmann, 2008). In response to sustained depolarization by intracellular current injection, subplate neurons are capable of firing overshooting and repetitive action potentials at frequencies exceeding 40 Hz (**Figures 2C and 3A**). Similar results were obtained from subplate neurons in acute neocortical slices harvested postmortem from human fetal brain at gestational week 16–22 (Moore et al., 2009).

In comparison to other neurons in the immature cerebral cortex, subplate cells also reveal the most mature properties in action potential characteristics and in the biophysical properties of voltage-dependent sodium and calcium currents. These observations have been made in developing rodent (Luhmann et al., 2000) as well as in human cerebral cortex (Moore et al., 2009), indicating that these relatively mature functional properties enable subplate cells to transmit afferent neuronal activity faithfully to the developing cortical plate.

Intracellular labeling of single subplate cells with fluorescent dyes or biocytin revealed an extensive neuronal network of dye-coupled neurons in the subplate and cortical plate (**Figure 2A**). In newborn rats, on average about nine neurons are dye-coupled to a single subplate cell and these gap junction coupled networks are often organized in a columnar manner (Dupont et al., 2006). Whole-cell patch-clamp recordings from pairs of dye-coupled subplate neurons (**Figure 2B**) allowed a more detailed electrophysiological characterization of the electrical synapses connecting subplate neurons (**Figure 2C**). The average coupling conductance amounted to about 1.2 nS (Dupont et al., 2006). These data indicate that subplate cells are strongly coupled via electrical synapses and form a functional columnar syncytium with neurons located in the cortical plate. It is tempting to speculate that this early columnar organization results from the radial, column-like neuronal migration of newly generated neurons into the developing neocortex (Noctor et al., 2004), which is also controlled by gap junctional coupling (Elias et al., 2007).

SUBPLATE NEURONS ARE WELL INTEGRATED IN THE DEVELOPING NEOCORTICAL NETWORK

Electrophysiological and optical imaging recordings further support the hypothesis that subplate neurons are well integrated in the developing cerebral cortex. *In vitro* intracellular recordings

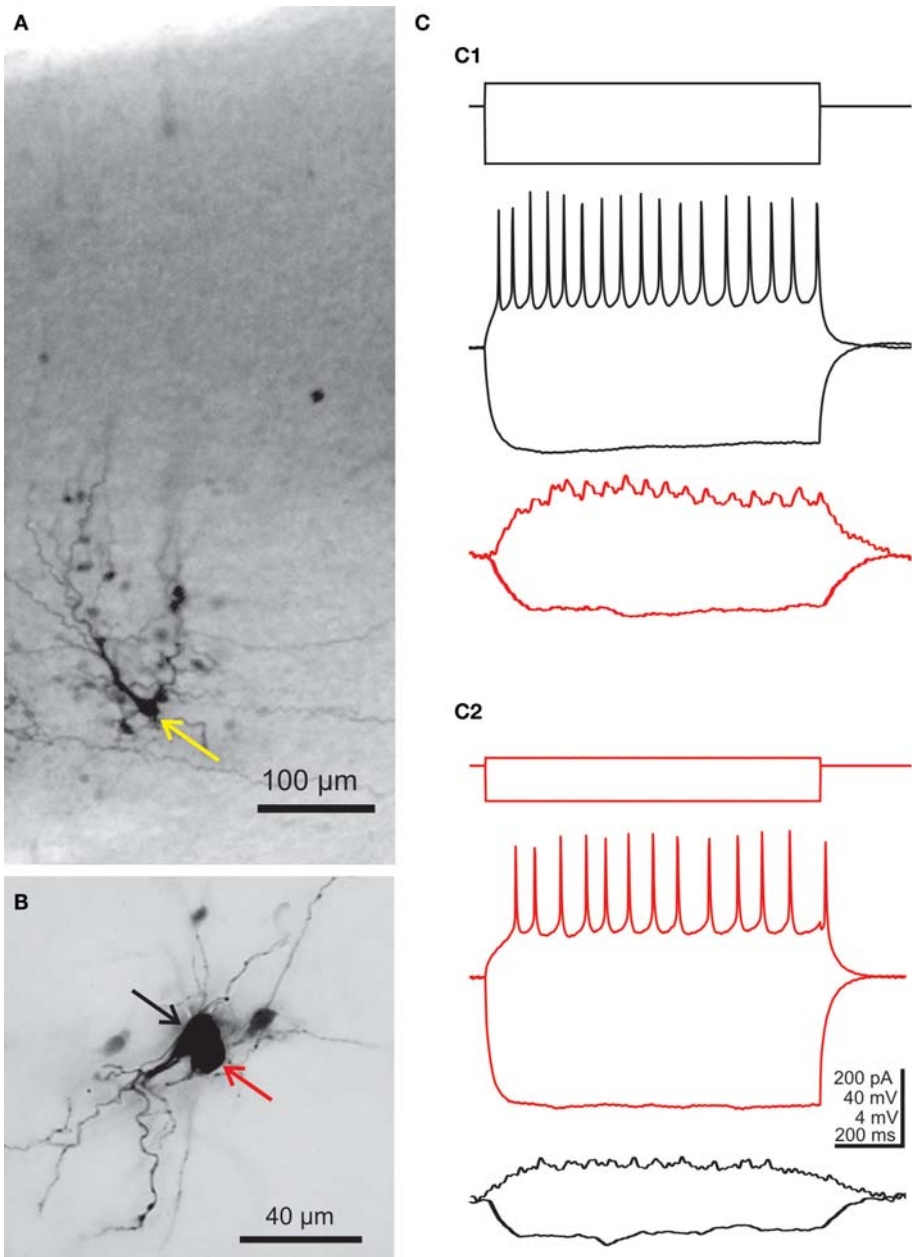

FIGURE 2 | Morphological and electrophysiological evidence for electrical coupling between subplate neurons. (A) Intracellular biocytin staining of one subplate neuron (yellow arrow) in the P3 mouse neocortex results in columnar labeling of several dye-coupled cells in the subplate and in the cortical plate. **(B)** Photograph of two simultaneously recorded P1 subplate neurons, which were both stained with biocytin. **(C)** Paired current-clamp recordings of the cells shown in **(B)**. Black traces were obtained from the cell marked by black arrow in **(B)** and red traces are from the cell marked by red arrow. **(C1)** Injection of a hyperpolarizing or suprathreshold depolarizing current pulse into the cell marked by black arrow causes voltage deflections in the gap junction coupled neuron. **(C2)** Vice versa, injection of a hyperpolarizing or suprathreshold depolarizing current pulse into the cell marked by red arrow causes voltage deflections in the other neuron. Cortical surface in **(A)** and **(B)** is up, action potentials in **(C)** are truncated. The hyperpolarizing responses in the lower traces are averages of four recordings. Reproduced and modified with permission from (Dupont et al., 2006).

and current-source density analyses in late embryonic and early postnatal kitten visual cortex demonstrated that subplate neurons receive functional excitatory synaptic inputs from axons that course in the developing white matter (Friauf et al., 1990; Friauf and Shatz, 1991). Subplate cells in newborn rat somatosensory cortical slices reveal a substantial amount of spontaneous postsynaptic currents (sPSCs) with different kinetics and pharmacological profile demonstrating that subplate neurons receive functional synaptic inputs mediated by AMPA, NMDA and $GABA_A$ receptors (Hanganu et al., 2001). A more detailed analysis of the synaptic inputs onto subplate cells could be obtained by electrical stimulation of specific afferent axonal projections (**Figures 3B,C**) (Staiger et al., 1999).

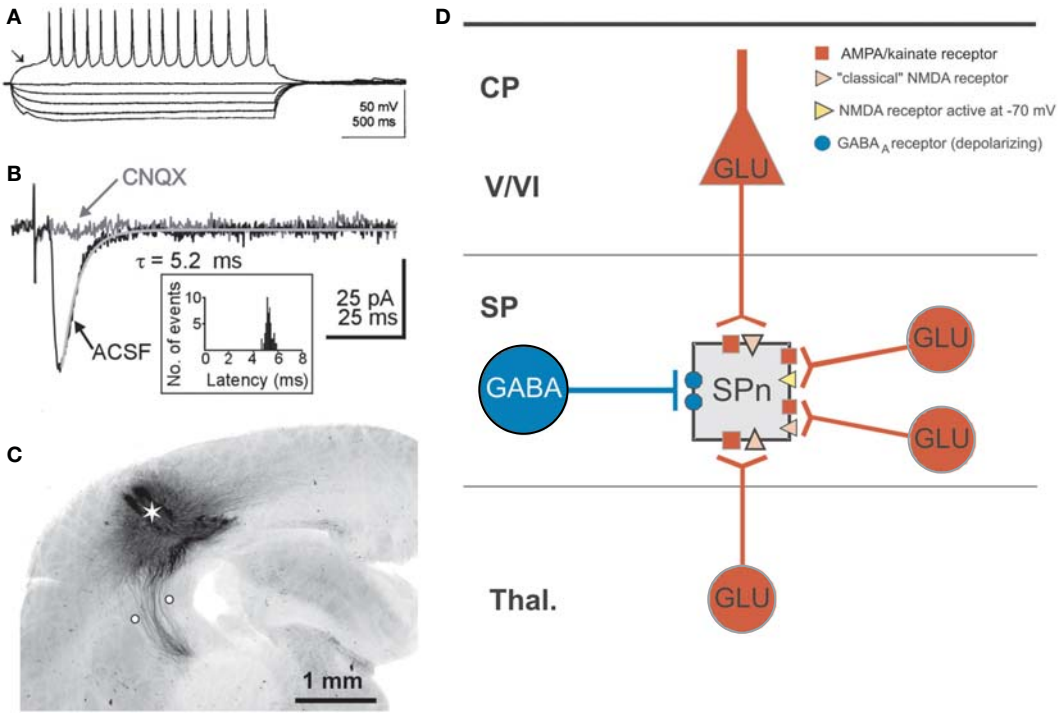

FIGURE 3 | Electrophysiological properties of subplate neurons and their synaptic integration in the developing cerebral cortex. (A) Typical action potential firing pattern of a subplate neuron in the cerebral cortex of a P1 rat. Note repetitive discharge pattern and presence of a prominent I_A current (arrow). **(B)** Excitatory postsynaptic currents (EPSCs) recorded in a P3 subplate cell at a holding potential of –70 mV and elicited by selective electrical stimulation of the thalamocortical input (see C). Synaptic responses were obtained under control conditions in normal extracellular bathing solution and after application of 10 μM CNQX to block AMPA/kainate receptors. The EPSC decay could be fitted by a monoexponential function (gray line). The inset shows the unimodal latency distribution of 50 thalamocortical EPSCs recorded from one SPn. **(C)** Photomicrograph of biocytin-stained thalamocortical projections in a 400-μm thick coronal slice from a P1 rat. Asterisk shows extracellular injection site of biocytin crystal and white circles mark position of bipolar electrode to selectively activate the thalamocortical input. **(D)** Schematic diagram illustrating the distribution of postsynaptic receptors on a subplate neuron for monosynaptic inputs arising from the thalamus (Thal.), the cortical plate (CP) including layers V/VI and within the subplate (SP). Note that synaptic inputs from other subplate neurons can lead to activation of depolarizing $GABA_A$ receptors and NMDA receptors activated at negative membrane potentials. Reproduced and modified with permission from (Luhmann et al., 2000) **(A)** (Hanganu et al., 2002) **(B–D)**.

Brief electrical stimulation of the thalamocortical projection elicits in immature rat and cat subplate neurons a fast and reliable excitatory postsynaptic potential/current (EPSP/C), which can blocked by the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Friauf et al., 1990; Hanganu et al., 2002). Upon membrane depolarization thalamocortical activation elicits a CNQX-insensitive component, which can be blocked by the NMDA receptor antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), demonstrating that the thalamic input also activates functional NMDA receptors on subplate cells (Hanganu et al., 2002). These electrophysiological observations from single subplate neurons are further supported by current source-density analyses performed in late embryonic and early postnatal kitten (Friauf and Shatz, 1991) and rat cerebral cortex (Molnár et al., 2003), demonstrating that subplate cells in these species receive a functional thalamic input at earliest stages of neocortical development. Recording of optical images with voltage-sensitive dyes in neocortical slices of prenatal rats have shown that there is a few days delay between the arrival of thalamocortical axons at the subplate at E16 and the appearance of functional thalamocortical synaptic transmission at E19 (Higashi et al., 2002).

Beside a glutamatergic thalamocortical input with relatively mature functional properties (short delay, fast kinetics, reliable responses), subplate cells in newborn rodents receive additional intracortical synaptic inputs from various presynaptic sources (**Figure 3D**). Local glutamatergic synaptic inputs arise from pyramidal neurons in the cortical plate and from glutamatergic subplate cells, which both activate postsynaptic AMPA/kainate and NMDA receptors (Hanganu et al., 2002). This intra-subplate glutamatergic input differs from the thalamocortical input. Whereas the intra-subplate input reveals a pronounced facilitation when repetitively activated at 10–40 Hz, the thalamocortical input is rather stable or suppressed at these stimulation frequencies (**Figure 4**) (Hirsch and Luhmann, 2008). Both synaptic inputs also differ in their molecular and developmental profile. The intra-subplate synaptic input can sustain high stimulation frequencies and may boost the thalamocortical input, thereby enhancing activity in the developing neocortical circuit (Hirsch and Luhmann, 2008). Anatomical studies in rodent and human immature cerebral cortex indicate that glutamatergic synaptic inputs most likely also arise from other neocortical sources via corticocortical connections (Ivy and Killackey, 1981; Kostovic

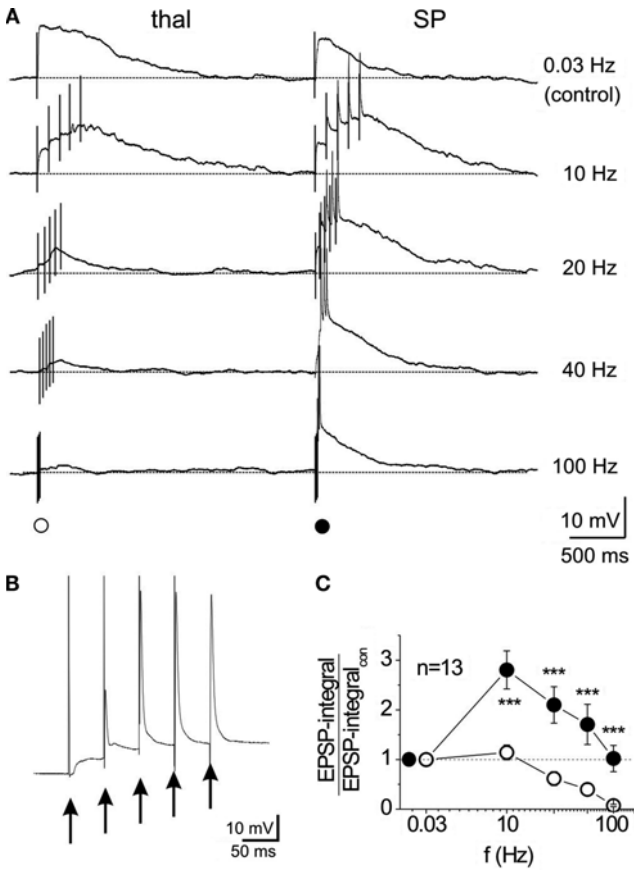

FIGURE 4 | Input-specific differences in temporal summation of NMDA receptor-mediated EPSPs recorded in subplate neurons of the newborn mouse cerebral cortex. Recordings were performed in the presence of CNQX and bicuculline methiodide to isolate NMDA receptor-mediated EPSPs. **(A)** Five successive stimuli to the thalamic (left) or subplate (right) input reveals prominent temporal summation and repetitive action potentials (truncated) of the subplate input at frequencies between 10 and 40 Hz. All recordings were obtained at a membrane potential of –80 mV. **(B)** Synaptic response to 20-Hz subplate stimulation at higher resolution [same trace as in **(A)**]. **(C)** Quotient between NMDA receptor-EPSP integral and control integral (determined at 0.03 Hz) for 13 subplate neurons. Reproduced with permission from Hirsch and Luhmann (2008).

and Jovanov-Milosevic, 2006), but the functional properties of these long-distance synaptic inputs onto subplate cells are currently unknown.

Subplate cells in rodents receive a GABAergic synaptic input from neighbouring GABAergic neurons located in the subplate and probably also in the cortical plate (**Figure 3D**) (Hanganu et al., 2002). However, as in other immature brain structures, this GABAergic input most likely has a pure excitatory postsynaptic effect (Figure 6 in Hanganu et al., 2002; for review Ben-Ari et al., 2007). An excitatory effect has been demonstrated in subplate cells also for glycine and taurine (Kilb et al., 2008).

Subplate neurons are not only well integrated in the developing cortical circuit, but during certain developmental periods they also receive a very selective input from neuromodulatory brain structures. Both in primates (for review (Rakic, 1995) as well as in rodents (Calarco and Robertson, 1995; Mechawar and Descarries, 2001) the subplate is specifically innervated by cholinergic fibers arising from the basal forebrain and from monoaminergic inputs. The activation of postsynaptic nicotinic acetylcholine receptors elicits in subplate cells a marked depolarization which is largely mediated by the activation of alpha4/beta2 receptors (Hanganu and Luhmann, 2004). The responsiveness to activation of muscarinic acetylcholine receptors (mAChR) is more complex and reveals a remarkable oscillatory discharge mode of subplate cells (Hanganu et al., 2009).

OSCILLATORY PROPERTIES OF SUBPLATE CELLS

Application of muscarine to neocortical slices from newborn rats induces in subplate cells repetitive burst discharges with burst frequencies in the range of 20 Hz (**Figure 5A**) (Hanganu et al., 2009). Similar burst patterns can be recorded from subplate neurons when the tissue concentration of acetylcholine is raised by application of the cholinesterase inhibitor neostigmine (**Figure 5B**). These electrophysiological data are further supported by calcium imaging experiments, which demonstrate that activation of muscarinic receptors induces repetitive Ca^{2+}-transients which are highly coordinated within the subplate (Figure 1 in Hanganu et al., 2009). Subplate cells, which express the m1–m5 subunits of the mAChR, are instantly and massively excited upon activation of postsynaptic muscarinic receptors and switch into an oscillatory burst firing mode (**Figure 5**). Since subplate cells are densely coupled via electrical (**Figure 2**) and chemical synapses (**Figure 3D**), these cellular oscillations are synchronized and amplified within the intra-subplate network. Gap junctional coupling, depolarizing GABA actions and a tonic non-synaptic GABA release contribute to the generation and maintenance of the cholinergic network oscillations (Figure 9 in Hanganu et al., 2009).

GABAergic SUBPLATE CELLS DRIVE EARLY CORTICAL NETWORKS

The crucial role of the subplate in generating cholinergic network oscillations is supported by experiments on thick (800–1000 μm) neocortical slices from newborn rodents. Only slices with an intact subplate reveal network oscillations upon activation of muscarinic receptors with carbachol (Dupont et al., 2006). When the subplate is removed, slices do not show any carbachol-induced network oscillations (**Figure 6**). Furthermore, selective electrical stimulation of the subplate in thick neocortical slices from newborn mice using a multi-electrode array (MEA) also evokes large-scale network oscillations (**Figure 7**) (Sun and Luhmann, 2007). In a recent study on mouse neocortical slice cultures, Moody and coworkers suggested that the subplate may act as a pacemaker region to generate propagating waves of spontaneous activity in the neonatal cerebral cortex (Lischalk et al., 2009). Early neocortical network oscillations may be triggered by the subplate (Dupont et al., 2006; Sun and Luhmann, 2007) or by the sensory input, i.e. the retina (Hanganu et al., 2006) or the whiskers (Yang et al., 2009). At early developmental stages, the sensory input from the periphery firstly reaches the subplate and is subsequently transmitted to the cortex (Friauf and Shatz, 1991). During this developmental period, intracortical and sensory driven activity patterns may interact. At later stages, the thalamocortical input directly innervates layer IV.

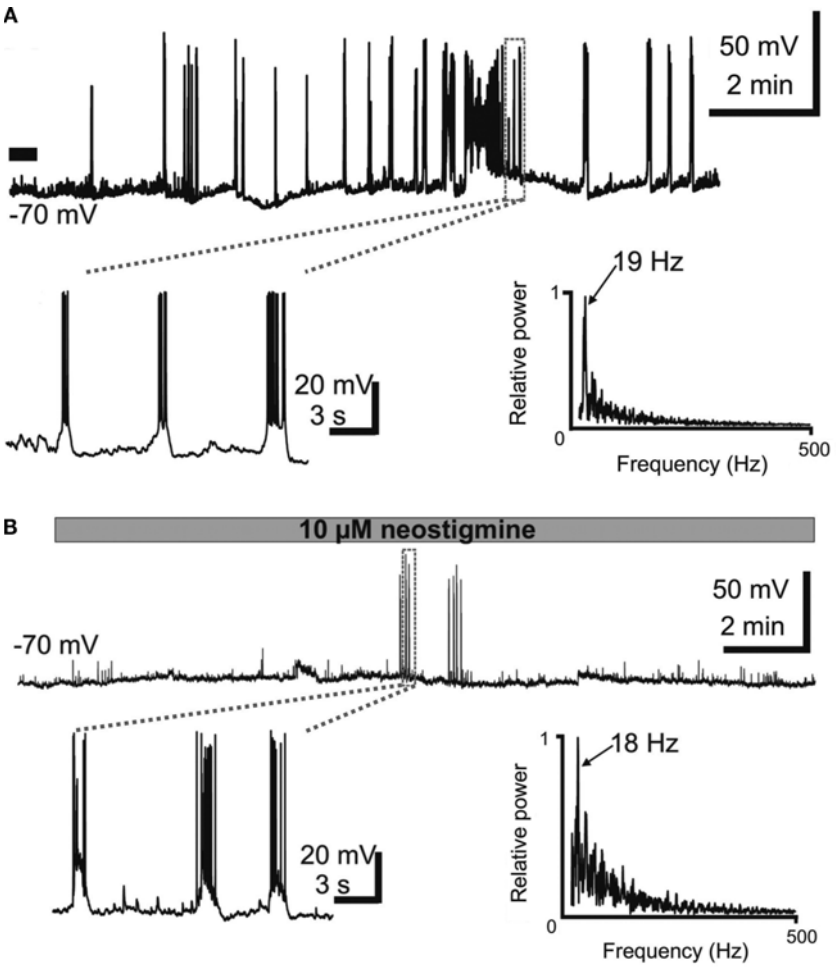

FIGURE 5 | Subplate neurons in newborn rat cerebral cortex reveal repetitive burst discharges upon activation of postsynaptic cholinergic receptors. (A) Current-clamp whole-cell recording from a P3 subplate cell at a holding potential of –70 mV. Note the long-lasting burst discharge after a single 30-s long bath application of muscarine (marked by a black bar). The recording marked by the dashed box is shown at higher magnification below. Frequency power spectrum of the action potential discharge within one representative burst from the trace above. **(B)** Current-clamp whole-cell recording from a P2 subplate neuron at a holding potential of –70 mV displaying the effects of 10 µM neostigmine, which blocks endogenous acetylcholine esterase and thereby increases the concentration of acetylcholine in the neocortical slice. Burst discharges appeared 10 min after the beginning of neostigmine application. The recording marked by the dashed box is displayed at higher magnification. Frequency power spectrum of the action potential discharge within burst from the upper trace in **(B)**. Note the similar frequency of the discharge in muscarine- and neostigmine-induced burst discharges. Reproduced and modified with permission from Hanganu et al. (2009).

Electrophysiological and calcium imaging recordings in acute neocortical slices from newborn rodents (Hanganu et al., 2009) as well as calcium imaging data obtained in neocortical cell cultures (Voigt et al., 2001) indicate that GABAergic subplate cells play a central role in generating this neuronal network activity. Voigt et al. (2001) estimated that a minimal number of two GABAergic subplate neurons per square millimeter are required for the occurrence of synchronous network activity. The unique structural and functional properties enable subplate cells to receive, synchronize and amplify the afferent and intrinsic synaptic inputs. It is tempting to speculate on the downstream effects of these early network oscillations in cortical maturation? It has been demonstrated that brain derived neurotrophic factor (BDNF) is released from synaptically localized secretory granules following burst stimulation at 20–50 Hz (Balkowiec and Katz, 2000; Hartmann et al., 2001) (for review Lessmann et al., 2003). If subplate-driven oscillations contribute to the local secretion of BDNF, the released BDNF may strengthen the synaptic connectivity between neurons in these early ensembles. Beside strengthening synaptic connections via local BDNF release, subplate-driven oscillations may also influence programmed cell death during early developmental stages. We recently demonstrated that the rate of apoptosis in organotypic slice cultures of the neonatal mouse cerebral cortex is regulated by electrical activity patterns, which resemble in many aspects the subplate-driven activity (Heck et al., 2008). This activity-dependent regulation of neuronal apoptosis was at least partly mediated by activation of the BDNF receptor TrkB, indicating that subplate-driven network oscillations may also influence programmed cell death via activity-dependent BDNF release.

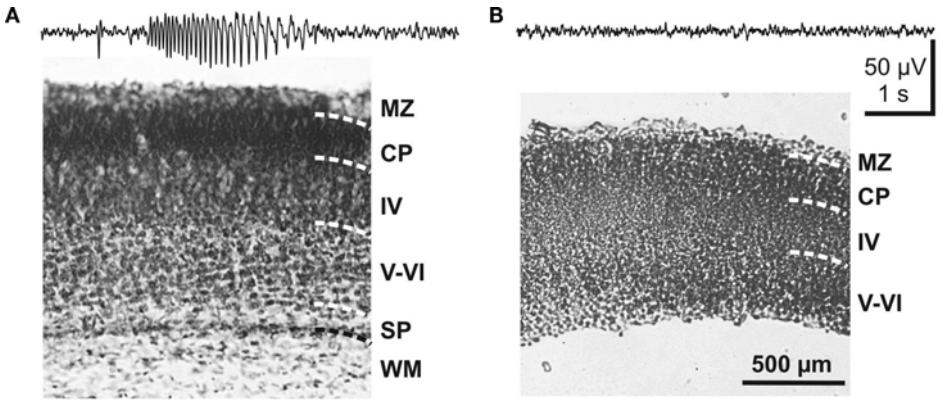

FIGURE 6 | Role of the subplate in triggering carbachol-induced network oscillations in the neonatal mouse cerebral cortex. (A) Field potential response to carbachol application in a coronal 800-µm thick cortical slab preparation with an intact subplate as shown in Nissl stained section below. **(B)** Lack of carbachol-induced activity in a cortical slab preparation, in which the subplate was eliminated. The absence of the subplate was verified by Nissl staining. Reproduced with permission from Dupont et al. (2006).

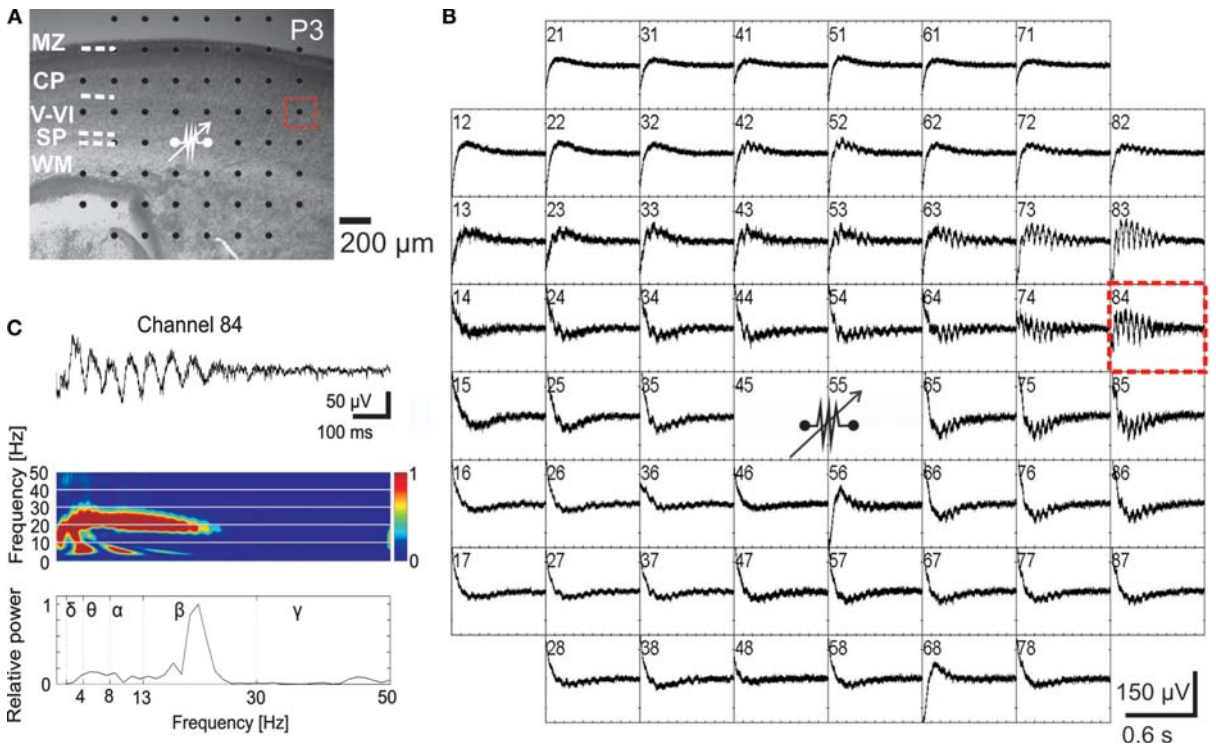

FIGURE 7 | Local electrical stimulation of the subplate elicits propagating network oscillations in the developing cerebral cortical network of the newborn mouse. (A) Photograph of P3 mouse Nissl-stained coronal slice with cortical layers and position of 60-channel multi-electrode array (MEA). **(B)** MEA recordings from the neocortical slice shown in **(A)** with site of bipolar stimulation in the subplate (80 µA, 200-µs duration, 50 Hz, 10 times). Spacing between electrodes is 200 µm [see scale bar in **(A)**]. **(C)** Recording at electrode number 84 [dotted red rectangle in **(A)**] shown at higher resolution with corresponding wavelet analysis and Fourier spectrum. Reproduced with permission from Sun and Luhmann (2007).

A large proportion of newly generated GABAergic interneurons arising from the medial ganglionic eminence and pyramdial neurons from the ventricular zone must migrate through the subplate on their way to the developing cortical plate (Kriegstein and Noctor, 2004). Therefore the subplate may also have a profound influence on the migration pattern. GABAergic and glutamatergic subplate neurons partly release their neurotransmitter in a paracrine, non-synaptic manner (Hanganu et al., 2009), thereby regulating the neuronal migration pattern (Reiprich et al., 2005; Manent et al., 2005, 2006; Heck et al., 2007) (for review Manent and Represa, 2007).

Neurotransmitter release in the subplate may rise substantially during oscillatory network activity, thereby activating low affinity or extrasynaptic receptors causing alterations in neuronal migration (Denter et al., 2009).

On the basis of the currently available data we suggest the following model (**Figure 8**): During early cortical development, in most mammals before birth, subplate cells with relatively mature structural properties (elaborated dendritic tree, complex axonal projections, mature symmetrical and asymmetrical synapses) receive a functional glutamatergic synaptic input from specific thalamic nuclei and a selective input from neuromodulatory systems (e.g. cholinergic inputs from the basal forebrain). In rodents, subplate neurons are densely interconnected via electrical and chemical synapses and upon activation of muscarinic (and probably also other metabotropic receptors (Wagner and Luhmann, 2006) discharge in repetitive ~20 Hz bursts (lower inset in **Figure 8**). GABAergic subplate neurons releasing GABA in a synaptic (1 in **Figure 8**) and tonic non-synaptic manner (2 in **Figure 8**) facilitate the generation of oscillatory network activity (Hanganu et al., 2009). Intra-subplate connections arising from glutamatergic subplate cells may boost the subplate activity in the 10–40 Hz frequency range (Hirsch and Luhmann, 2008). Subplate cells are dye-coupled to cortical plate neurons in a columnar manner (Dupont et al., 2006) and faithfully transmit synchronized 10–20 Hz network oscillations generated and amplified in the subplate to the cortical plate and marginal zone (upper inset in **Figure 8**) (Dupont et al., 2006; Wagner and Luhmann, 2006; Sun and Luhmann, 2007).

Elimination or interruption of the subplate prevents the generation of the subplate-driven synchronized activity patterns and disturbs the maturation of the columnar architecture (Ghosh and Shatz, 1992; Kanold et al., 2003; Kanold and Shatz, 2006). Under normal conditions and with further development, subplate cells disappear by apoptosis (Ferrer et al., 1990; Price et al., 1997; Arias et al., 2002) or transform into white matter interstitial cells (Valverde and Facal-Valverde, 1988; Valverde et al., 1995) or layer VIb (layer VII) neurons (Woo et al., 1991; Reep, 2000). In the normal mature cerebral cortex, subplate neurons have lost their capabilities to receive and amplify incoming neuronal activity and to generate synchronized network oscillations. However, as already suggested by Jones (1995), disturbances in the pattern of programmed cell death in the subplate may cause a failure to establish normal patterns of connections in the overlying cerebral cortex, leading to long-term neurological deficits such as schizophrenia. Abnormal placement of (surviving subplate?) neurons in the white matter and atypical circuits have been observed in the prefrontal cortex of schizophrenic patients (Akbarian et al., 1996; Kirkpatrick et al., 1999; Bunney and Bunney, 2000). It has been further suggested that subplate-like neurons may persist in cortical dysplasia and contribute to the manifestation of pharmacoresistant epilepsy in adults (Cepeda et al., 2007). Interestingly in the resectioned human tissue GABA application induced depolarizing postsynaptic responses and spontaneous GABAergic synaptic potentials even elicited action potentials (Cepeda et al., 2007).

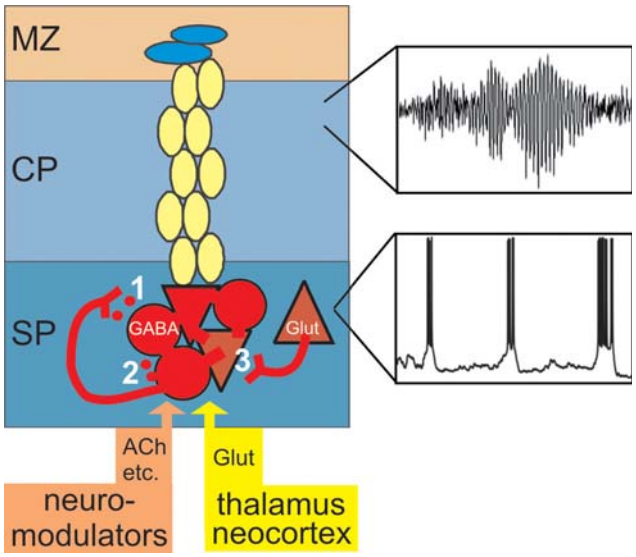

FIGURE 8 | Amplification of afferent and intracortical activity by the subplate. During early cortical development the subplate receives a transient and selective synaptic input from neuromodulatory systems, from the sensory periphery via specific thalamic nuclei and via corticocortical fibers from other neocortical areas. Subplate cells are capable to fire repetitive action potentials **(Figure 3A)** and upon activation of cholinergic postsynaptic receptors discharge in repetitive bursts (lower inset and **Figure 5**). GABAergic subplate neurons release GABA in an activity-dependent (1) and tonic (2) manner thereby inducing a postsynaptic excitatory response. Glutamatergic subplate neurons boost neuronal network activity in the 10–40 Hz frequency range. Subplate neurons are not only coupled via these excitatory synaptic circuits, but also via gap junctions **(Figure 2)**. Chemical and electrical synapses contribute to the generation of subplate-driven oscillatory activity which is transmitted to the gap junctional coupled network in the cortical plate **(Figure 2A)** and marginal zone and induces 10–20 Hz neocortical network oscillations (upper inset).

In summary, experimental data indicate that subplate cells, most likely GABAergic subplate neurons, play an important role in the generation of early synchronized network activity and in the normal development of the neocortical network and columnar architecture. Clinical evidence suggests that subplate cells in the mature cortex contribute to the manifestation of abnormal neuronal circuits, pathological activities and long-term neurological deficits.

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REFERENCES

Aboitiz, F., and Montiel, J. (2007). Origin and evolution of the vertebrate telencephalon, with special reference to the mammalian neocortex. *Adv. Anat. Embryol. Cell Biol.* 193, 1–112.

Akbarian, S., Kim, J. J., Potkin, S. G., Hetrick, W. P., Bunney, W. E. Jr., and Jones, E. G. (1996). Maldistribution of interstitial neurons in prefrontal white matter of the brains of schizophrenic patients. *Arch. Gen. Psychiatry* 53, 425–436.

Allendoerfer, K. L., and Shatz, C. J. (1994). The subplate, a transient neocortical structure: Its role in the development of connections between thalamus and cortex. *Annu. Rev. Neurosci.* 17, 185–218.

Aoki, C. (1997). Postnatal changes in the laminar and subcellular distribution of NMDA-R1 subunits in the cat visual cortex as revealed by immuno-electron microscopy. *Dev. Brain Res.* 98, 41–59.

Arias, M. S. Baratta, J., Yu, J., and Robertson, R. T. (2002). Absence of selectivity in the loss of neurons from the developing cortical subplate of the rat. *Dev. Brain Res.* 139, 331–335.

Balkowiec, A., and Katz, D. M. (2000). Activity-dependent release of endogenous brain-derived neurotrophic

factor from primary sensory neurons detected by ELISA in situ. *J. Neurosci.* 20, 7417–7423.

Ben-Ari, Y., Gaiarsa, J. L., Tyzio, R., and Khazipov, R. (2007). GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol. Rev.* 87, 1215–1284.

Bunney, W. E., and Bunney, B. G. (2000). Evidence for a compromised dorsolateral prefrontal cortical parallel circuit in schizophrenia. *Brain Res. Rev.* 31, 138–146.

Bystron, I., Blakemore, C., and Rakic, P. (2008). Development of the human cerebral cortex: Boulder Committee revisited. *Nat. Rev. Neurosci.* 9, 110–122.

Calarco, C. A., and Robertson, R. T. (1995). Development of basal forebrain projections to visual cortex: DiI studies in rat. *J. Comp. Neurol.* 354, 608–626.

Catalano, S. M., Chang, C. K., and Shatz, C. J. (1997). Activity-dependent regulation of NMDAR1 immunoreactivity in the developing visual cortex. *J. Neurosci.* 17, 8376–8390.

Cepeda, C., Andre, V. M., Wu, N., Yamazaki, I., Uzgil, B., Vinters, H. V., Levine, M. S., and Mathern, G. W. (2007). Immature neurons and GABA networks may contribute to epileptogenesis in pediatric cortical dysplasia. *Epilepsia* 48(Suppl. 5), 79–85.

Chun, J. J., Nakamura, M. J., and Shatz, C. J. (1987). Transient cells of the developing mammalian telencephalon are peptide-immunoreactive neurons. *Nature* 325, 617–620.

Chun, J. J., and Shatz, C. J. (1988). Redistribution of synaptic vesicle antigens is correlated with the disappearance of a transient synaptic zone in the developing cerebral cortex. *Neuron* 1, 297–310.

Clancy, B., and Cauller, L. J. (1999). Widespread projections from subgriseal neurons (Layer VII) to layer I in adult rat cortex. *J. Comp. Neurol.* 407, 275–286.

Crandall, J. E., and Caviness, V. S. J. (1984). Axon strata of the cerebral wall in embryonic mice. *Brain Res.* 316, 185–195.

De Carlos, J. A., and O'Leary, D. D. M. (1992). Growth and targeting of subplate axons and establishment of major cortical pathways. *J. Neurosci.* 12, 1194–1211.

Del Río, J. A., Martínez, A., Auladell, C., and Soriano, E. (2000). Developmental history of the subplate and developing white matter in the murine neocortex. Neuronal organization and relationship with the main afferent systems at embryonic perinatal stages. *Cereb. Cortex* 10, 784–801.

Denter, D., Heck, N., Riedemann, T., Kilb, W., and Luhmann, H. J. (2009). GABAc receptors are functionally expressed in the intermediate zone and regulate radial migration in the embryonic mouse neocortex. *Neuroscience.* (submitted).

Dupont, E., Hanganu, I. L., Kilb, W., Hirsch, S., and Luhmann, H. J. (2006). Rapid developmental switch in the mechanisms driving early cortical columnar networks. *Nature* 439, 79–83.

Elias, L. A., Wang, D. D., and Kriegstein, A. R. (2007). Gap junction adhesion is necessary for radial migration in the neocortex. *Nature* 448, 901–907.

Ferrer, I., Bernet, E., Soriano, E., Del Rio, T., and Fonseca, M. (1990). Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. *Neuroscience* 39, 451–458.

Finney, E. M., Stone, J. R., and Shatz, C. J. (1998). Major glutamatergic projection from subplate into visual cortex during development. *J. Comp. Neurol.* 398, 105–118.

Friauf, E., McConnell, S. K., and Shatz, C. J. (1990). Functional synaptic circuits in the subplate during fetal and early postnatal development of cat visual cortex. *J. Neurosci.* 10, 2601–2613.

Friauf, E., and Shatz, C. J. (1991). Changing patterns of synaptic input to subplate and cortical plate during development of visual cortex. *J. Neurophysiol.* 66, 2059–2071.

Furuta, A., and Martin, L. J. (1999). Laminar segregation of the cortical plate during corticogenesis is accompanied by changes in glutamate receptor expression. *J. Neurobiol.* 39, 67–80.

Ghosh, A., Antonini, A., McConnell, S. K., and Shatz, C. J. (1990). Requirements of subplate neurons in the formation of thalamocortical connections. *Nature* 347, 179–181.

Ghosh, A., and Shatz, C. J. (1992). Involvement of subplate neurons in the formation of ocular dominance columns. *Science* 255, 1441–1443.

Hanganu, I. L., Ben-Ari, Y., and Khazipov, R. (2006). Retinal waves trigger spindle bursts in the neonatal rat visual cortex. *J. Neurosci.* 26, 6728–6736.

Hanganu, I. L., Kilb, W., and Luhmann, H. J. (2001). Spontaneous synaptic activity of subplate neurons in neonatal rat somatosensory cortex. *Cereb. Cortex* 11, 400–410.

Hanganu, I. L., Kilb, W., and Luhmann, H. J. (2002). Functional synaptic projections onto subplate neurons in neonatal rat somatosensory cortex. *J. Neurosci.* 22, 7165–7176.

Hanganu, I. L., and Luhmann, H. J. (2004). Functional nicotinic acetylcholine receptors on subplate neurons in neonatal rat somatosensory cortex. *J. Neurophysiol.* 92, 189–198.

Hanganu, I. L., Okabe, A., Lessmann, V., and Luhmann, H. J. (2009). Cellular mechanisms of subplate-driven and cholinergic input-dependent network activity in the neonatal rat somatosensory cortex. *Cereb. Cortex* 19, 89–105.

Hartmann, M., Heumann, R., and Lessmann, V. (2001). Synaptic secretion of BDNF after high-frequency stimulation of glutamatergic synapses. *EMBO J.* 20, 5887–5897.

Heck, N., Golbs, A., Riedemann, T., Sun, J. J., Lessmann, V., and Luhmann, H. J. (2008). Activity-dependent regulation of neuronal apoptosis in neonatal mouse cerebral cortex. *Cereb. Cortex* 18, 1335–1349.

Heck, N., Kilb, W., Reiprich, P., Kubota, H., Furukawa, T., Fukuda, A., and Luhmann, H. J. (2007). GABA-A receptors regulate neocortical neuronal migration in vitro and in vivo. *Cereb. Cortex* 17, 138–148.

Herrmann, K. (1996). Differential distribution of AMPA receptors and glutamate during pre- and postnatal development in the visual cortex of ferrets. *J. Comp. Neurol.* 375, 1–17.

Herrmann, K., Antonini, A., and Shatz, C. J. (1994). Ultrastructural evidence for synaptic interactions between thalamocortical axons and subplate neurons. *Eur. J. Neurosci.* 6, 1729–1742.

Hevner, R. F., Daza, R. A., Englund, C., Kohtz, J., and Fink, A. (2004). Postnatal shifts of interneuron position in the neocortex of normal and reeler mice: evidence for inward radial migration. *Neuroscience* 124, 605–618.

Higashi, S., Molnár, Z., Kurotani, T., and Toyama, K. (2002). Prenatal development of neural excitation in rat thalamocortical projections studied by optical recording. *Neuroscience* 115, 1231–1246.

Higo, S., Udaka, N., and Tamamaki, N. (2007). Long-range GABAergic projection neurons in the cat neocortex. *J. Comp. Neurol.* 503, 421–431.

Hirsch, S., and Luhmann, H. J. (2008). Pathway-specificity in N-methyl-d-aspartate receptor-mediated synaptic inputs onto subplate neurons. *Neuroscience* 153, 1092–1102.

Hoerder-Suabedissen, A., Wang, W. Z., Lee, S., Davies, K. E., Goffinet, A., Rakic, S., Parnavelas, J., Reim, K., Nicolic, M., Paulsen, O., and Molnár, Z. (2009). Novel markers reveal subpopulations of subplate neurons in the murine cerebral cortex. *Cereb. Cortex* 19, 1738–1750.

Huntley, G. W., De Blas, A. L., and Jones, E. G. (1990). $GABA_A$ receptor immunoreactivity in adult and developing monkey sensory-motor cortex. *Exp. Brain Res.* 82, 519–535.

Ivy, G. O., and Killackey, H. P. (1981). The ontogeny of the distribution of callosal projection neurons in the rat parietal cortex. *J. Comp. Neurol.* 195, 367–389.

Jones, E. G. (1995). Cortical development and neuropathology in schizophrenia. *Ciba Found. Symp.* 193, 277–295.

Kanold, P. O., Kara, P., Reid, R. C., and Shatz, C. J. (2003). Role of subplate neurons in functional maturation of visual cortical columns. *Science* 301, 521–525.

Kanold, P. O., and Shatz, C. J. (2006). Subplate neurons regulate maturation of cortical inhibition and outcome of ocular dominance plasticity. *Neuron* 51, 627–638.

Kilb, W., Hanganu, I. L., Okabe, A., Sava, B. A., Shimizu-Okabe, C., Fukuda, A., and Luhmann, H. J. (2008). Glycine receptors mediate excitation of subplate neurons in neonatal rat cerebral cortex. *J. Neurophysiol.* 100, 698–707.

Kim, G. J., Shatz, C. J., and McConnell, S. K. (1991). Morphology of pioneer and follower growth cones in the developing cerebral cortex. *J. Neurobiol.* 22, 629–642.

Kirkpatrick, B., Conley, R. C., Kakoyannis, A., Reep, R. L., and Roberts, R. C. (1999). Interstitial cells of the white matter in the inferior parietal cortex in schizophrenia: an unbiased cell-counting study. *Synapse* 34, 95–102.

Kostovic, I., and Jovanov-Milosevic, N. (2006). The development of cerebral connections during the first 20–45 weeks' gestation. *Semin. Fetal Neonatal Med.* 11, 415–422.

Kostovic, I., Judas, M., Rados, M., and Hrabac, P. (2002). Laminar organization of the human fetal cerebrum revealed by histochemical markers and magnetic resonance imaging. *Cereb. Cortex* 12, 536–544.

Kostovic, I., and Molliver, M. E. (1974). A new interpretation of the laminar development of cerebral cortex: synaptogenesis in different layers of neopallium in the human fetus. *Anat. Rec.* 178, 395.

Kostovic, I., and Rakic, P. (1980). Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. *J. Neurocytol.* 9, 219–242.

Kostovic, I., and Rakic, P. (1990). Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque

monkey and human brain. *J. Comp. Neurol.* 297, 441–470.

Kriegstein, A. R., and Noctor, S. C. (2004). Patterns of neuronal migration in the embryonic cortex. *Trends Neurosci.* 27, 392–399.

Lavdas, A. A., Grigoriou, M., Pachnis, V., and Parnavelas, J. G. (1999). The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. *J. Neurosci.* 19, 7881–7888.

Lessmann, V., Gottmann, K., and Malcangio, M. (2003). Neurotrophin secretion: current facts and future prospects. *Prog. Neurobiol.* 69, 341–374.

Lischalk, J. W., Easton, C. R., and Moody, W. J. (2009). Bilaterally propagating waves of spontaneous activity arising from discrete pacemakers in the neonatal mouse cerebral cortex. *Dev. Neurobiol.* 69, 407–414.

Luhmann, H. J., Reiprich, R. A., Hanganu, I. L., and Kilb, W. (2000). Cellular physiology of the neonatal rat cerebral cortex: Intrinsic membrane properties, sodium and calcium currents. *J. Neurosci. Res.* 62, 574–584.

Lund, R. D., and Mustari, M. J. (1977). Development of the geniculocortical pathway in rats. *J. Comp. Neurol.* 173, 289–306.

Luskin, M. B., and Shatz, C. J. (1985). Studies of the earliest generated cells of the cat's visual cortex: cogeneration of subplate and marginal zones. *J. Neurosci.* 5, 1062–1075.

Manent, J. B., Demarque, M., Jorquera, I., Pellegrino, C., Ben-Ari, Y., Aniksztejn, L., and Represa, A. (2005). A noncanonical release of GABA and glutamate modulates neuronal migration. *J. Neurosci.* 25, 4755–4765.

Manent, J. B., Jorquera, I., Ben-Ari, Y., Aniksztejn, L., and Represa, A. (2006). Glutamate acting on AMPA but not NMDA receptors modulates the migration of hippocampal interneurons. *J. Neurosci.* 26, 5901–5909.

Manent, J. B., and Represa, A. (2007). Neurotransmitters and brain maturation: early paracrine actions of GABA and glutamate modulate neuronal migration. *Neuroscientist* 13, 268–279.

McConnell, S. K., Ghosh, A., and Shatz, C. J. (1989). Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science* 245, 978–982.

McConnell, S. K., Ghosh, A., and Shatz, C. J. (1994). Subplate pioneers and the formation of descending connections from cerebral cortex. *J. Neurosci.* 14, 1892–1907.

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.

Meinecke, D. L., and Rakic, P. (1992). Expression of GABA and $GABA_A$ receptors by neurons of the subplate zone in developing primate occipital cortex: evidence for transient local circuits. *J. Comp. Neurol.* 317, 91–101.

Molnár, Z. (1998). Development of Thalamocortical Connections. Berlin, Heidelberg, Springer.

Molnár, Z., Kurotani, T., Higashi, S., Yamamoto, N., and Toyama, K. (2003). Development of functional thalamocortical synapses studied with current source-density analysis in whole forebrain slices in the rat. *Brain Res. Bull.* 60, 355–371.

Moore, A. R., Filipovic, R., Mo, Z., Rasband, M. N., Zecevic, N., and Antic, S. D. (2009). Electrical excitability of early neurons in the human cerebral cortex during the second trimester of gestattion. *Cereb. Cortex* 19, 1795–1805.

Mrzljak, L., Uylings, H. B., Van Eden, C. G., and Judas, M. (1990). Neuronal development in human prefrontal cortex in prenatal and postnatal stages. *Prog. Brain Res.* 85, 185–222.

Mrzljak, L., Uylings, H. B. M., Kostovic, I., and Van Eden, C. G. (1992). Prenatal development of neurons in the human prefrontal cortex. II. A quantitative Golgi study. *J. Comp. Neurol.* 316, 485–496.

Noctor, S. C., Martinez-Cerdeno, V., Ivic, L., and Kriegstein, A. R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat. Neurosci.* 7, 136–144.

Price, D. J., Aslam, S., Tasker, L., and Gillies, K. (1997). Fates of the earliest generated cells in the developing murine neocortex. *J. Comp. Neurol.* 377, 414–422.

Rakic, P. (1977). Prenatal development of the visual system in rhesus monkey. *Philos. Trans. R Soc. Lond., B, Biol. Sci.* 278, 245–260.

Rakic, P. (1983). Geniculo-cortical connections in primates: normal and experimentally altered development. *Prog. Brain Res.* 58, 393–404.

Rakic, P. (1995). A small step for the cell, a giant leap for mankind: A hypothesis of neocortical expansion during evolution. *Trends Neurosci.* 18, 383–388.

Reep, R. L. (2000). Cortical layer VII and persistent subplate cells in mammalian brains. *Brain Behav. Evol.* 56, 212–234.

Reiprich, P., Kilb, W., and Luhmann, H. J. (2005). Neonatal NMDA receptor blockade disturbs neuronal migration in rat somatosensory cortex in vivo. *Cereb. Cortex* 15, 349–358.

Schlumpf, M., Richards, J. G., Lichtensteiger, W., and Möhler, H. (1983). An autoradiographic study of the prenatal development of benzodiazepine-binding sites in rat brain. *J. Neurosci.* 3, 1478–1487.

Staiger, J. F., Kötter, R., Zilles, K., and Luhmann, H. J. (1999). Connectivity in the somatosensory cortex of the adolescent rat: an in vitro biocytin study. *Anat. Embryol.* 199, 357–365.

Sun, J. J., and Luhmann, H. J. (2007). Spatio-temporal dynamics of oscillatory network activity in the neonatal mouse cerebral cortex. *Eur. J. Neurosci.* 26, 1995–2004.

Tomioka, R., Okamoto, K., Furuta, T., Fujiyama, F., Iwasato, T., Yanagawa, Y., Obata, K., Kaneko, T., and Tamamaki, N. (2005). Demonstration of long-range GABAergic connections distributed throughout the mouse neocortex. *Eur. J. Neurosci.* 21, 1587–1600.

Uylings, H. B. M., Van Eden, C. G., Parnavelas, J. G., and Kalsbeek, A. (1990). The prenatal and postnatal development of rat cerebral cortex. In The Cerebral Cortex of the Rat, B. Kolb and R. C. Tees, eds (Cambridge, MIT Press), pp. 35–76.

Valverde, F., Facal Valverde, M. V., Santacana, M., and Heredia, M. (1989). Development and differentiation of early generated cells of sublayer VIb in the somatosensory cortex of the rat: a correlated Golgi and autoradiographic study. *J. Comp. Neurol.* 290, 118–140.

Valverde, F., and Facal-Valverde, M. V. (1988). Postnatal development of interstitial (subplate) cells in the white matter of the temporal cortex of kittens: a correlated Golgi and electron microscopic study. *J. Comp. Neurol.* 269, 168–192.

Valverde, F., López-Mascaraque, L., Santacana, M., and De Carlos, J. A. (1995). Persistence of early-generated neurons in the rodent subplate: Assessment of cell death in neocortex during the early postnatal period. *J. Neurosci.* 15, 5014–5024.

Voigt, T., Opitz, T., and De Lima, A. D. (2001). Synchronous oscillatory activity in immature cortical network is driven by GABAergic preplate neurons. *J. Neurosci.* 21, 8895–8905.

Wagner, J., and Luhmann, H. J. (2006). Activation of metabotropic glutamate receptors induces propagating network oscillations in the intact cerebral cortex of the newborn mouse. *Neuropharmacology* 51, 848–857.

Wahle, P., Lübke, J., and Naegele, J. R. (1994). Inverted pyramidal neurons and interneurons in cat cortical subplate zone are labelled by monoclonal antibody SP1. *Eur. J. Neurosci.* 6, 1167–1178.

Wahle, P., Meyer, G., Wu, J. Y., and Albus, K. (1987). Morphology and axon terminal pattern of glutamate decarboxylase- immunoreactive cell types in the white matter of the cat occipital cortex during early postnatal development. *Brain Res.* 433, 53–61.

Woo, T. U., Beale, J. M., and Finlay, B. L. (1991). Dual fate of subplate neurons in a rodent. *Cereb. Cortex* 1, 433–443.

Yang, J. W., Hanganu-Opatz, I. L., Sun, J. J., and Luhmann, H. J. (2009). Three patterns of oscillatory activity differentially synchronize developing neocortical networks *in vivo*. *J. Neurosci.* 29, 9011–9025.

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Primate-specific origins and migration of cortical GABAergic neurons

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Gamma-aminobutyric-acidergic (GABAergic) cells form a very heterogeneous population of neurons that play a crucial role in the coordination and integration of cortical functions. Their number and diversity increase through mammalian brain evolution. Does evolution use the same or different developmental rules to provide the increased population of cortical GABAergic neurons? In rodents, these neurons are not generated in the pallial proliferative zones as glutamatergic principal neurons. They are produced almost exclusively by the subpallial proliferative zones, the ganglionic eminence (GE) and migrate tangentially to reach their target cortical layers. The GE is organized in molecularly different subdomains that produce different subpopulations of cortical GABAergic neurons. In humans and non-human primates, in addition to the GE, cortical GABAergic neurons are also abundantly generated by the proliferative zones of the dorsal telencephalon. Neurogenesis in ventral and dorsal telencephalon occurs with distinct temporal profiles. These dorsal and ventral lineages give rise to different populations of GABAergic neurons. Early-generated GABAergic neurons originate from the GE and mostly migrate to the marginal zone and the subplate. Later-generated GABAergic neurons, originating from both proliferative sites, populate the cortical plate. Interestingly, the pool of GABAergic progenitors in dorsal telencephalon produces mainly calretinin neurons, a population known to be significantly increased and to display specific features in primates. We conclude that the development of cortical GABAergic neurons have exclusive features in primates that need to be considered in order to understand pathological mechanisms leading to some neurological and psychiatric diseases.

Keywords: interneurons, neurogenesis, tangential migration, ganglionic eminence, ventricular zone, glutamic acid decarboxylase

INTRODUCTION

The cerebral cortex, including the hippocampal formation, is composed of two main classes of neurons: principal and non-principal cells. The first and the more numerous class (around 80% of the neurons) corresponds mainly to pyramidal cells, which are excitatory glutamatergic neurons (for reviews see DeFelipe and Jones, 1988; Spruston, 2008). The second class is formed for its vast majority by gamma-aminobutyric acid (GABA) neurons (Freund and Buzsaki, 1996; Huang et al., 2007; Ascoli et al., 2008; Burkhalter, 2008). GABA is the major inhibitory neurotransmitter in adult brain (Krnjevic and Schwartz, 1967). These GABAergic non-principal neurons have been also referred as interneurons because most of them have an axon that remains restricted to the local structure. However, several types of such so-called interneurons have an axon that project to distant brain regions (Ribak et al., 1986; Toth and Freund, 1992; Jinno et al., 2007; Tomioka and Rockland, 2007; for review see Jinno, 2009).

These GABAergic neurons form a very heterogenous population that plays a crucial role in regulating the activity of neuronal networks and complex interactions among principal cells (Somogyi and Klausberger, 2005; Skaggs et al., 2007). Therefore, the coordination and integration of cortical functions depends on the number and diversity of GABAergic neurons.

In fact, dysfunction or cell death of several specific types of GABAergic neurons has been described as a hallmark of various psychiatric and neurological disorders, such as schizophrenia (for reviews see Lewis et al., 2005; Gonzalez-Burgos and Lewis, 2008; Maldonado-Aviles et al., 2009) and epilepsy (Houser, 1999; DeFelipe, 2004; Andrioli et al., 2007) in which defects in neurogenesis or migration can be predisposing factors (Powell et al., 2003; Poluch et al., 2008; for reviews see Levitt et al., 2004; Kostovic et al., 2007; Leviton and Gressens, 2007; Metin et al., 2008). Understanding mechanisms that regulate the development of GABAergic neurons is a crucial pre-requisite step to assess pathological processes that can take place during development and lead to such neurological and psychiatric diseases.

Up to date most of our knowledge regarding GABAergic neuron development are based on studies performed in rodents. However, differences in cortical GABAergic circuitry exist between rodents and primates. Although many types of cortical interneurons appear to be common to all species, some types in primates, like the double bouquet cells (DeFelipe and Jones, 1988; Yanez et al., 2005; DeFelipe et al., 2006; Melchitzky and Lewis, 2008; Povysheva et al., 2008; Cruz et al., 2009; Jones, 2009; Zaitsev et al., 2009), or interneurons of cortical layer I (Rakic and Zecevic, 2003; Kostovic et al., 2005;

Meyer, 2007; Bystron et al., 2008), display more elaborate features. These types may represent evolutionary specializations. In addition, several studies suggest an increased proportion of cortical GABAergic neurons between rodents and primates (Hendry et al., 1987; Gabbott and Bacon, 1996; DeFelipe, 2002; Zaitsev et al., 2009). Such increases in the number and diversity of GABAergic neurons have been suggested to be closely related to the tremendous increased brain complexity that occurs during mammalian evolution (Jones, 2009; Pierani and Wassef, 2009; Rakic, 2009; Rakic et al., 2009).

Whereas there is a consensus for all species examined that principal glutamatergic neurons originate in the local ventricular (VZ) and subventricular zones (SVZ) of the dorsal telencephalon (pallium) and migrate along radial glia to their target cortical layer (Rakic, 1971; Noctor et al., 2001; for reviews see Rakic, 2006; Bystron et al., 2008; Metin et al., 2008; Rakic, 2009; Rakic et al., 2009), this seems not to be the case for GABAergic neurons (Molnar et al., 2006; Wonders and Anderson, 2006; Metin et al., 2008; Petanjek et al., 2008). In rodents, there are compelling evidences that cortical GABAergic neurons (**Figure 1**) are not generated in these cortical (pallial) proliferative zones. They are produced in proliferative zones of the ventral (basal) telencephalon (subpallium), the ganglionic eminence (GE). From this region, newly born GABAergic neurons migrate tangentially into the cortex (for review see Wonders and Anderson, 2006). Surprisingly, data obtained in the human (Letinic et al., 2002; Rakic and Zecevic, 2003; Fertuzinhos et al., 2009) and monkey cortex (Petanjek et al., 2009) point out significant evolutionary changes with respect to the origin of cortical GABAergic neurons. Therefore, the aim of this paper is to update the literature in this field (see Petanjek et al., 2008), giving an overview of the main data about the origin and migration of cortical GABAergic neurons in rodents and primates.

IN RODENTS GABAergic NEURONS ORIGINATE EXCLUSIVELY FROM THE GANGLIONIC EMINENCE AND MIGRATE TANGENTIALLY TO THE CORTEX

Golgi-impregnation studies have first described tangentially migrating cells in the fetal telencephalon (Stensaas, 1968). Increased attention started when it was established that these cells indeed correspond to GABAergic neurons (Van Eden et al., 1989; DeDiego et al., 1994). Although the stream of those cells was observed all along the intermediate zone (IZ) of the dorsal telencephalon, from the GE to the most medial part of dorsal hippocampus, it was difficult at this time to conceive

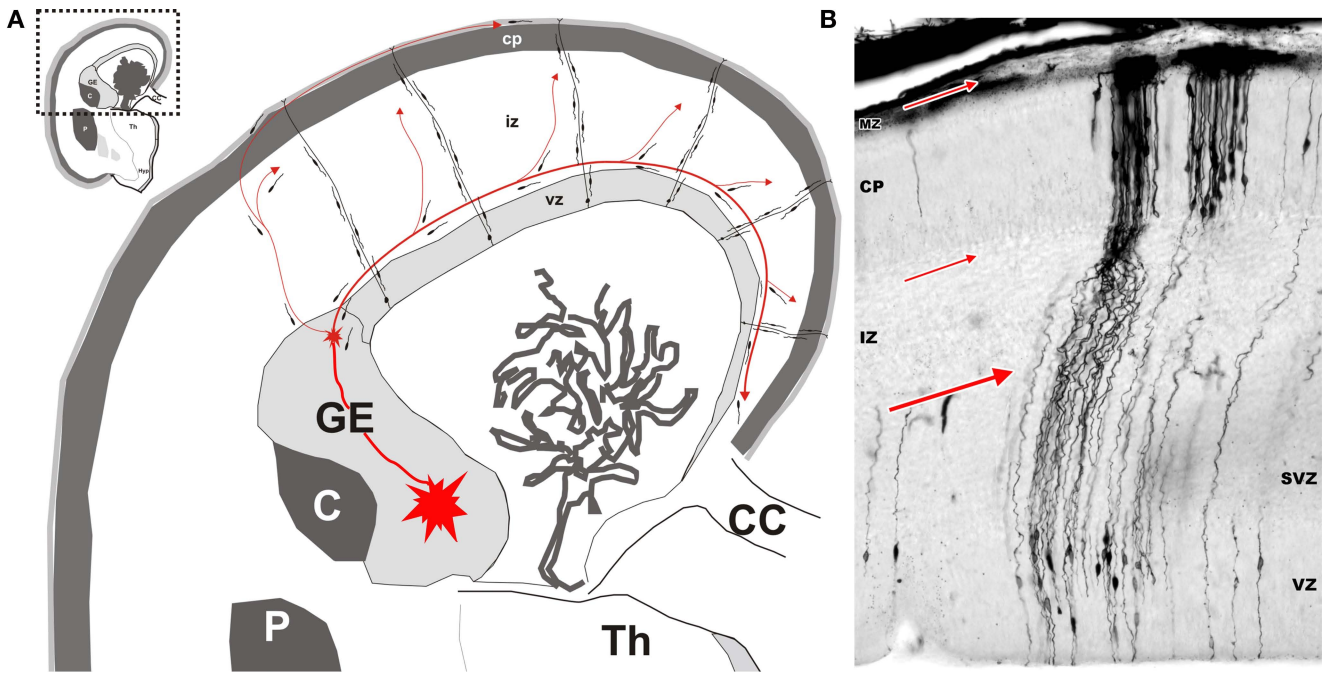

FIGURE 1 | Origin and migratory routes differ between the two major classes of cortical neurons. (A) Schematic drawing of migratory routes for cortical neurons in the dorsal telencephalon (pallium). Principal (pyramidal) neurons (excitatory glutamatergic neurons) originate locally in the proliferative ventricular (VZ) and subventricular (SVZ) zones of the dorsal telencephalon. Early-generated neurons display a bipolar morphology and migrate orthogonal to the surface of the brain along radially oriented glial fibres (radial migration). According to data obtained in rodents, hippocampal and cortical GABAergic neurons originate in the ventral proliferative zones, i.e. ganglionic eminence (GE) and migrate tangentially to their target cortical regions (red arrowed lines). The largest stream follows the lower intermediate zone (IZ)/upper subventricular zone (SVZ). In the marginal zone (MZ), the stream of migrating neurons forms a subpial granular layer. Numerous tangentially oriented neurons are present below the cortical plate (CP), in the subplate. Migratory routes are shown on schematized frontal section of human fetus at postconceptual week 15 (Th – thalamus; C – caudate nucleus; P – putamen; CC – corpus callosum). **(B)** Microphotography of a Golgi impregnated section through the whole thickness of dorsal telencephalon in a human fetus at postconceptual week 10. Bipolar migrating, prospective principal neurons are mostly observed in the VZ and SVZ. Numerous radial glia cells extend their processes from ventricle to pia. Numerous prospective pyramidal neurons at the beginning of differentiation are present in the cortical plate. Red arrows indicate major locations and direction of tangential migration. Modified by permission from Kostovic et al. (2007).

that cortical GABAergic neurons could originate from outside the cortical proliferative zones (DeDiego et al., 1994). Lineage experiments later established that clones of GABAergic neurons are tangentially dispersed, whereas radially arranged clones are formed primarily of glutamatergic neurons (Tan et al., 1998; Noctor et al., 2001; for reviews see Molnar et al., 2006; Dehay and Kennedy, 2007; Metin et al., 2008; Petanjek et al., 2008; Javaherian and Kriegstein, 2009). Gorski et al. (2002) further demonstrated that cortical glutamatergic neurons are produced in proliferative zones of the dorsal telencephalon (pallium), but not GABAergic cells. They suggested that GABAergic neurons originate from progenitors located outside the pallium. *In vitro* studies directly established that in rodents GABAergic neurons migrate tangentially from the ventral telencephalon (i.e. GE) into the cortex. These migrating neurons follow several routes to reach their target regions (**Figure 1**). The major stream of tangentially migrating GABAergic neurons is present at the border of IZ and SVZ (lower part of IZ and upper part of SVZ). In addition, smaller streams of migrating cells are present in the subplate (SP) and the upper part of marginal zone (MZ) (Anderson et al., 1997; Pleasure et al., 2000; Heng et al., 2007; Metin et al., 2007; for reviews see Molnar et al., 2006; Wonders and Anderson, 2006; Metin et al., 2008). Evidence that GABAergic neurons originate mainly from progenitors in the GE was provided by studies using several lines of knockout mice for genes expressed specifically in the ventral telencephalon. A marked reductions in the number of neocortical GABAergic neurons is observed at birth in all these lines including mutants for the transcription factors Dlx1 and Dlx2 (Anderson et al., 1997; Pleasure et al., 2000; Wonders and Anderson, 2005; Long et al., 2009), Nkx2.1 (Sussel et al., 1999; Pleasure et al., 2000; Butt et al., 2008; Nobrega-Pereira et al., 2008) and Mash1 (Casarosa et al., 1999; Horton et al., 1999; Guillemot, 2007; Long et al., 2009). Together these studies have clearly established that in rodents almost all cortical GABAergic neurons originate from the GE and migrate tangentially to their target cortical layers.

Interestingly, there are compelling evidences that different subdomains within the GE generate different populations of cortical GABAergic neurons. The GE displays two major divisions: the medial and lateral GE (Sidman and Rakic, 1973; O'Rahilly and Müller, 2006; Petanjek et al., 2008). Recent findings demonstrated the existence of two additional components, the caudal and septal divisions (Xu et al., 2004; Ghanem et al., 2007; Long et al., 2009; Petanjek et al., 2009). *In vitro* studies using focal electroporation into small, defined regions of the GE, showed that neurons from the medial part migrate laterally and spread widely throughout the cortex, whereas most neurons from the caudal part migrate toward the most caudal end of the telencephalon (Ang et al., 2003; Liodis et al., 2007; Kanatani et al., 2008; Zhao et al., 2008; for reviews see Wonders and Anderson, 2006; Metin et al., 2008; Wonders et al., 2008). The vast majority of cortical GABAergic neurons are provided by the medial GE and at a lower extent by the caudal GE. However, significant, although smaller numbers of cortical GABAergic neurons originate from the lateral GE, especially at later stages (Xu et al., 2004; Wonders and Anderson, 2006; Wonders et al., 2008). Among the different subpopulations of GABAergic neurons, most of somatostatin-expressing neurons and all parvalbumin-containing cells derive from the medial GE whereas the majority of calretinin-containing cells and half of the NPY-expressing neurons arise from the caudal GE. Even within the medial GE, the different subpopulations of cortical GABAergic neurons have distinct spatial origins. Somatostatin cells are primarily generated within the dorsal part of the medial GE whereas parvalbumin neurons are provided by the ventral part of the medial GE (Wonders et al., 2008). Therefore, the GE is organized in molecularly different subdomains that produce different subpopulations of cortical GABAergic neurons (Nery et al., 2002; Bellion and Metin, 2005; Bielle et al., 2005; Butt et al., 2005, 2007; Cobos et al., 2006; Wonders and Anderson, 2006; Flames et al., 2007; Fogarty et al., 2007; Ghanem et al., 2007; Guillemot, 2007; Kohwi et al., 2007; Liodis et al., 2007; Molyneaux et al., 2007; Batista-Brito et al., 2008; Bayatti et al., 2008; Du et al., 2008; Garcia-Moreno et al., 2008; Kanatani et al., 2008; Zhao et al., 2008; Carney et al., 2009; Long et al., 2009). In addition to spatial diversity in the origin of GABAergic neuron subpopulations, the generation of these different subgroups may occur at specific time windows (Butt et al., 2005, 2008; Ghanem et al., 2007; Batista-Brito et al., 2008; Cai et al., 2009; Sousa et al., 2009). However, the precise spatio-temporal pattern of production, for the different GABAergic subtypes, has yet to be established.

IN PRIMATES THE DORSAL TELENCEPHALON IS A MAJOR SOURCE FOR CORTICAL GABAergic NEURONS

Several studies have provided evidence that mammalian evolution uses different developmental rules to afford for cortical GABAergic neurons to an expanding neocortex. In human and non-human primates, cortical GABAergic neurons are produced not only by the GE (as in rodents), but also massively by the proliferative zones of the dorsal telencephalon (Letinic et al., 2002; Rakic and Zecevic, 2003; Fertuzinhos et al., 2009; Petanjek et al., 2009).

Letinic et al. (2002) were first to demonstrate the existence of two distinct lineages of cortical GABAergic neurons using retroviral labelling in organotypic slice cultures of the fetal human forebrain. One lineage originates from the GE as in rodents but give rise to only 35% of the cortical GABAergic neurons in human. The second that represents 65% of cortical GABAergic neurons originates from progenitors within the proliferative VZ and SVZ of the dorsal telencephalon. In this study, the authors suggested that the dorsal telencephalic origin of cortical GABAergic neurons might be human-specific (Letinic et al., 2002), but we demonstrated more recently that this occurs also in the macaque monkey (*Macaca rhesus* and *Macaca fascicularis*) (Petanjek et al., 2009).

Both in human and macaque, neurogenesis of GABAergic neurons within the ventral and dorsal telencephalon occurs with distinct temporal profiles. Whereas at early stages of primate fetal development, GE is an exclusive site of origin for cortical GABAergic neurons, later on the dorsal telencephalic proliferative layers are the major source for these neurons (Letinic et al., 2002; Petanjek et al., 2009). In the human fetal brain Mash1, a transcription factor known to be involved in the early specification of GABAergic neurons (Meredith and Johnson, 2000; Letinic et al., 2002; Britz et al., 2006; Guillemot, 2007; Long et al., 2009), was expressed only by the progenitors in the GE from postconceptional week (PCW) 10 to PCW13 (Letinic et al., 2002). In the macaque fetal brain (**Figure 2**), progenitors labeled for the GABA

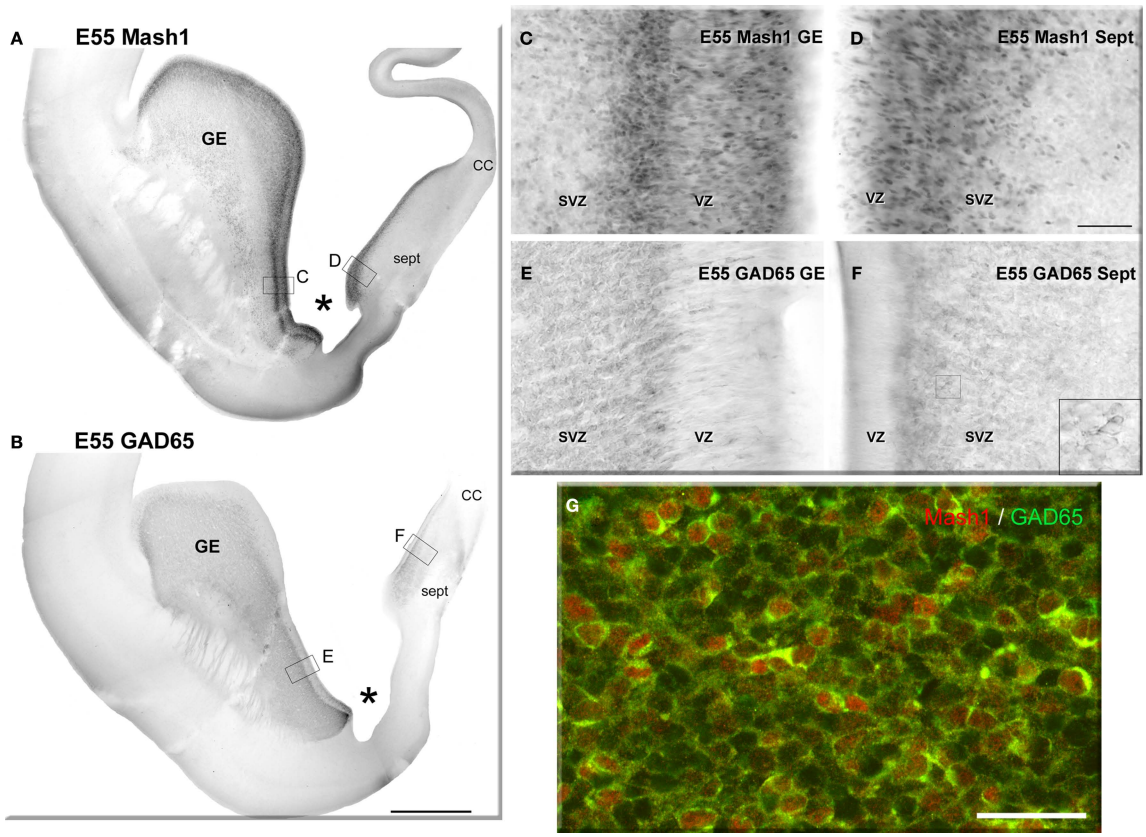

FIGURE 2 | Neurogenesis of GABAergic neurons in the ventral telencephalon of a monkey fetus at embryonic day (E) 55. (A,B) Adjacent sections labeled for Mash1 **(A)** and GAD65 **(B)** from a E55 fetal brain at a rostral level in which the lateral ventricle is dilated (paleocortical ventricle indicated by a star). **(A)** Progenitors labeled for Mash1 were present mainly in the ganglionic eminence (GE) [box magnified in panel **(C)**] and septal (Sept) proliferative zone [box magnified in panel **(D)**]. **(B)** GAD65-containing cells were observed in the same regions of the ventral telencephalon as Mash1, the GE and the Sept [boxes enlarged in panels **(E)** and **(F)**]. **(C–F)** Figures correspond to magnified regions indicated by boxes in **(A,B)**. **(C)** In the GE many cells were labeled for Mash1 in the ventricular (VZ) and the subventricular zone (SVZ). The intensity of labeling was much higher in the VZ than in the SVZ. **(D)** In the prospective septal region (Sept), numerous cells highly labeled for Mash1 were observed in the VZ whereas the cells in the SVZ displayed a moderate level of labeling. **(E)** Many cells labeled for GAD65 were observed in the GE. These cells were mainly located in the SVZ, and only a few cells were present in the VZ. **(F)** Many cells labeled for GAD65 were present in the SVZ of the Sept. Box: magnified region. **(G)** Confocal micrographs of an adjacent coronal section double-labeled for Mash1 (red, nuclear) and GAD65 (green, cytoplasmic) showing that in the SVZ of GE numerous Mash1 containing progenitors were labeled for GAD65. Scale bars: 1 mm **(A,B)**, 50 μm **(C–G)**, 10 μm [box in **(F)**]. Abbreviation: CC – corpus callosum. Modified by permission from Petanjek et al. (2009).

synthesizing enzyme, glutamic acid decarboxylase 65 (GAD65) and Mash1 were present almost exclusively in the GE and the septal VZ/SVZ (septal eminence) from embryonic day (E) 47 to E55 (Petanjek et al., 2009).

At these stages of fetal development, large contingent of GAD65-containing cells with morphological features of tangentially oriented migrating neurons were present in the upper SVZ and lower IZ as well as in the SP and MZ of the dorsal telencephalon (**Figure 3**). Such migratory pathways of GABAergic neurons from the GE to the prospective cortical region were observed also in human from PCW10 to PCW13 (Letinic et al., 2002). So, during the early fetal period both in monkey (E47–E55) and in human (PCW10 – PCW13), migratory cortical GABAergic neurons arise from the GE.

Later on during fetal development (E64–E75 in monkey; PCW15–PCW20 in human), cortical GABAergic neurons are, in addition to the GE, massively generated by the proliferative regions of the dorsal telencephalon. In macaque, numerous Mash1-containing progenitors coexpressing GAD65 were present in the SVZ and VZ of the entire dorsal telencephalon (**Figure 4**). These double-labeled cells displayed features of proliferative cells. They were clearly morphologically different from adjacent post-mitotic GAD65-containing migrating neurons. Many migrating GAD65-containing neurons displayed nontangential orientation. This is in contrast to earlier stage (E47–E55), when almost all migrating neurons were tangentially oriented (**Figure 3**). Similar patterns of GABAergic neuron progenitors and migrating GABAergic neurons were described in human fetuses (Letinic et al., 2002).

Furthermore, our results strongly support the view that the progenitors of GABAergic neurons in the cortical (dorsal) proliferative zones are generated locally, rather than arriving from the GE. Our main argument is that at E47–E55, no Mash1-containing cells were observed in the dorsal telencephalic wall close to the GE whereas onset of Mash1 expression was already observed in the VZ/SVZ of the most dorsal part of the telencephalon

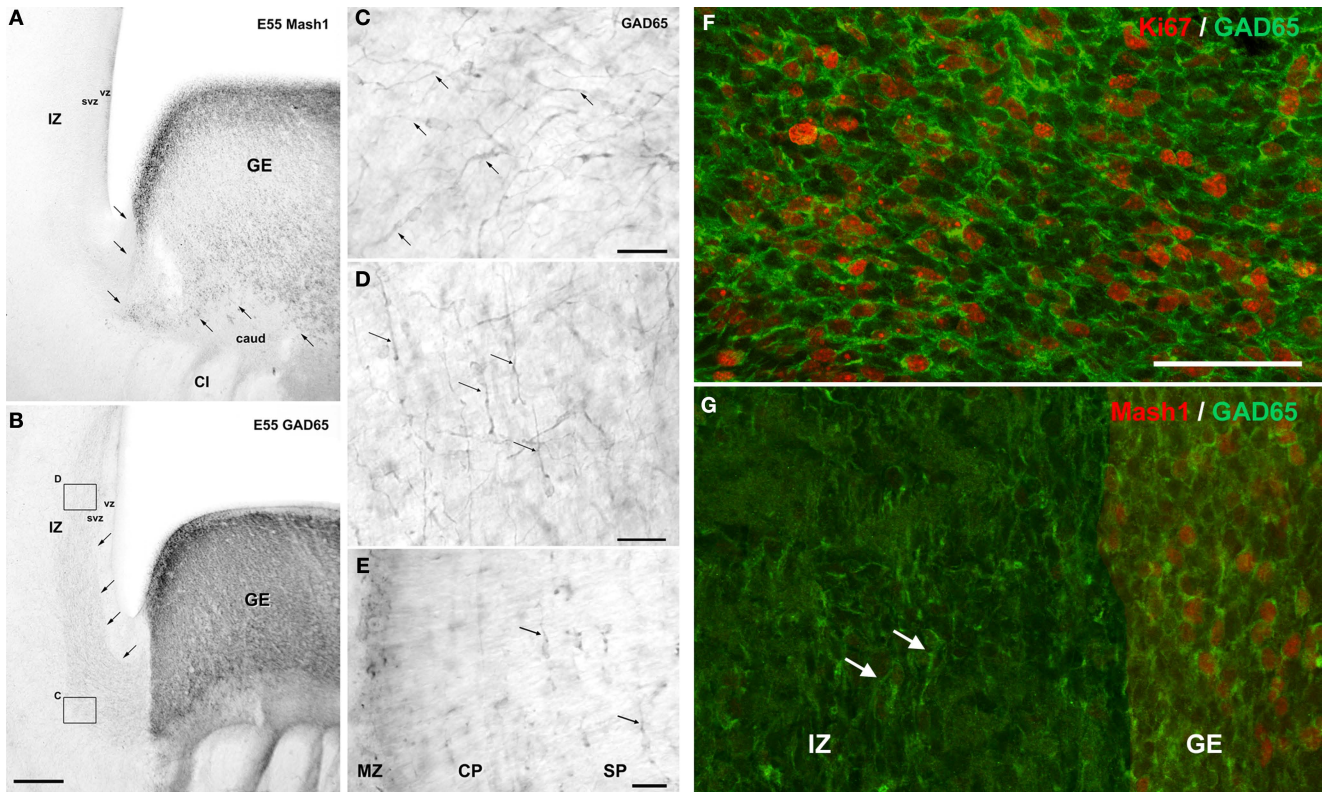

FIGURE 3 | Tangential migration of GABAergic neurons in coronal sections of a monkey fetus at embryonic day (E) 55. (A) Mash1 containing progenitors were present in the ganglionic eminence (GE) but not in the neighboring structures including the caudate nucleus (caud), capsula interna (CI), and adjacent zones of the dorsal telencephalon: ventricular zone (VZ), subventricular zone (SVZ) and intermediate zone (IZ). Arrows indicated the border between GE and adjacent structures. **(B)** GAD65 containing progenitors were present almost exclusively in the GE. In addition a stream of post-mitotic-like, GAD65-containing neurons extending from the GE to dorsal telencephalon was observed in the lower IZ/upper SVZ. Arrows indicate the deeper border of this stream. Boxes: magnified regions in panels **(C)** and **(D)**. **(C)** GAD65-containing cells with a characteristic morphology of post-mitotic migrating neurons, including a leading process (arrows) leaving the GE. **(D)** In the IZ and upper SVZ of dorsal telencephalon, the majority of GAD65 migrating neurons were tangentially oriented (arrows). **(E)** Tangentially oriented GAD65 migrating neurons are also observed in the subplate (SP) (arrows), cortical plate (CP) and marginal zone (MZ) of the dorsal telencephalon. **(F,G)** Confocal micrographs of an adjacent coronal section double-labeled for Ki67 **(F)** or Mash1 **(G)** (red, nuclear) and GAD65 (green, cytoplasmic). At E55, in the SVZ of the GE many GAD65-labeled cells were labeled for Ki67 **(F)** and therefore corresponded to proliferating cells. In contrast none of the migrating GAD65-containing cells in the IZ displayed detectable level of Ki67 or Mash1 **(G)** and therefore likely corresponded to early post-mitotic neurons. Scale bars: 350 μm **(A,B)**, 50 μm **(F,G)**, 20 μm, **(C–E)**. Modified by permission from Petanjek et al. (2009).

(**Figures 5 and 6B**). This pattern of Mash1 expression in dorsal progenitors indicates that these progenitors did not migrate from the GE. However, at earlier stage (E47–E55), the Mash1-containing cells in the dorsal telencephalon did not contain GAD65 and thus may not have a GABAergic phenotype. We cannot exclude the possibility that these Mash1-containing progenitors generate other types of cells such as oligodendrocytes (Jakovcevski and Zecevic, 2005; Guillemot, 2007; Parras et al., 2007) or reflect a regulation of the progenitor division mode not related with specification of GABAergic neurons (Britz et al., 2006). However, at E64 only few Mash1-containing cells express PDGFR-α, a marker of oligodendrocyte progenitors, and the vast majority of Mash1-containing cells in the VZ/SVZ of the entire dorsal telencephalon expresses GAD65 (Petanjek et al., 2009). Thus, the expression of Mash1 in the dorsal telencephalon at E47–E55 corresponds likely to symmetrically dividing prospective GABAergic precursors (Rakic, 2009; Rakic et al., 2009) and marks the onset of GABAergic neurogenesis in this region.

Therefore, in human and monkey, cortical GABAergic neurons are generated by two distinct sources: the GE and the proliferative zone of the dorsal telencephalon. In addition, the dorsal proliferative zone is not only an additional but also a major pool of GABAergic progenitors in primates (**Figure 7**).

THE DORSAL AND VENTRAL SITES OF NEUROGENESIS PRODUCE DIFFERENT POPULATIONS OF CORTICAL GABAergic NEURONS

In the macaque, the dorsal and ventral lineage of GABAergic neurons displayed different cortical distributions. Early-generated GABAergic neurons from the GE populated mainly the MZ and the SP. Indeed, as early as E47, morphologically differentiated GAD-containing neurons were observed first in the MZ and the SP just below the cortical plate (CP). Their number increased in the SP with the development of this layer at a period when progenitors of GABAergic neurons are mainly observed in the ventral telencephalon (Petanjek et al., 2009). A similar pattern

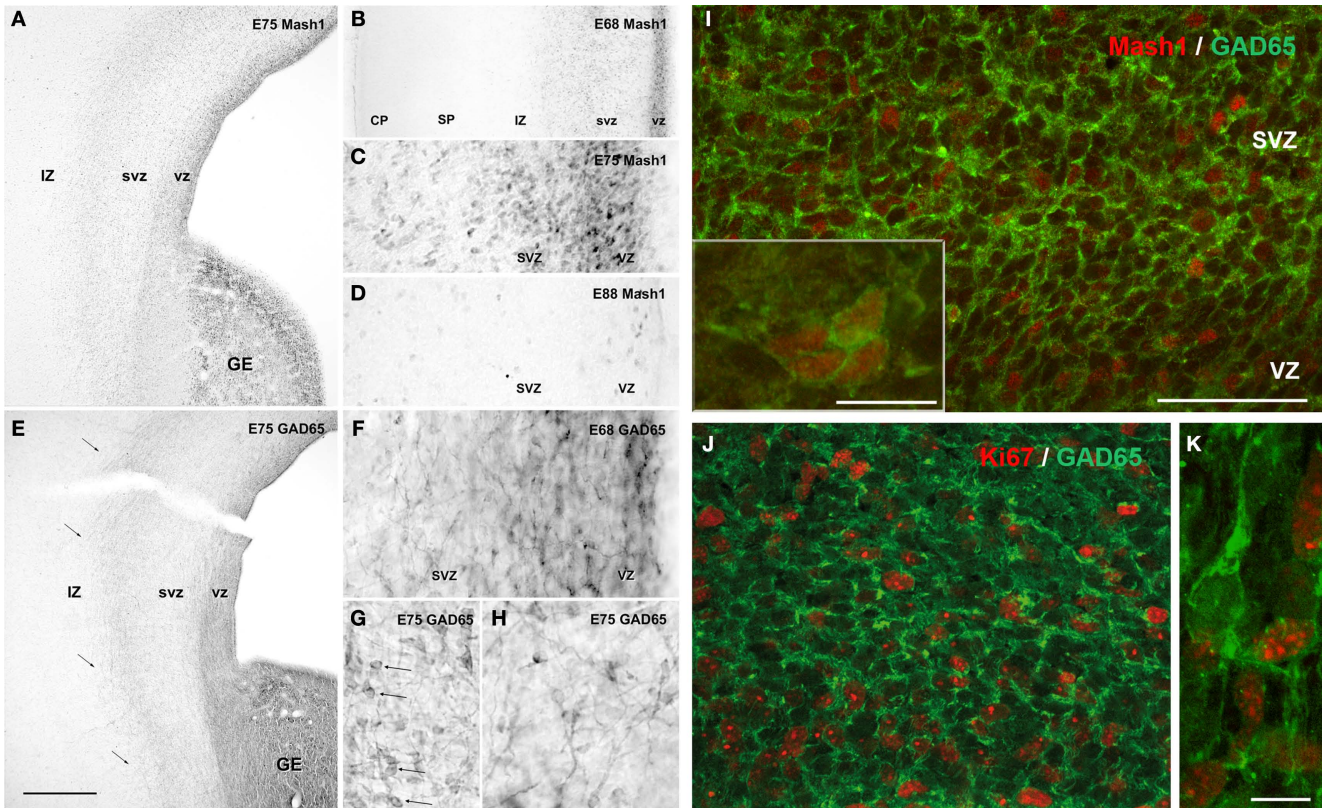

FIGURE 4 | Neurogenesis of GABAergic neurons in the ventral and dorsal telencephalon of monkey fetuses at embryonic day (E) 68, E75 and E88. **(A–D)** Mash1 expression. **(A)** At E75, Mash1 containing progenitors were present in the ganglionic eminence (GE) as well as in ventricular (VZ) and subventricular (SVZ) zone of the entire dorsal telencephalon. In both areas, the intensity of labeling for Mash1 decreased from the VZ to the SVZ. **(B)** At E68, the same pattern of labeling for Mash1 is already present in the VZ and SVZ of the dorsal telencephalon. No labeling is observed in the upper part of intermediate zone (IZ), the subplate (SP) and the cortical plate (CP). **(C)** Higher magnification of panel **(A)**. The numerous Mash1 highly labeled cells located in the VZ and SVZ of the dorsal telencephalon. **(D)** At E88, end of neurogenesis, the intensity of labeling for Mash1 was strongly decreased as compared to E68 **(B)** and E75 **(A,C)**. **(E–H)** GAD65 expression. **(E)** At E75, GAD65-containing progenitors were present in the GE, but also in the proliferative VZ and SVZ of the dorsal telencephalon in contrast to earlier stages. The stream of migrating GAD65-containing neurons observed at E55 [compare with panel **(B)** in **Figure 3**] in the lower part of IZ and upper part of SVZ was still present (arrows). **(F)** At E68, similar labeling was already observed. In contrast to previous stages many migrating-like post-mitotic neurons with one leading process were now observed in the VZ and SVZ of the dorsal telencephalon. **(G)** At E75, in addition to migrating-like post-mitotic neurons, many round-shaped cells without any process were labeled for GAD65 (arrows). These cells displayed a similar morphology to that observed in the GE and septal region. **(H)** At E75, many migrating-like GAD65-neurons in the SVZ of the dorsal telencephalon displayed multidirectional orientations. **(I–J)** Confocal micrographs of an adjacent coronal section double-labeled for Mash1 **(I)** or Ki67 **(J,K)** (red, nuclear) and GAD65 (green, cytoplasmic). **(I)** At E75, in the dorsal telencephalon, most Mash1-containing cells of SVZ also contained GAD65, whereas many Mash1-labeled cells in the VZ did not. Insert: aggregates of cells double-labeled for Mash1 and GAD65 were frequently observed in the SVZ of the dorsal telencephalon, suggesting cells undergoing division. **(J)** At E75 in the SVZ of the dorsal telencephalon [compare with panel **(G)**] most GAD65-containing cells were labeled for Ki67 and therefore corresponded to progenitors of GABAergic neurons. **(K)** Migrating-like GAD65-containing cells in the IZ of the dorsal telencephalon were not labeled for Ki67. Scale bars: 550 μm **(A,E)**, 250 μm **(B)**, 60 μm **(C,D)** and 30 μm **(F–H)**; 50 μm **(I,J)**, 10 μm [insert **(I,K)**]. Modified by permission from Petanjek et al. (2009).

of early-generated GABAergic neurons was described in human fetuses (Zecevic and Milosevic, 1997; Meyer et al., 2000; Rakic and Zecevic, 2003; Meyer, 2007). It was shown that SP neurons as well as early-generated neurons in the hippocampal MZ are functional and play a crucial role in early-generated network activity (Khazipov et al., 2001; Hanganu et al., 2002; Moore et al., 2009). In human and non-human primates GABAergic neurons in the CP are generated in both GE and dorsal proliferative zones. In macaque, from E64–E75 onwards, GABAergic neurons penetrate into the CP following an inside-out gradient. Their number increases during a period when progenitors of GABAergic neurons are massively present in proliferative regions of the entire dorsal telencephalon. In human, two thirds of CP interneurons are generated in the dorsal telencephalon (Letinic et al., 2002).

A recent study from Fertuzinhos et al. (2009) investigates the proportion of different subpopulations of GABAergic neurons in brains of human fetuses or infants affected by holoprosencephaly. This syndrome is characterized by severe striatal hypoplasia and atrophy of the GE (Judas et al., 2003). Interestingly, in holoprosencephaly, the numbers of cortical GABAergic neurons expressing nitric oxide synthase, neuropeptide Y or somatostatin were significantly reduced in comparison to healthy infants. In contrast, calretinin-containing neurons were present in normal

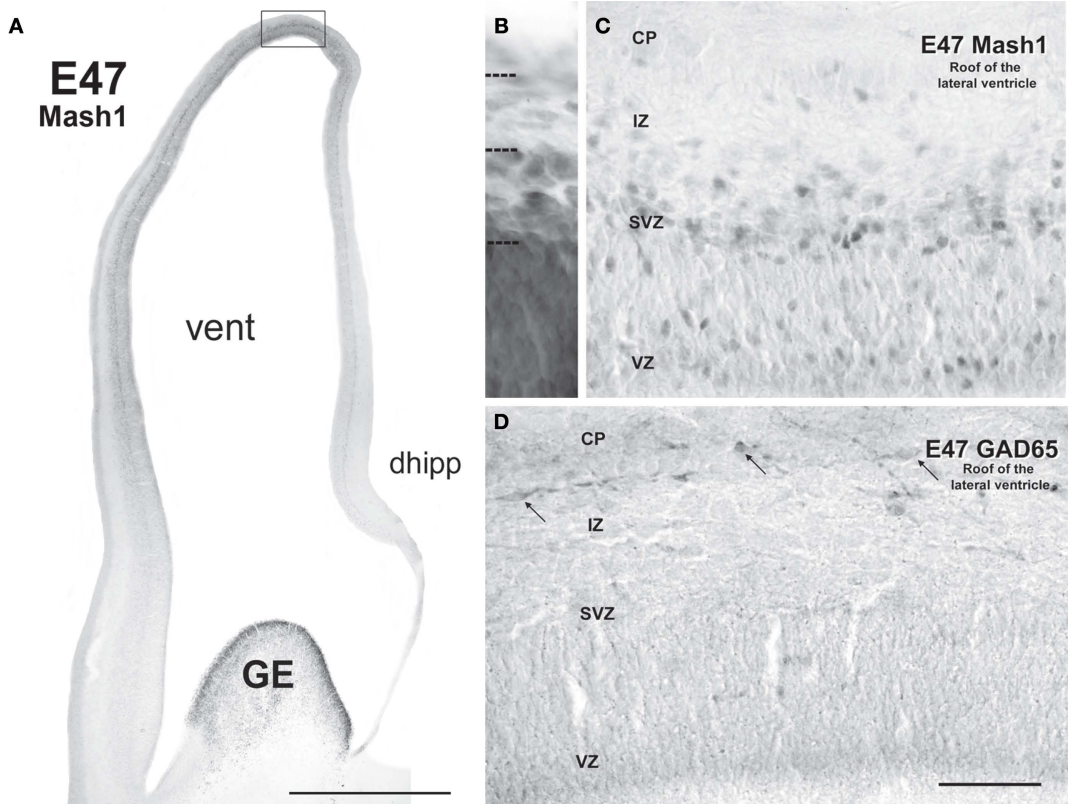

FIGURE 5 | Mash1 and GAD65 labeling patterns in coronal sections of monkey fetal telencephalon at embryonic day (E) 47 suggest that neurogenesis of GABAergic neurons is induced locally. (A) At E47 Mash1 was highly expressed in the proliferative zone of the ventral telencephalon, the ganglionic eminence (GE). Mash1 immunoreactivity was also observed in the proliferative zones of the dorsal telencephalon. It followed a clear dorso-ventral decreasing gradient in the labeling intensity within the entire cortical wall. Moderate level of labeling was observed in the most dorsal part of the telencephalon [indicated by square and magnified in panel **(C)**]. The intensity of staining decreased in the lateral and medial wall. No Mash1 immunolabeling was observed close to the GE, nor in the most ventral part of the medial telencephalic wall, that corresponds at this level to dorsal hippocampus (dhipp). **(B)** Cresyl violet labeled section. Different cellular zones of the most dorsal part of the dorsal telencephalon are delineated. **(C)** Some cells moderately labeled for Mash1 were observed in the proliferative zones of the most dorsal part of the dorsal telencephalon. Most of these Mash1 labeled cells were present in the upper part of subventricular zone (SVZ), on the border with the intermediate zone (IZ). Some cells were also observed in the deepest part of ventricular zone (VZ). **(D)** In contrast to Mash1 no GAD65-containing cells were observed in the VZ and SVZ of the most dorsal part of the dorsal telencephalon at E47. However in this region, some GAD65-containing cells with a characteristic morphology of early post-mitotic migrating neurons, including a leading process, and tangentially oriented, were observed in the IZ and the cortical plate (CP) (arrows). Vent – lateral ventricle. Scale bars: 1 mm **(A)**, 50 μm **(B–D)**. Modified by permission from Petanjek et al. (2009).

numbers as well as principal neurons. These findings show that, in human, nitric oxide synthase-, neuropeptide Y- and somatostatin-containing neurons originate from the GE whereas calretinin neurons are generated by the dorsal telencephalon.

It is interesting to note that GABAergic neurons identified as missing or significantly reduced (Fertuzinhos et al., 2009) are those normally found in greatest numbers in derivatives of the MZ and SP (Kostovic and Rakic, 1990; Delalle et al., 1997; Rakic and Zecevic, 2003; Meyer, 2007; Kostovic et al., 2008; Petanjek et al., 2009). In addition, the population of calretinin neurons, generated mainly in the dorsal telencephalon in human, corresponds to the population of GABAergic neurons which proportion increases dramatically in primates (Hendry et al., 1987; Gabbott and Bacon, 1996; Zaitsev et al., 2009) and displays primate-specific subtypes such as double bouquet cells (DeFelipe and Jones, 1988; DeFelipe, 2002; Yanez et al., 2005; DeFelipe et al., 2006; Jones, 2009).

Altogether, these studies favor the hypothesis that dorsal production occurs principally as an answer to an increased evolutionary need for specific classes of cortical GABAergic neurons.

MASSIVE DORSAL ORIGIN OF CORTICAL GABAergic NEURONS IS PRIMATE-SPECIFIC

Whereas in primates a large majority of cortical GABAergic neurons are produced in the proliferative regions of the dorsal telencephalon (**Figure 7**), in rodents cortical GABAergic neurons are generated almost exclusively in the GE (**Figure 6A**). A puzzling question is whether this dorsal telencephalic origin of cortical GABAergic neurons is primate-specific or reflects an enhancement of preexisting developmental mechanisms.

We have to stress that mammalian species examined until now included only mouse, rat, ferret, monkey and human. However, in favor of the later hypothesis, a production of GABAergic neurons in

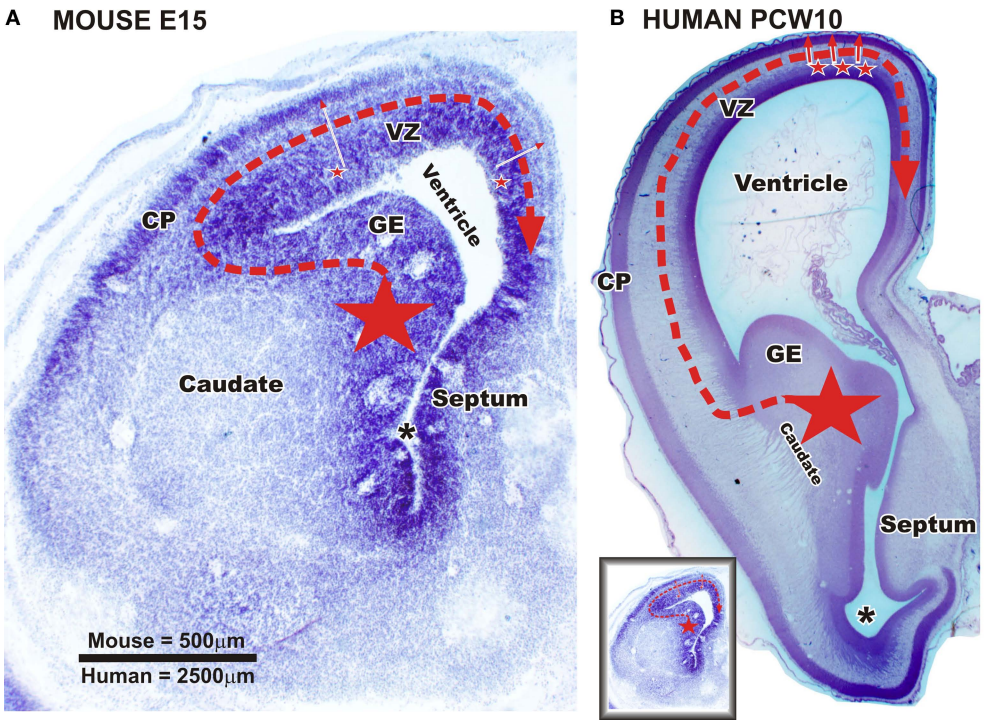

FIGURE 6 | Sources of cortical GABAergic neuron in a mouse fetus and in primate fetuses at early developmental stage (embryonic day (E) 47 to E55 in macaque/postconceptional week (PCW) 10 to PCW13 in human). Nissl staining of frontal brain sections through telencephalic vesicle at the level of septum and paleocortical ventricle (asterix) in a mouse fetus at embryonic day (E) 15 [panel **(A)** and in a human fetus at postconceptional week 10 panel **(B)**]. **(A)** In the rodent, the vast majority of cortical and hippocampal GABAergic neurons are produced by ganglionic eminence (GE) (large star). The neurons migrate tangentially in the cortex (red dashed arrow). A very small percentage of the GABAergic neurons is supposed to be also produced in dorsal proliferative zones (small stars – full red arrows). **(B)** In the monkey and human brain, the GE is also an important source of cortical GABAergic neurons (large star). Post-mitotic neurons migrate tangentially into the cortex (red dashed arrow). Data from macaque monkey (Petanjek et al., 2009) suggest that already at this ages (see below) a production of GABAergic neurons started in the proliferative zones of most dorsal part of the pallium (small stars – full red arrows). VZ – ventricular and subventricular zone; CP – cortical plate. Scale bar: 500 μm **(A)**, 2500 μm **(B)**. Small figure [left down in panel **(B)**] is a mouse section at E15 **(A)** reduced in size to be comparable with human fetus at PCW10 **(B)**.

dorsal proliferative zones has been reported in mouse (**Figure 6A**), but it accounts for a very small fraction of neurons present at maturity (Xu et al., 2004; Molnar et al., 2006; Wonders and Anderson, 2006). In this species, the progenitor cells of the dorsal telencephalon are able to generate GABA-expressing neurons *in vitro* (He et al., 2001). However, in the ferret, that has a convoluted neocortex and displays a 2–3 times longer neurogenesis compared to mouse and rat, very few (less than 5%) cortical GABAergic neurons originated from proliferative zones of the dorsal telencephalon (Anderson et al., 2002). This is about the proportion obtained in rodents (Xu et al., 2004; Molnar et al., 2006; Wonders and Anderson, 2006). However, the massive neurogenesis of cortical GABAergic neurons from dorsal telencephalon is most likely primate-specific.

During tangential migration, prospective GABAergic neurons move parallel to the surface of the brain. They pass by a complex route between numerous growing axon bundles and cross regional boundaries. The length of their trajectory is greater than that of radially migrating neurons (**Figure 1**). Therefore, the mechanisms of cellular interactions enabling proper positioning and specification of GABAergic neurons are more complex than for radially migrating neurons (Metin et al., 2006, 2008). One can suggests that dorsal production of cortical GABAergic neurons in primates might occur in order to facilitate migration routes through an expanding neocortex (**Figures 6 and 7**). An exclusive ventral telencephalic origin of cortical GABAergic neurons in primates would imply extremely long and complex migratory routes for such neurons (**Figure 6B**; compare the real size between embryonic mouse and human fetus). In keeping with this hypothesis, it might be expected that the percentage of GABAergic neurons produced dorsally will increase in larger brains in order to keep migratory routes shorter and simpler. However, the data obtained in ferret (Anderson et al., 2002), which displays a convoluted neocortex significantly larger compared to rodents but shows limited cortical GABAergic neurons generated by the dorsal telencephalon such as described in mouse, do not support this hypothesis. Extensive dorsal production of GABAergic neurons in primates can be related to an increased need in number and/or specific types for GABAergic neurons in brains with more complex cortical circuitries (Fertuzinhos et al., 2009; Jones, 2009).

The massive neurogenesis of cortical GABAergic neurons from dorsal telencephalon suggests distinct properties of dorsal telencephalic progenitors in primates compared to rodents. Interestingly, it was demonstrated that the proliferative behavior of cortical neuronal precursors during neurogenesis differs between rodents and primates. This leads to significant differences in morphology and function of their dorsal proliferative zones (Smart et al., 2002; Kriegstein et al., 2006; Dehay and Kennedy, 2007; Noctor et al., 2007, 2008; Bystron

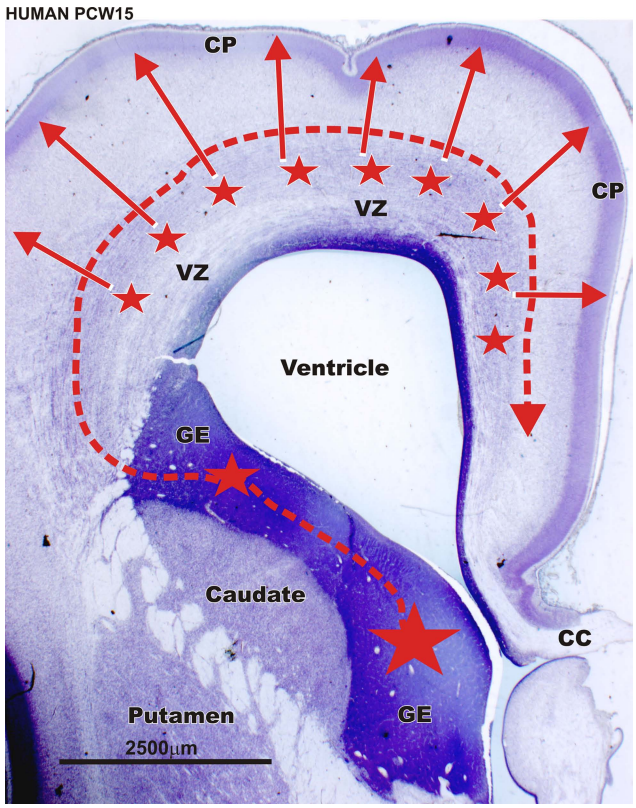

FIGURE 7 | Sources of cortical GABAergic neuron in the the primate during middle fetal stage. Nissl staining of frontal brain sections through rostral part of the telencephalic vesicle in a human fetus at postconceptional week (PCW) 15 (at a level of the anterior horn). In human (PCW15 – PCW20) as in macaque monkey [embryonic day (E) 64 to E75] later during gestation, GABAergic neurons are produced massively by the proliferative zones of the dorsal telencephalon (smaller stars – full red arrows). According to Letinic et al. (2002) these dorsal proliferatives zones generate 65% of the cortical GABAergic neurons. This massive dorsal production of GABAergic neurons is primate-specific. VZ – ventricular and subventricular zone; CP – cortical plate; CC – corpus callosum. Scale bar: 2500 μm.

et al., 2008; Fish et al., 2008; Javaherian and Kriegstein, 2009; Tabata et al., 2009). In the human embryo, in comparison to the rodent embryo, there is a dramatic increase in surface area and thickness of the VZ at the earliest stage of proliferation (Carney et al., 2007). In comparison to other species, the primate SVZ is larger and more complex. It displays a different type of cellular organization (Smart et al., 2002; Zecevic et al., 2005; Kriegstein et al., 2006). There is a new, outer compartment within the SVZ, which displays a number of unique features, and exists much longer during development (Molnar et al., 2006; Dehay and Kennedy, 2007; Bystron et al., 2008; Kostovic and Vasung 2009; Jovanov-Milosevic et al., 2009). So, it is not unreasonable to suggest that the increase in complexity, or even the specific cellular and laminar organization of dorsal proliferative zones in primates are significantly connected to the massive production of GABAergic neurons. However, no data to prove this hypothesis are currently available.

CONCLUDING REMARKS

There has been an impressive amount of research over past years in the field of GABAergic neuron development. The vast majority of this work has been done in rodents, birds and reptiles. These studies give us insight into molecular and cellular mechanisms of GABAergic neuron specification, proper positioning and differentiation. In higher mammals, especially primates, the amount of research in this field is in general disproportionally low. Primate data have pointed out significant changes in the origin (**Figures 6B and 7**), specification and migration of cortical GABAergic neurons compared to lower mammals (**Figure 6A**). They strongly support the view that our understanding of GABAergic neuron development in the human brain can not be assessed only on studies performed in rodents.

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REFERENCES

Anderson, S. A., Eisenstat, D. D., Shi, L., and Rubenstein, J. L. (1997). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* 278, 474–476.

Anderson, S. A., Kaznowski, C. E., Horn, C., Rubenstein, J. L., and McConnell, S. K. (2002). Distinct origins of neocortical projection neurons and interneurons *in vivo*. *Cereb. Cortex* 12, 702–709.

Andrioli, A., Alonso-Nanclares, L., Arellano, J. I., and DeFelipe, J. (2007). Quantitative analysis of parvalbumin-immunoreactive cells in the human epileptic hippocampus. *Neuroscience* 149, 131–143.

Ang, E. S., Jr., Haydar, T. F., Gluncic, V., and Rakic, P. (2003). Four-dimensional migratory coordinates of GABAergic interneurons in the developing mouse cortex. *J. Neurosci.* 23, 5805–5815.

Ascoli, G. A., Alonso-Nanclares, L., Anderson, S. A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., Buzsaki, G., Cauli, B., Defelipe, J., Fairen, A., Feldmeyer, D., Fishell, G., Fregnac, Y., Freund, T. F., Gardner, D., Gardner, E. P., Goldberg, J. H., Helmstaedter, M., Hestrin, S., Karube, F., Kisvarday, Z. F., Lambolez, B., Lewis, D. A., Marin, O., Markram, H., Munoz, A., Packer, A., Petersen, C. C., Rockland, K. S., Rossier, J., Rudy, B., Somogyi, P., Staiger, J. F., Tamas, G., Thomson, A. M., Toledo-Rodriguez, M., Wang, Y., West, D. C., and Yuste, R. (2008). Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nat. Rev. Neurosci.* 9, 557–568.

Batista-Brito, R., Machold, R., Klein, C., and Fishell, G. (2008). Gene expression in cortical interneuron precursors is prescient of their mature function. *Cereb. Cortex* 18, 2306–2317.

Bayatti, N., Moss, J. A., Sun, L., Ambrose, P., Ward, J. F., Lindsay, S., and Clowry, G. J. (2008). A molecular neuroanatomical study of the developing human neocortex from 8 to 17 postconceptional weeks revealing the early differentiation of the subplate and subventricular zone. *Cereb. Cortex* 18, 1536–1548.

Bellion, A., and Metin, C. (2005). Early regionalisation of the neocortex and the medial ganglionic eminence. *Brain Res. Bull.* 66, 402–409.

Bielle, F., Griveau, A., Narboux-Neme, N., Vigneau, S., Sigrist, M., Arber, S., Wassef, M., and Pierani, A. (2005). Multiple origins of Cajal-Retzius cells at the borders of the developing pallium. *Nat. Neurosci.* 8, 1002–1012.

Britz, O., Mattar, P., Nguyen, L., Langevin, L. M., Zimmer, C., Alam, S., Guillemot, F., and Schuurmans, C. (2006). A role for proneural genes in the maturation of cortical progenitor cells. *Cereb. Cortex* 16(Suppl. 1), i138–i151.

Burkhalter, A. (2008). Many specialists for suppressing cortical excitation. *Front. Neurosci.* 2, 155–167. doi:10.3389/neuro.01.026.2008.

Butt, S. J., Cobos, I., Golden, J., Kessaris, N., Pachnis, V., and Anderson, S. (2007). Transcriptional regulation of cortical interneuron development. *J. Neurosci.* 27, 11847–11850.

Butt, S. J., Fuccillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J. G., and Fishell, G. (2005). The temporal and

spatial origins of cortical interneurons predict their physiological subtype. *Neuron* 48, 591–604.

Butt, S. J., Sousa, V. H., Fuccillo, M. V., Hjerling-Leffler, J., Miyoshi, G., Kimura, S., and Fishell, G. (2008). The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. *Neuron* 59, 722–732.

Bystron, I., Blakemore, C., and Rakic, P. (2008). Development of the human cerebral cortex: boulder committee revisited. *Nat. Rev. Neurosci.* 9, 110–122.

Cai, Y., Xiong, K., Chu, Y., Luo, D. W., Luo, X. G., Yuan, X. Y., Struble, R. G., Clough, R. W., Spencer, D. D., Williamson, A., Kordower, J. H., Patrylo, P. R., and Yan, X. X. (2009). Doublecortin expression in adult cat and primate cerebral cortex relates to immature neurons that develop into GABAergic subgroups. *Exp. Neurol.* 216, 342–356.

Carney, R. S., Bystron, I., Lopez-Bendito, G., and Molnar, Z. (2007). Comparative analysis of extra-ventricular mitoses at early stages of cortical development in rat and human. *Brain Struct. Funct.* 212, 37–54.

Carney, R. S., Cocas, L. A., Hirata, T., Mansfield, K., and Corbin, J. G. (2009). Differential regulation of telencephalic pallial-subpallial boundary patterning by pax6 and gsh2. *Cereb. Cortex* 19, 745–759.

Casarosa, S., Fode, C., and Guillemot, F. (1999). Mash1 regulates neurogenesis in the ventral telencephalon. *Development* 126, 525–534.

Cobos, I., Long, J. E., Thwin, M. T., and Rubenstein, J. L. (2006). Cellular patterns of transcription factor expression in developing cortical interneurons. *Cereb. Cortex* 16 (Suppl. 1), i82–i88.

Cruz, D. A., Lovallo, E. M., Stockton, S., Rasband, M., and Lewis, D. A. (2009). Postnatal development of synaptic structure proteins in pyramidal neuron axon initial segments in monkey prefrontal cortex. *J. Comp. Neurol.* 514, 353–367.

DeDiego, I., Smith-Fernandez, A., and Fairen, A. (1994). Cortical cells that migrate beyond area boundaries: characterization of an early neuronal population in the lower intermediate zone of prenatal rats. *Eur. J. Neurosci.* 6, 983–997.

DeFelipe, J. (2002). Cortical interneurons: from Cajal to 2001. *Prog. Brain Res.* 136, 215–238.

DeFelipe, J. (2004). Cortical microanatomy and human brain disorders: epilepsy. *Cortex* 40, 232–233.

DeFelipe, J., Ballesteros-Yanez, I., Inda, M. C., and Munoz, A. (2006). Double-bouquet cells in the monkey and human cerebral cortex with special reference to areas 17 and 18. *Prog. Brain Res.* 154, 15–32.

DeFelipe, J., and Jones, E. G. (1988). Cajal on the Cerebral Cortex, An Annotated Translation of the Complete Writings. New York, Oxford University Press.

Dehay, C., and Kennedy, H. (2007). Cell-cycle control and cortical development. *Nat. Rev. Neurosci.* 8, 438–450.

Delalle, I., Evers, P., Kostovic, I., and Uylings, H. B. (1997). Laminar distribution of neuropeptide Y-immunoreactive neurons in human prefrontal cortex during development. *J. Comp. Neurol.* 379, 515–522.

Du, T., Xu, Q., Ocbina, P. J., and Anderson, S. A. (2008). NKX2.1 specifies cortical interneuron fate by activating Lhx6. *Development* 135, 1559–1567.

Fertuzinhos, S., Krsnik, Z., Kawasawa, Y. I., Rasin, M. R., Kwan, K. Y., Chen, J. G., Judas, M., Hayashi, M., and Sestan, N. (2009). Selective depletion of molecularly defined cortical interneurons in human holoprosencephaly with severe striatal hypoplasia. *Cereb. Cortex* 19, 2196–2207.

Fish, J. L., Dehay, C., Kennedy, H., and Huttner, W. B. (2008). Making bigger brains-the evolution of neural-progenitor-cell division. *J. Cell. Sci.* 121, 2783–2793.

Flames, N., Pla, R., Gelman, D. M., Rubenstein, J. L., Puelles, L., and Marin, O. (2007). Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. *J. Neurosci.* 27, 9682–9695.

Fogarty, M., Grist, M., Gelman, D., Marin, O., Pachnis, V., and Kessaris, N. (2007). Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. *J. Neurosci.* 27, 10935–10946.

Freund, T. F., and Buzsaki, G. (1996). Interneurons of the hippocampus. *Hippocampus* 6, 347–470.

Gabbott, P. L., and Bacon, S. J. (1996). Local circuit neurons in the medial prefrontal cortex (areas 24a,b,c, 25 and 32) in the monkey: II. Quantitative areal and laminar distributions. *J. Comp. Neurol.* 364, 609–636.

Garcia-Moreno, F., Lopez-Mascaraque, L., and de Carlos, J. A. (2008). Early telencephalic migration topographically converging in the olfactory cortex. *Cereb. Cortex* 18, 1239–1252.

Ghanem, N., Yu, M., Long, J., Hatch, G., Rubenstein, J. L., and Ekker, M. (2007). Distinct cis-regulatory elements from the Dlx1/Dlx2 locus mark different progenitor cell populations in the ganglionic eminences and different subtypes of adult cortical interneurons. *J. Neurosci.* 27, 5012–5022.

Gonzalez-Burgos, G., and Lewis, D. A. (2008). GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. *Schizophr. Bull.* 34, 944–961.

Gorski, J. A., Talley, T., Qiu, M., Puelles, L., Rubenstein, J. L., and Jones, K. R. (2002). Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J. Neurosci.* 22, 6309–6314.

Guillemot, F. (2007). Cell fate specification in the mammalian telencephalon. *Prog. Neurobiol.* 83, 37–52.

Hanganu, I. L., Kilb, W., and Luhmann, H. J. (2002). Functional synaptic projections onto subplate neurons in neonatal rat somatosensory cortex. *J. Neurosci.* 22, 7165–7176.

He, W., Ingraham, C., Rising, L., Goderie, S. and Temple, S. (2001). Multipotent stem cells from the mouse basal forebrain contribute GABAergic neurons and oligodendrocytes to the cerebral cortex during embryogenesis. *J. Neurosci.* 21, 8854–8862.

Hendry, S. H., Schwark, H. D., Jones, E. G., and Yan, J. (1987). Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. *J. Neurosci.* 7, 1503–1519.

Heng, J. I., Moonen, G., and Nguyen, L. (2007). Neurotransmitters regulate cell migration in the telencephalon. *Eur. J. Neurosci.* 26, 537–546.

Horton, S., Meredith, A., Richardson, J. A., and Johnson, J. E. (1999). Correct coordination of neuronal differentiation events in ventral forebrain requires the bHLH factor MASH1. *Mol. Cell. Neurosci.* 14, 355–369.

Houser, C. R. (1999). Neuronal loss and synaptic reorganization in temporal lobe epilepsy. *Adv. Neurol.* 79, 743–761.

Huang, Z. J., Di Cristo, G., and Ango, F. (2007). Development of GABA innervation in the cerebral and cerebellar cortices. *Nat. Rev. Neurosci.* 8, 673–686.

Jakovcevski, I., and Zecevic, N. (2005). Sequence of oligodendrocyte development in the human fetal telencephalon. *Glia* 49, 480–491.

Javaherian, A., and Kriegstein, A. (2009). A Stem Cell Niche for Intermediate Progenitor Cells of the Embryonic Cortex. *Cereb. Cortex* 19, i70–i77.

Jinno S. (2009). Structural organization of long-range GABAergic projection system of the hippocampus. *Front. Neuroanat.* 3, 13. doi: 10.3389/neuro.05.013.2009.

Jinno, S., Klausberger, T., Marton, L. F., Dalezios, Y., Roberts, J. D., Fuentealba, P., Bushong, E. A., Henze, D., Buzsaki, G., and Somogyi, P. (2007). Neuronal diversity in GABAergic long-range projections from the hippocampus. *J. Neurosci.* 27, 8790–8804.

Jones, E. G. (2009). The origins of cortical interneurons: mouse versus monkey and human. *Cereb. Cortex* 19, 1953–1956.

Jovanov-Milosevic, N., Culjat, M., and Kostovic, I. (2009). Growth of the human corpus callosum: modular and laminar morphogenetic zones. *Front. Neuroanat.* 3, 6. doi:10.3389/neuro.05.006.2009.

Judas, M., Rasin, M. R., Kruslin, B., Kostovic, K., Jukic, D., Petanjek, Z., and Kostovic, I. (2003). Dendritic overgrowth and alterations in laminar phenotypes of neocortical neurons in the newborn with semilobar holoprosencephaly. *Brain Dev.* 25, 32–39.

Kanatani, S., Yozu, M., Tabata, H., and Nakajima, K. (2008). COUP-TFII is preferentially expressed in the caudal ganglionic eminence and is involved in the caudal migratory stream. *J. Neurosci.* 28, 13582–13591.

Khazipov, R., Esclapez, M., Caillard, O., Bernard, C., Khalilov, I., Tyzio, R., Hirsch, J., Dzhala, V., Berger, B., and Ben-Ari, Y. (2001). Early development of neuronal activity in the primate hippocampus in utero. *J. Neurosci.* 21, 9770–9781.

Kohwi, M., Petryniak, M. A., Long, J. E., Ekker, M., Obata, K., Yanagawa, Y., Rubenstein, J. L., and Alvarez-Buylla, A. (2007). A subpopulation of olfactory bulb GABAergic interneurons is derived from Emx1- and Dlx5/6-expressing progenitors. *J. Neurosci.* 27, 6878–6891.

Kostovic, I., Jovanov-Milosevic, N., Krsnik, Z., Petanjek, Z., and Judas, M. (2005). Laminar organization of the marginal zone in the human fetal cortex. *Neuroembryol. Aging* 3, 19–26.

Kostovic, I., Jovanov-Milosevic, N., and Petanjek, Z. (2007). Neuronal migration and cortical migratory disorders. *Paediatr. Croat.* 51, 179–190.

Kostovic, I., Judas, M., and Petanjek, Z. (2008). Structural development of the human prefrontal cortex. In Handbook of Developmental Cognitive Neuroscience, C. A. Nelson, and M. Luciana, eds (Cambridge, MIT Press), pp. 213–235.

Kostovic, I., and Rakic, P. (1990). Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J. Comp. Neurol.* 297, 441–470.

Kostovic, I., and Vasung, L. (2009). Insights from *in vitro* fetal magnetic resonance imaging of cerebral development. *Semin. Perinatol.* 33, 220–233.

Kriegstein, A., Noctor, S., and Martinez-Cerdeno, V. (2006). Patterns of neural stem and progenitor cell division may underlie evolutionary cortical expansion. *Nat. Rev. Neurosci.* 7, 883–890.

Krnjevic, K., and Schwartz, S. (1967). The action of gamma-aminobutyric acid on cortical neurones. *Exp. Brain Res.* 3, 320–336.

Letinic, K., Zoncu, R., and Rakic, P. (2002). Origin of GABAergic neurons in the human neocortex. *Nature* 417, 645–649.

Leviton, A., and Gressens, P. (2007). Neuronal damage accompanies perinatal white-matter damage. *Trends Neurosci.* 30, 473–478.

Levitt, P., Eagleson, K. L., and Powell, E. M. (2004). Regulation of neocortical interneuron development and the implications for neurodevelopmental disorders. *Trends Neurosci.* 27, 400–406.

Lewis, D. A., Hashimoto, T., and Volk, D. W. (2005). Cortical inhibitory neurons and schizophrenia. *Nat. Rev. Neurosci.* 6, 312–324.

Liodis, P., Denaxa, M., Grigoriou, M., Akufo-Addo, C., Yanagawa, Y., and Pachnis, V. (2007). Lhx6 activity is required for the normal migration and specification of cortical interneuron subtypes. *J. Neurosci.* 27, 3078–3089.

Long, J. E., Cobos, I., Potter, G. B., and Rubenstein, J. L. (2009). Dlx1 and Mash1 transcription factors control MGE and CGE patterning and differentiation through parallel and overlapping pathways. *Cereb. Cortex* 19, i96–i106.

Maldonado-Aviles, J. G., Curley, A. A., Hashimoto, T., Morrow, A. L., Ramsey, A. J., O'Donnell, P., Volk, D. W., and Lewis, D. A. (2009). Altered markers of tonic inhibition in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Am. J. Psychiatry* 166, 450–459.

Melchitzky, D. S., and Lewis, D. A. (2008). Dendritic-targeting GABA neurons in monkey prefrontal cortex: comparison of somatostatin- and calretinin-immunoreactive axon terminals. *Synapse* 62, 456–465.

Meredith, A., and Johnson, J. E. (2000). Negative autoregulation of Mash1 expression in CNS development. *Dev. Biol.* 222, 336–346.

Metin, C., Alvarez, C., Moudoux, D., Vitalis, T., Pieau, C., and Molnar, Z. (2007). Conserved pattern of tangential neuronal migration during forebrain development. *Development* 134, 2815–2827.

Metin, C., Baudoin, J. P., Rakic, S., and Parnavelas, J. G. (2006). Cell and molecular mechanisms involved in the migration of cortical interneurons. *Eur. J. Neurosci.* 23, 894–900.

Metin, C., Vallee, R. B., Rakic, P., and Bhide, P. G. (2008). Modes and mishaps of neuronal migration in the mammalian brain. *J. Neurosci.* 28, 11746–11752.

Meyer, G. (2007). Genetic control of neuronal migrations in human cortical development. *Adv. Anat. Embryol. Cell Biol.* 189, 1–111.

Meyer, G., Schaaps, J. P., Moreau, L., and Goffinet, A. M. (2000). Embryonic and early fetal development of the human neocortex. *J. Neurosci.* 20, 1858–1868.

Molnar, Z., Metin, C., Stoykova, A., Tarabykin, V., Price, D. J., Francis, F., Meyer, G., Dehay, C., and Kennedy, H. (2006). Comparative aspects of cerebral cortical development. *Eur. J. Neurosci.* 23, 921–934.

Molyneaux, B. J., Arlotta, P., Menezes, J. R., and Macklis, J. D. (2007). Neuronal subtype specification in the cerebral cortex. *Nat. Rev. Neurosci.* 8, 427–437.

Moore, A. R., Filipovic, R., Mo, Z., Rasband, M. N., Zecevic, N., and Antic, S. D. (2009). Electrical excitability of early neurons in the human cerebral cortex during the second trimester of gestation. *Cereb. Cortex* 19, 1795–1805.

Nery, S., Fishell, G., and Corbin, J. G. (2002). The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. *Nat. Neurosci.* 5, 1279–1287.

Nobrega-Pereira, S., Kessaris, N., Du, T., Kimura, S., Anderson, S. A., and Marin, O. (2008). Postmitotic Nkx2-1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. *Neuron* 59, 733–745.

Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S., and Kriegstein, A. R. (2001). Neurons derived from radial glial cells establish radial units in neocortex. *Nature* 409, 714–720.

Noctor, S. C., Martinez-Cerdeno, V., and Kriegstein, A. R. (2007). Contribution of intermediate progenitor cells to cortical histogenesis. *Arch. Neurol.* 64, 639–642.

Noctor, S. C., Martinez-Cerdeno, V., and Kriegstein, A. R. (2008). Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. *J. Comp. Neurol.* 508, 28–44.

O'Rahilly, R., and Müller, F. (2006). The Embryonic Human Brain: An Atlas of Developmental Stages. Hoboken, NJ, Willey-Liss.

Parras, C. M., Hunt, C., Sugimori, M., Nakafuku, M., Rowitch, D., and Guillemot, F. (2007). The proneural gene Mash1 specifies an early population of telencephalic oligodendrocytes. *J. Neurosci.* 27, 4233–4242.

Petanjek, Z., Berger, B., and Esclapez, M. (2009). Origins of cortical GABAergic neurons in the cynomolgus monkey. *Cereb. Cortex* 19, 249–262.

Petanjek, Z., Dujmovic, A., Kostovic, I., and Esclapez, M. (2008). Distinct origin of GABA-ergic neurons in forebrain of man, nonhuman primates and lower mammals. *Coll Antropol* 32(Suppl. 1), 9–17.

Pierani, A., and Wassef, M. (2009). Cerebral cortex development: From progenitors patterning to neocortical size during evolution. *Dev. Growth Differ.* 51, 325–342.

Pleasure, S. J., Anderson, S., Hevner, R., Bagri, A., Marin, O., Lowenstein, D. H., and Rubenstein, J. L. (2000). Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. *Neuron* 28, 727–740.

Poluch, S., Jablonska, B., and Juliano, S. L. (2008). Alteration of interneuron migration in a ferret model of cortical dysplasia. *Cereb. Cortex* 18, 78–92.

Povysheva, N. V., Zaitsev, A. V., Rotaru, D. C., Gonzalez-Burgos, G., Lewis, D. A., and Krimer, L. S. (2008). Parvalbumin-positive basket interneurons in monkey and rat prefrontal cortex. *J. Neurophysiol.* 100, 2348–2360.

Powell, E. M., Campbell, D. B., Stanwood, G. D., Davis, C., Noebels, J. L., and Levitt, P. (2003). Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction. *J. Neurosci.* 23, 622–631.

Rakic, P. (1971). Guidance of neurons migrating to the fetal monkey neocortex. *Brain Res.* 33, 471–476.

Rakic, P. (2006). A century of progress in corticoneurogenesis: from silver impregnation to genetic engineering. *Cereb. Cortex* 16(Suppl. 1), i3–i17.

Rakic, P. (2009). Evolution of the neocortex: a perspective from developmental biology. *Nat. Rev. Neurosci.* 10, 724–735.

Rakic, P., Ayoub, A. E., Breunig, J. J., and Dominguez, M. H. (2009). Decision by division: making cortical maps. *Trends Neurosci.* 32, 291–301.

Rakic, S., and Zecevic, N. (2003). Emerging complexity of layer I in human cerebral cortex. *Cereb. Cortex* 13, 1072–1083.

Ribak, C. E., Seress, L., Peterson, G. M., Seroogy, K. B., Fallon, J. H., and Schmued, L. C. (1986). A GABAergic inhibitory component within the hippocampal commissural pathway. *J. Neurosci.* 6, 3492–3498.

Sidman, R. L., and Rakic, P. (1973). Neuronal migration, with special reference to developing human brain: a review. *Brain Res.* 62, 1–35.

Skaggs, W. E., McNaughton, B. L., Permenter, M., Archibeque, M., Vogt, J., Amaral, D. G., and Barnes, C. A. (2007). EEG sharp waves and sparse ensemble unit activity in the macaque hippocampus. *J. Neurophysiol.* 98, 898–910.

Smart, I. H., Dehay, C., Giroud, P., Berland, M., and Kennedy, H. (2002). Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb. Cortex* 12, 37–53.

Somogyi, P., and Klausberger, T. (2005). Defined types of cortical interneurone structure space and spike timing in the hippocampus. *J. Physiol.* 562, 9–26.

Sousa, V. H., Miyoshi, G., Hjerling-Leffler, J., Karayannis, T., and Fishell, G. (2009). Characterization of Nkx6-2-derived neocortical interneuron lineages. *Cereb. Cortex* 19, i1–i10.

Spruston, N. (2008). Pyramidal neurons: dendritic structure and synaptic integration. *Nat. Rev. Neurosci.* 9, 206–221.

Stensaas, L. J. (1968). The development of hippocampal and dorsolateral pallial regions of the cerebral hemisphere in fetal rabbits. *J. Comp. Neurol.* 132, 93–108.

Sussel, L., Marin, O., Kimura, S., and Rubenstein, J. L. (1999). Loss of Nk × 2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* 126, 3359–3370.

Tabata, H., Kanatani, S., and Nakajima, K. (2009). Differences of migratory behavior between direct progeny of apical progenitors and basal progenitors in the developing cerebral cortex. *Cereb. Cortex* 19, 2092–2105.

Tan, S. S., Kalloniatis, M., Sturm, K., Tam, P. P., Reese, B. E., and Faulkner-Jones, B. (1998). Separate progenitors for radial and tangential cell dispersion during development of the cerebral neocortex. *Neuron* 21, 295–304.

Tomioka, R., and Rockland, K. S. (2007). Long-distance corticocortical GABAergic neurons in the adult monkey white and gray matter. *J. Comp. Neurol.* 505, 526–538.

Toth, K., and Freund, T. F. (1992). Calbindin D28k-containing nonpyramidal cells in the rat hippocampus: their immunoreactivity for GABA and projection to the medial septum. *Neuroscience* 49, 793–805.

Van Eden, C. G., Mrzljak, L., Voorn, P., and Uylings, H. B. (1989). Prenatal development of GABA-ergic neurons in the neocortex of the rat. *J. Comp. Neurol.* 289, 213–227.

Wonders, C., and Anderson, S. (2005). Beyond migration: Dlx1 regulates interneuron differentiation. *Nat. Neurosci.* 8, 979–981.

Wonders, C. P., and Anderson, S. A. (2006). The origin and specification of cortical interneurons. *Nat. Rev. Neurosci.* 7, 687–696.

Wonders, C. P., Taylor, L., Welagen, J., Mbata, I. C., Xiang, J. Z., and Anderson, S. A. (2008). A spatial bias for the origins of interneuron subgroups within the medial ganglionic eminence. *Dev. Biol.* 314, 127–136.

Xu, Q., Cobos, I., De La Cruz, E., Rubenstein, J. L., and Anderson, S. A. (2004). Origins of cortical interneuron subtypes. *J. Neurosci.* 24, 2612–2622.

Yanez, I. B., Munoz, A., Contreras, J., Gonzalez, J., Rodriguez-Veiga, E., and DeFelipe, J. (2005). Double bouquet cell in the human cerebral cortex and a comparison with other mammals. *J. Comp. Neurol.* 486, 344–360.

Zaitsev, A. V., Povysheva, N. V., Gonzalez-Burgos, G., Rotaru, D., Fish, K. N., Krimer, L. S., and Lewis, D. A. (2009). Interneuron diversity in layers 2–3 of monkey prefrontal cortex. *Cereb. Cortex* 19, 1597–1615.

Zecevic, N., Chen, Y., and Filipovic, R. (2005). Contributions of cortical subventricular zone to the development of the human cerebral cortex. *J. Comp. Neurol.* 491, 109–122.

Zecevic, N., and Milosevic, A. (1997). Initial development of gamma-aminobutyric acid immunoreactivity in the human cerebral cortex. *J. Comp. Neurol.* 380, 495–506.

Zhao, Y., Flandin, P., Long, J. E., Cuesta, M. D., Westphal, H., and Rubenstein, J. L. (2008). Distinct molecular pathways for development of telencephalic interneuron subtypes revealed through analysis of Lhx6 mutants. *J. Comp. Neurol.* 510, 79–99.

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Two separate subtypes of early non-subplate projection neurons in the developing cerebral cortex of rodents

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The preplate of the cerebral cortex contains projection neurons that connect the cortical primordium with the subpallium. These are collectively named pioneer neurons. After preplate partition, most of these pioneer neurons become subplate neurons. Certain preplate neurons, however, never associate with the subplate but rather with the marginal zone. In the present overview, we propose a novel classification of non-subplate pioneer neurons in rodents into two subtypes. In rats, the neurons of the first subtype are calbindin$^+$ (CB), calretinin$^+$ (CR) and L1$^+$ and are situated in the upper part of the preplate before its partition. Neurons of the second subtype are TAG-1$^+$ and are located slightly deeper to the previous population in the preplate. After the preplate partition, the CB$^+$, CR$^+$ and L1$^+$ neurons remain in the marginal zone whereas TAG-1$^+$ neurons become transiently localized in the upper cortical plate. In mice, by contrast, calcium binding proteins did not label pioneer neurons. We define in mice two subtypes of non-subplate pioneer neurons, either L1$^+$ or TAG-1$^+$/cntn2$^+$. We propose these to be the homologues of the two subtypes of non-subplate pioneer neurons of rats. The anatomical distribution of these neuron populations is similar in rats and mice. The two populations of non-subplate pioneer neurons differ in their axonal projections. Axons of L1$^+$ pioneer neurons project to the ganglionic eminences and the anterior preoptic area, but avoid entering the posterior limb of the internal capsule towards the thalamus. Axons of TAG-1$^+$ pioneer neurons project to the lateral parts of the ganglionic eminences at the early stages of cortical histogenesis examined.

Keywords: preplate, marginal zone, pioneer neurons, calcium binding proteins, TAG1/cntn2, L1, rat, mouse

INTRODUCTION

At the onset of corticogenesis, early-differentiated neurons form a primitive neuropil, the "primordial plexiform layer" (Marín-Padilla, 1971), now commonly known as the preplate. Thereafter, the cortical plate develops within the preplate, so that preplate neurons become redistributed between the subplate and the marginal zone. The preplate and its derivatives contain projection neurons, collectively named pioneer neurons, whose axonal arborizations establish the earliest corticofugal projection systems during cortical development. These neurons have traditionally been associated with the subplate. However, certain preplate neurons never associate with the subplate but instead with the marginal zone.

Previous studies of this laboratory have described in rats (Meyer et al., 1998, 1999; Soria et al., 1999; Soria and Fairén, 2000; López-Bendito et al., 2002a,b) and mice (Morante-Oria et al., 2003) both neurochemical and functional features of these projection neurons of the marginal zone. A clear unifying picture has nevertheless not yet emerged, in part because interspecies differences have frustrated any attempt at convincingly classifying these neurons. Therefore, we decided to systematically survey early corticogenesis in rats and mice to further characterize such pioneer neurons. In particular, proteins that are strongly expressed in these neurons facilitated populational analyses of corticofugal axon projections by immunohistochemistry, as an alternative approach to axonal tract-tracing methodologies.

These distinguishing marker proteins included the calcium binding proteins calbindin (CB) and calretinin (CR) in rats (Meyer et al., 1998; Soria and Fairén, 2000). Although CR decorates pioneer cells in the human preplate (Meyer et al., 2000), surprisingly, none of these two calcium binding proteins label early projecting neurons in mice (our own unpublished data). Thus, we searched for alternative immunohistochemical markers to try to identify the homologues in mice of the calcium binding protein-expressing pioneer neurons of rats. We used antibodies to the neural cell adhesion and recognition molecules L1 and TAG-1/cntn2/axonin1, found previously to label pioneer cells in mice (Morante-Oria et al., 2003). Differences in the expression of these molecules on the cell surfaces helped us to define two major groups of early projection neurons common to rats and mice, one expressing L1 and the second expressing TAG-1/cntn2. On this basis, we analyzed the distribution of these two classes of pioneer neurons in the cortical primordium and the possible existence of differential patterns of corticofugal axonal projections.

The axons of L1$^+$ neurons project massively to the ganglionic eminences and the anterior preoptic area, but avoid entering the posterior limb of the internal capsule that leads to the dorsal thalamus. The axons of TAG-1$^+$ neurons remain restricted to the lateral regions of the basal forebrain at the early stages of cortical histogenesis. Additionally, we analyzed the anatomical relationships of these axonal projections with the migrating GABAergic interneurons. By this, we intended to investigate if axonophilic guidance could

be a possible mechanism for interneuron migration. In preparing the present overview, we have hypothesized that the populations of marginal zone pioneer neurons could be more common across mammals that hitherto accepted. We have essayed to build up a framework for future rigorous studies of the preplate and marginal zone of mammals, including primates and humans. Understanding the bases of the neuronal organization of the early cortical neuropil is of importance for understanding the possible conservation in mammalian evolution of certain neuronal populations found in early non-human fetuses; for example, the so-called pioneer plate (Meyer et al., 2000) and the predecessor cells (Bystron et al., 2006). We thus (1) introduce here the concept of an unexpected diversity among non-subplate projection neurons and (2) highlight the importance of markers that can be used in a rigorous comparison across species.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Pregnant rats and mice were maintained at the animal facilities of the Servicio de Experimentación Animal, Universidad Miguel Hernández. We used Wistar rats, wild type C57Bl6/J mice, heterozygous GAD67-GFP knock-in mice and heterozygous cntn2-EGFP (GENSAT) transgenic mice. GAD67-GFP mice were obtained by targeting the cDNA encoding EGFP to the GAD67 locus by homologous recombination. These animals show fluorescent labeling of GABAergic migratory interneurons (Tamamaki et al., 2003). cntn2-EGFP (GENSAT) mice contain multiple copies of a modified BAC in which EGFP reporter gene was inserted immediately upstream of the coding sequence of the *cntn2* gene (Gong et al., 2003). Animals were kept on a 12-h light/dark cycle with constant ambient temperature (21 ± 1°C) and humidity (55 ± 9%). Food and water were available *ad libitum*. Experiments were carried out in accordance with the guidelines of the European Union (2003/65/CE) and Spanish regulations (BOE 252/34367-91, 2005) for the use of laboratory animals and were approved by the Comité Ético de Experimentación y Bienestar Animal, Universidad Miguel Hernández.

IMMUNOHISTOCHEMISTRY

Midday of the vaginal plug or sperm positivity was considered as embryonic day 0.5 (E0.5). Timed-pregnant dams were anaesthetized by i.p. injection of ketamine (Imalgène 1000, 100 mg/ml, 110 mg/kg) and xylazine (Xilagesic 2%, 10 mg/kg) and the embryos were extracted by caesarean section. We used brains of E13.5 ($n = 14$) and E15.5 ($n = 14$) Wistar rats; E12.5 ($n = 15$) and E13.5 ($n = 15$) wild type C57Bl6/J mice; E12.5 ($n = 15$) and E13.5 ($n = 15$) GAD67-GFP knock-in mice and E12.5 ($n = 10$) and E13.5 ($n = 10$) cntn2-EGFP transgenic mice. Embryos were fixed in 4% paraformaldehyde in PBS. One hundred-µm-thick vibratome sections or twelve-µm-thick cryostat sections were obtained. Immunohistochemistry protocols were as detailed in Morante-Oria et al. (2003). Primary antibodies included rabbit polyclonal antibodies to CB (Swant, 1:2000), CR (Swant, 1:2000), Tbr-1 (gift from R. Hevner, 1:1000), TAG-1 (gift K. Takeuchi, Nagoya, Japan, 1:1,000) and L1 (gift from F. G. Rathjen, 1:1000); chicken polyclonal antibody to GFP (Aves Labs, 1:4000); mouse monoclonal antibodies 12E3 to PSA-NCAM (gift from T. Seki, 1:1,000), and 4D7 to TAG-1/cntn2 (DSHB, Dodd et al., 1988, 1:40). Secondary antibodies were conjugated to Alexa Fluor 488, 546 or 647 (Molecular Probes). Zenon rabbit IgG labeling kits (Molecular Probes) were used for immunostaining with multiple rabbit primary antibodies as per manufacturer's instructions.

CONFOCAL MICROSCOPY AND IMAGE MANIPULATION

Imaging was performed with a Leica TCS SL confocal microscope. Plates were prepared using Adobe Photoshop CS2, and mosaic reconstructions were done using the Photomerge tool of this software package.

RESULTS

CALCIUM BINDING PROTEINS CALBINDIN AND CALRETININ CO-LABEL IN RATS A SINGLE POPULATION OF PREPLATE PIONEER NEURONS THAT END UP IN THE MARGINAL ZONE

Non-subplate pioneer neurons are early generated, long projecting neurons of the cortical primordium that do not associate with the subplate at any stage of development. In previous studies, Meyer et al. (1998) and Soria and Fairén (2000) described such neurons in rat embryos, as forming two separate cell populations characterized by the expression of either one of the calcium binding proteins CB or CR. We decided to reassess this issue using the enhanced sensitivity and selectivity afforded by confocal microscopy. Here, we present evidence suggesting that projecting neurons associated with the marginal zone co-express the two calcium binding proteins and thus form a unique cell population.

In the E15.5 rat we identified densely packed CR^+ subpial cells (**Figure 1**; arrowheads in **Figure 1D**) that most likely were Cajal-Retzius cells (Weisenhorn et al., 1994; Meyer et al., 1998). More deeply in the preplate was a compact layer of CB^+ and CR^+ cells. Within this band, the most superficial cells in the upper preplate co-expressed both labels, and the deepest ones (**Figure 1D**) only expressed CB. When the preplate was split at the lateral sectors of the neocortex primordium by the arrival of the first cortical plate neurons, CB^+/CR^+ neurons separated as in the opening of a zipper (**Figures 1C,E and 3B′,C′**). The lateralmost edge of the cortex showed that the preplate has already divided into marginal zone and subplate; this lateral extreme of the neocortex primordium contained far more CB^+/CR^+ cells than the medial regions (**Figures 1A–C**). CB^+/CR^+ cells accumulated in the marginal zone, whereas most cells expressing CB only ("CB^+-only cells") accumulated in the subplate (**Figures 1C,E**) and to a lesser degree in the marginal zone (**Figure 1E**).

In summary, CB^+/CR^+ cells in rats were apparent as a unique cell population located first in the upper preplate and thereafter in the marginal zone (**Table 1**). In the preplate, another population was made up of CB^+ cells interspersed among the CB^+/CR^+ cells. Most of these CB^+-only cells ended up in the subplate. The radial distribution of the cells could better be represented by a semiquantitative analysis. At levels similar to that shown in **Figure 1D**, 32% of a total of 118 cells were CR^+ cells and were located within a range of 0–20 µm from the pia; 33% were CB^+/CR^+ cells and were located at 10–40 µm from the pia, and 35% were CB^+-only cells, located at 20–60 µm from the pia. After the partition of the preplate, at a level similar to that of **Figure 1E**, 31% of a total of 87 cells were CR^+ cells located at 0–20 µm from the pia, 38% were CB^+/CR^+ cells located at 10–50 µm from the pia, and 31% were CB^+-only cells located

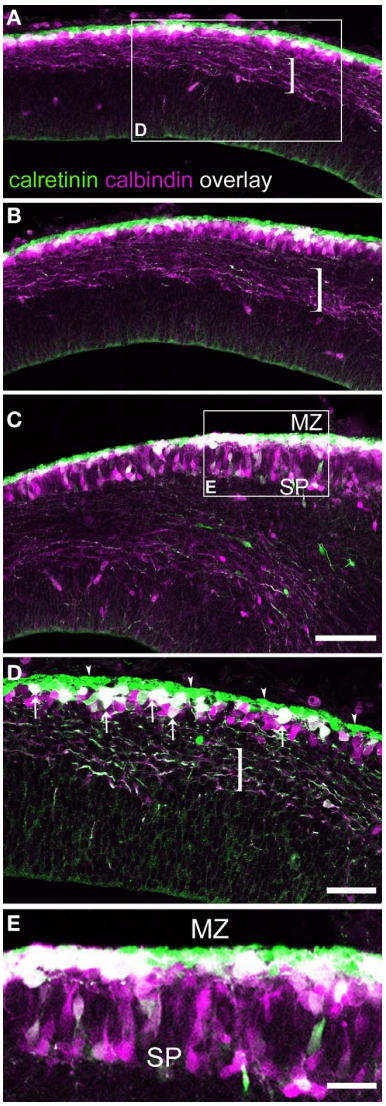

FIGURE 1 | Calcium binding protein expression in the preplate and its derivatives. E15.5 rat. **(A–C)** are views of single confocal sections of the preplate taken from medial to lateral, displaying a well known latero-medial developmental gradient. **(C)** shows the partition of the preplate. Brackets indicate pioneer axons emitted by pioneer neurons in the preplate. The majority of these axons co-expressed CB and CR. **(D)** From the boxed area in **(A):** arrowheads indicate a layer of densely packed CR^+ cells, interpreted as Cajal-Retzius cells. Co-expression of CB and CR appeared in white. Arrows point to double labeled, CB^+/CR^+ cells. Cells expressing CB only interspersed among CB^+/CR^+ cells. **(E)** From the boxed area in **(C):** when the preplate became partitioned into marginal zone and subplate, co-expression of CB and CR was more common in the marginal zone than in the subplate. MZ, marginal zone; SP, subplate. Calibration bars: **(A–C)** 100 μm; **(D)** 50 μm; **(E)** 30 μm.

at 10–110 μm from the pia. Note this range in radial distribution remained unchanged for CR^+ Cajal-Retzius cells and for CB^+/CR^+ cells, while that of CB^+-only cells reflected their massive translocation from the preplate to the subplate.

In mice, CB is a marker for migrating GABA interneurons at the earliest stages of cortical histogenesis (Anderson et al., 1997). We found that in rats as well, CB and CR decorated putative migrating interneurons (**Figures 3 and 8**). We verified that CB did not label projection, pioneer neurons in the mouse preplate but, contrary to the findings of García-Moreno et al. (2007), we observed CB expression in early migrating GAD67-GFP^+ interneurons that arrived to the preplate before its partition (**Figure 2**).

THE AXONAL PROJECTION OF CALCIUM BINDING PROTEIN-EXPRESSING PIONEER NEURONS IN RATS AVOID THE POSTERIOR LIMB OF THE INTERNAL CAPSULE

In our material, Z-stacks of confocal images showed that the corticofugal projection from calcium binding protein-expressing pioneer cells was more extensive than hitherto suspected (Liu and Graybiel, 1992; Meyer et al., 1998; see Figure 6 in Meyer et al., 1999). We show in **Figure 3A** a compact yet widely distributed CB^+ axonal bundle that entered the medial ganglionic eminence and the preoptic area. Significantly, these axons avoided the posterior limb of the internal capsule at the ages analyzed. Thus, we can conclude there is no projection to the dorsal thalamus. The bulk of CB^+ axons originated from cells at medial positions in the cortex, i.e. from cells in the undivided preplate (**Figures 3A,B,B′**); this was the same for CR^+ axons (**Figures 3C,C′**). The descending axonal bundle widened progressively as axons were recruited from medial to lateral regions (**Figure 3A**). Additionally, axons were seen to descend from cells in the lateral areas of neocortex primordium where the MZ and the subplate had already separated (**Figures 3B,C**).

ADHESION MOLECULES TAG-1 (cntn2) AND L1 IDENTIFIED TWO DIFFERENT POPULATIONS OF PIONEER NEURONS COMMON TO MICE AND RATS

The expression of two proteins belonging to the immunoglobulin superfamily of neural cell adhesion and recognition molecules was crucial to our effort to classify pioneer neurons in rodents. TAG-1 (transient axonal glycoprotein-1) belongs to the contactin family; in mice, it is known as contactin 2 (cntn2). TAG-1 is linked to the cell membrane by glycophosphatidyl inositol links. L1 belongs to the L1-CAM family and is a transmembrane protein.

We previously reported in mice the presence of pioneer neurons in the preplate that express TAG-1 and L1 (Morante-Oria et al., 2003). In particular, $TAG\text{-}1^+$ cell bodies formed a conspicuous population in the mouse preplate at E12.5 (**Figures 4A,A′**) that was continuous with similar cells in a territory located near the pallial-subpallial boundary, that corresponds to the early mantle of the lateral and ventral pallium, LP/VP (**Figure 4A″**; see also, Wolfer et al., 1994; Puelles et al., 2000; Denaxa et al., 2001; Morante-Oria et al., 2003). In the E13.5 mouse (**Figure 4B**), $TAG\text{-}1^+$ axons were more abundant than at previous stages. $TAG\text{-}1^+$ cells distributed medially within the preplate and laterally within the upper part of the cortical plate (**Figures 4B,B′** and **Table 1**; see Wolfer et al., 1994; Morante-Oria et al., 2003). $TAG\text{-}1^+$ axons traversed vertically the cortical plate (**Figures 4B,B′**) to join the main axonal bundle originating in the preplate at more medial regions. In E15.5 rats, the distribution of $TAG\text{-}1^+$ cells and their descending axons was similar (**Figures 5A,A″**). In rats, the descending projections from $TAG\text{-}1^+$ neurons partially overlapped with those of CB^+/CR^+ cells of the preplate and the MZ (**Figures 3 and 5A**). $TAG\text{-}1^+$ axons were located more laterally at the base of the neocortex, mainly within

Table 1 | The two basic subtypes of non-subplate pioneer neurons in mice and rats.

Cell type	Rodent species	Markers	Localization	
			Preplate stage	After preplate partition
Non-SP pioneer neurons: *L1 subtype*	Rat	CB and CR L1[1] Tbr1 No Reelin	Preplate	MZ
	Mouse	No CB, no CR L1[1] Tbr1 No Reelin	Preplate	MZ
Non-SP pioneer neurons: *TAG-1/cntn2 subtype*	Rat, mouse	TAG-1/cntn2 Tbr1 No Reelin	Preplate	Upper CP[2]

This Table summarizes the markers that were useful for defining two different types of non-subplate pioneer cells in rodents, based on the results shown in this study. CP, cortical plate; MZ, marginal zone; SP, subplate.

[1]L1 protein accumulated transiently in neuron perikarya, and then it translocated into the projecting axons.

[2]This is one important exception: pioneer TAG-1$^+$ cells were located first in the preplate and thereafter in the upper tier of the cortical plate and in the subplate (Wolfer et al., 1994; Morante-Oria et al., 2003; our present results).

the LP/VP mantle. The CB$^+$/CR$^+$ descending axons were more widespread in the MGE and reached more distant places in the MGE than the TAG-1$^+$ axons (**Figure 5A**).

Detailed pictures of the relationships between CB$^+$/CR$^+$ and TAG-1$^+$ cells in the preplate are shown in **Figures 5C–G**. As discussed before, the different types of calcium binding protein-expressing neurons in rats occupied typical radial positions within the cortical primordium. Thus, although CR immunoreactivity is not shown in this Figure, we consider that the CB$^+$ cells located close to the pia might belong to the CB$^+$/CR$^+$ population in the MZ, and the deepest ones to the CB$^+$-only cell population in the subplate. The images show that TAG-1$^+$ and CB$^+$ cells did not mutually overlap (**Figure 5G**, arrows) and, additionally, that TAG-1$^+$ cells are an important component of the subplate.

L1$^+$ cell bodies were conspicuous in mice until E12.5. At this stage, L1$^+$ cell bodies occupied all the extension of the LP/VP mantle (**Figure 4C′**) and the preplate (**Figure 4C″**). At E13.5, the protein had already translocated into axons (**Figures 4C,D,D′**). L1$^+$ axons formed a massive projection from the preplate towards the basal telencephalon both in mice (**Figures 4C,D**) and in rats (**Figure 5B**). In rats, the corticofugal L1$^+$ axons overlapped with the CB$^+$/CR$^+$ or CB$^+$-only axons in the ventral forebrain (**Figure 5B**). Different from the CB$^+$ or CR$^+$ axons, L1$^+$ axons also extended laterally into the LP/VP (**Figures 4 and 9**). L1 also is expressed by the growing thalamocortical axons (Fukuda et al., 1997), but these axons had not yet reached the ventral forebrain at the ages considered here. Thalamocortical L1$^+$ axons in the posterior limb of the internal capsule are shown in **Figure 5B**.

Given the different tissue distribution of TAG-1 and L1 immunoreactivities, we hypothesized that the expression of these two molecules might define two different subtypes of non-subplate pioneer neurons in the two rodent species analyzed here (**Table 1**). To analyze this possibility further, we compared the distribution of L1$^+$ and TAG-1$^+$ cells in the cortical primordium using cntn2-EGFP mice from GENSAT (**Figure 6**). We found that L1$^+$ cells occupied complementary locations to those of cntn2-EGFP$^+$ cells in the MZ and in the subplate. Initially, L1$^+$ cells were located in the preplate, below isolated cntn2-EGFP$^+$ cells residing subpially (**Figures 6A–E**, arrowheads). These subpial cntn2-EGFP$^+$ cells co-expressed Reelin (not shown; see also, Morante-Oria et al., 2003). They can thus be considered as candidate Cajal-Retzius cells, although recent microarray studies have not detected expression of the *cntn2* gene in Cajal-Retzius cells (Yamazaki et al., 2004). Leaving aside these cntn2-EGFP$^+$/Reelin$^+$ cells, other cntn2-EGFP$^+$ cells were deeper in the preplate, at the midway level in the dorsal cortex (**Figure 6B**). More laterally (**Figure 6C**), the cortical plate was defined by an accumulation of cntn2-EGFP$^+$ cells in its upper tier (**Figure 6C**, asterisks). At this level, L1$^+$ cells were located in the subplate (**Figure 6C**, open arrows) together with isolated TAG-1$^+$ neurons (arrows). Finally, shown in **Figure 6D**, a palisade of cntn2-EGFP$^+$ pioneer neurons was seen in the upper cortical plate (asterisks). At this level, some cntn2-EGFP$^+$ cells were in the subplate (**Figure 6D**, arrows), as shown also in **Figure 5G** for rat TAG-1$^+$ cells. It is our interpretation that the cntn2-EGFP$^+$ cells in the cortical plate correspond to the images shown in **Figure 4B′** of TAG-1$^+$ cells, which are in an identical anatomical localization. In further support of this, we show in **Figure 6E** that the cntn2-EGFP$^+$ cells in the cortical plate were surrounded by TAG-1 immunoreactivity, as would be expected from the extracellular disposition of TAG-1 molecules. Thus, these images must correspond to the cell profiles detected with TAG-1 antibodies in the same location in mice (see Figures 2E,F in Morante-Oria et al., 2003, and our **Figures 4B,B′**) and rats (**Figure 5A′**).

Finally, we tried to find a plausible correspondence between the pioneer cells expressing neural cell adhesion molecules in rats and mice, and those expressing calcium binding proteins in rats. The data reported so far indicated that TAG-1 would not likely be a candidate marker, so we turned our attention towards L1 as a

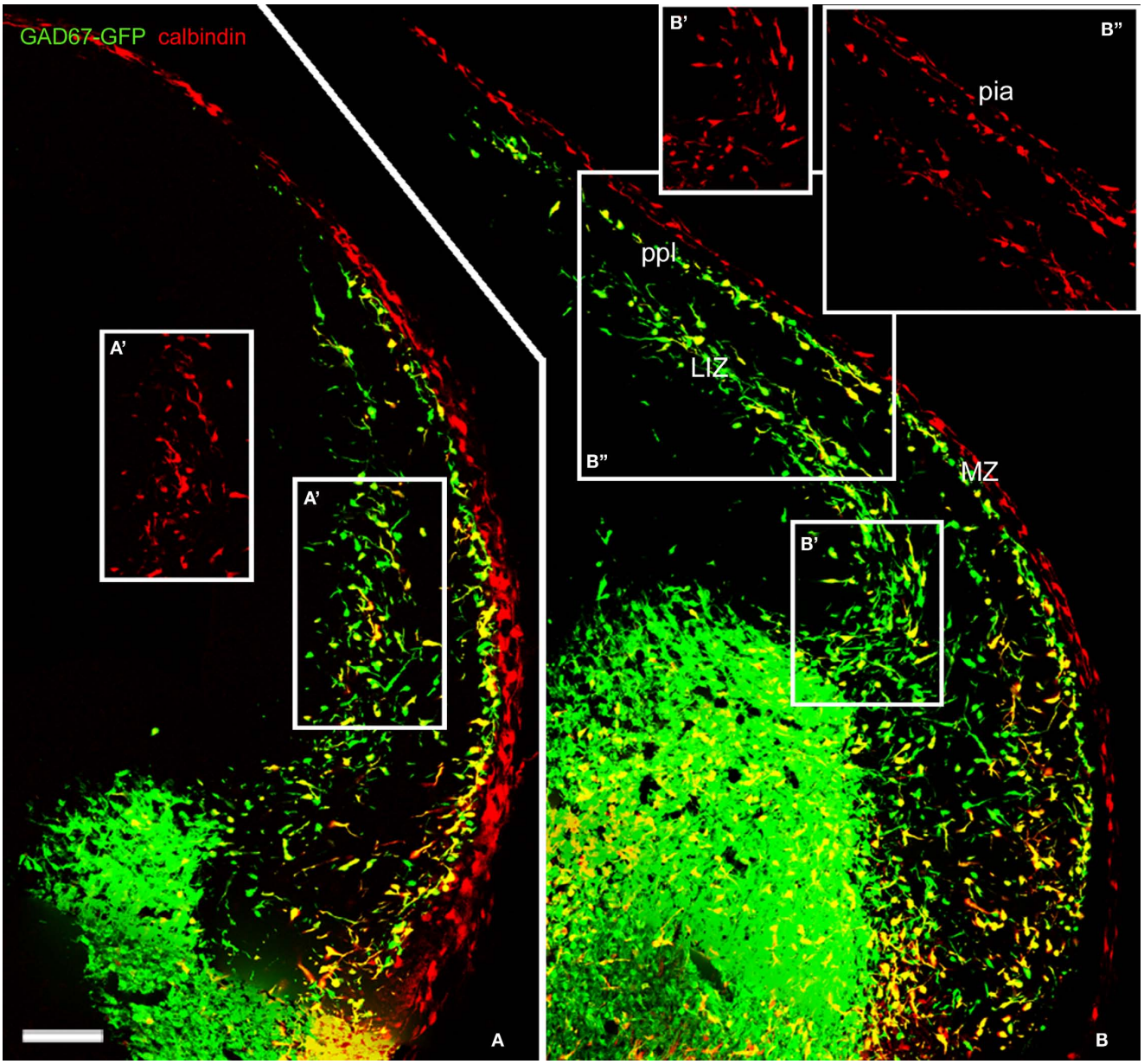

FIGURE 2 | Calbindin labeled migratory GABAergic interneurons in the E13.5 mouse preplate. Migratory interneurons, labeled by GAD67 promoter-driven GFP expression, were seen to leave the ventral forebrain in their way to the pallium, following well-defined migratory pathways at rostral **(A)** and more caudal levels **(B)**. CB immunoreactivity co-localized with EGFP in only part of the GAD67-GFP interneurons. Boxes represent CB^+ cells for comparison, at the lower intermediate zone **(A′,B′,B″)** and in the preplate **(B″)**. CB did not label pioneer neurons in mice. Note CB immunostaining at the pia (see Meyer et al., 1998). LIZ, lower intermediate zone; MZ, marginal zone. ppl, primordial plexiform zone or preplate. Photomontages of single confocal sections. Calibration bar: 100 μm.

possible complementary marker of CB^+/CR^+ cells in rats. In double immunostaining experiments, we observed that the superficial CB^+ neurons of the preplate co-expressed L1 (**Figure 7**). Given their anatomical location in the upper preplate, these CB^+ cells most likely correspond to the CB^+/CR^+ pioneer neurons described above. The finding that L1 labeled in rats a corticofugal axonal projection virtually identical to the projection of CB^+/CR^+ axons (**Figure 5B**) further reinforced the conclusion that L1 was an additional marker of CB^+/CR^+ cells in rats. Therefore, we postulate L1 as a marker of a subtype of non-subplate pioneer neurons common to rats and mice. We have summarized these data in **Table 1**.

PIONEER NEURONS EXPRESS Tbr1

The T-box transcription factor Tbr1 is expressed early after the differentiation of cortical progenitors, and is functionally important for corticogenesis. Tbr1 is expressed by Cajal-Retzius cells,

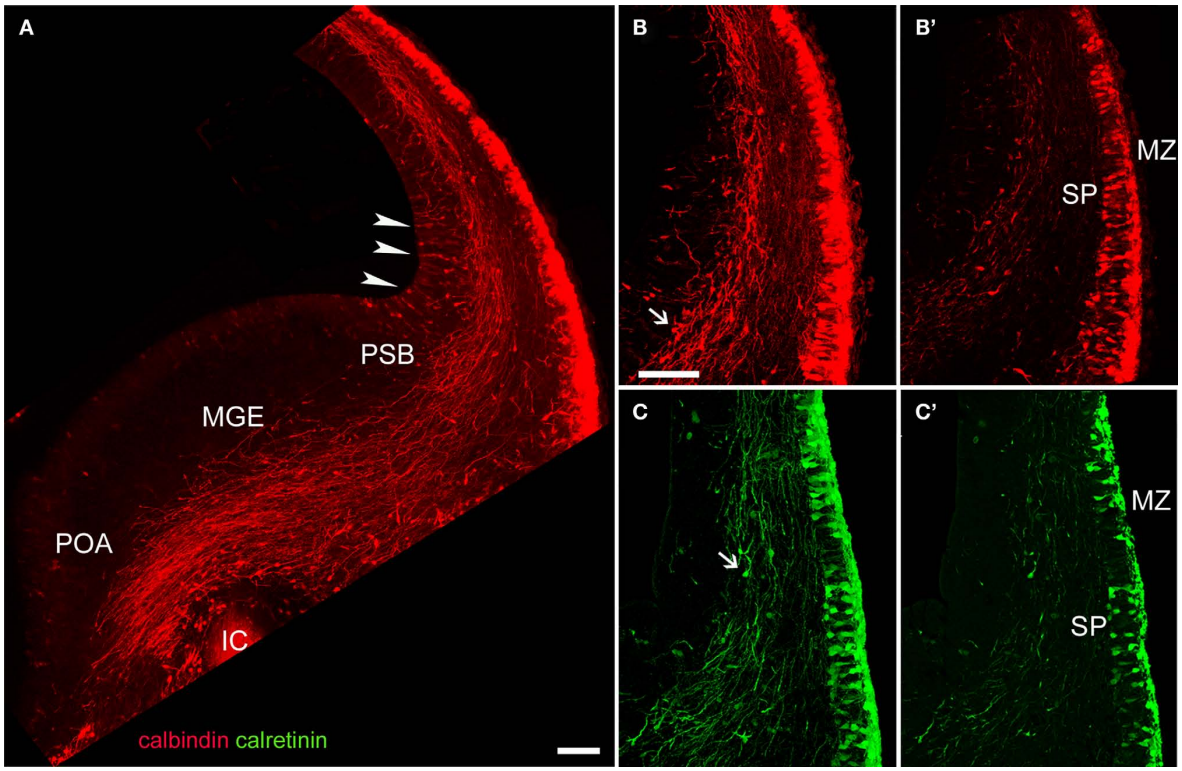

FIGURE 3 | CB and CR immunostaining of a corticofugal projection in E15.5 rats. (A) The dense corticofugal CB projection from the preplate avoided entering the posterior limb of the internal capsule (IC). Arrowheads indicate CB$^+$ cells in the cortical VZ, near the pallial-subpallial boundary (PSB). **(B–C′)** CR$^+$ cells were less abundant in subplate than CB cells. Note in **(B)** and **(C)** immunostained axons in apparent continuity with the already divided preplate. Arrows in **(B)** and **(C)** point to putative migrating interneurons. **(A–C)** are confocal Z-stacks, **(B′)** and **(C′)** are single confocal sections. ic, posterior limb of the internal capsule; MGE, medial ganglionic eminence; MZ, marginal zone; POA, anterior preoptic area; PSB, pallio-subpallial boundary; SP, subplate. Calibration bars: **(A)** 200 μm; **(B–C′)** 100 μm.

subplate cells and glutamatergic neurons, but not by GABAergic cells (Bulfone et al., 1995; Hevner et al., 2001, 2003). Since we characterized the CB$^+$/CR$^+$ cells of the rat preplate and marginal zone as projecting neurons, we wished to confirm that these cells co-expressed the T-box transcription factor Tbr1. This was indeed the case (**Figures 8A–C**), and we additionally observed that Tbr1 was expressed in the mouse preplate by pioneer neurons both of the L1 and the TAG-1 subtypes (**Figures 8D,E**).

MIGRATING INTERNEURONS ACQUIRE CLOSE RELATIONSHIPS WITH L1$^+$ AND TAG-1$^+$ CORTICOFUGAL AXONS

GABAergic interneurons migrate tangentially from the basal telencephalon to the cortical anlage along the intermediate zone and the marginal zone. Given the anatomical overlap of the migration path followed by these interneurons in the intermediate zone and that of the systems of corticofugal axons described here, we explored in detail if corticofugal axons could be a potential substrate for that migration. We used two different markers for migrating interneurons, i.e., GAD67 promoter-driven GFP expression in mice, and PSA-NCAM immunohistochemistry in rats. In E15.5 rats (**Figure 9A**), PSA-NCAM$^+$ interneurons maintained contact with L1$^+$ axons. In E13.5 mice (**Figure 9B**), the GAD67-GFP$^+$ migrating interneurons were also closely related to corticofugal L1$^+$ axons. Interestingly, in both species, the denser accumulation of migrating interneurons was associated with the fraction of L1$^+$ axons deployed in the medial part of the intermediate zone, i.e., those axons that distributed in the same territory than CB$^+$/CR$^+$ pioneer neurons in rats.

The lateral tier of L1$^+$ descending axons overlapped with descending TAG-1$^+$ axons (**Figure 9C**), and both sets of axons were associated with a sparser population of migrating interneurons. TAG-1$^+$ axons also occupied a territory crossed by migrating interneurons on their way to the cortical plate (**Figure 9C**). We are suggesting that TAG-1$^+$ vertically oriented axons in the cortical plate could serve as potential substrates for interneuron migration from the subplate or the MZ (see also **Figure 6E**, and Denaxa et al., 2001).

DISCUSSION

Recent large-scale gene expression analyses (Hoerder-Suabedissen et al., 2009; Osheroff and Hatten, 2009) have revived interest in the roles played by the early-differentiated neurons in the cortical primordium, especially by neurons of the subplate. The use of transgenic mouse lines allows selected neuron subpopulations to be identified and isolated for further analysis, including in-depth genetic analysis or axonal tracing of specific neuron populations with minimal manipulation of the embryos (Gong et al., 2003; Jacobs et al., 2007). These studies are further substantiating the concept that subplate neurons are heterogeneous and encompass different populations of neurons; namely, glutamatergic pioneer

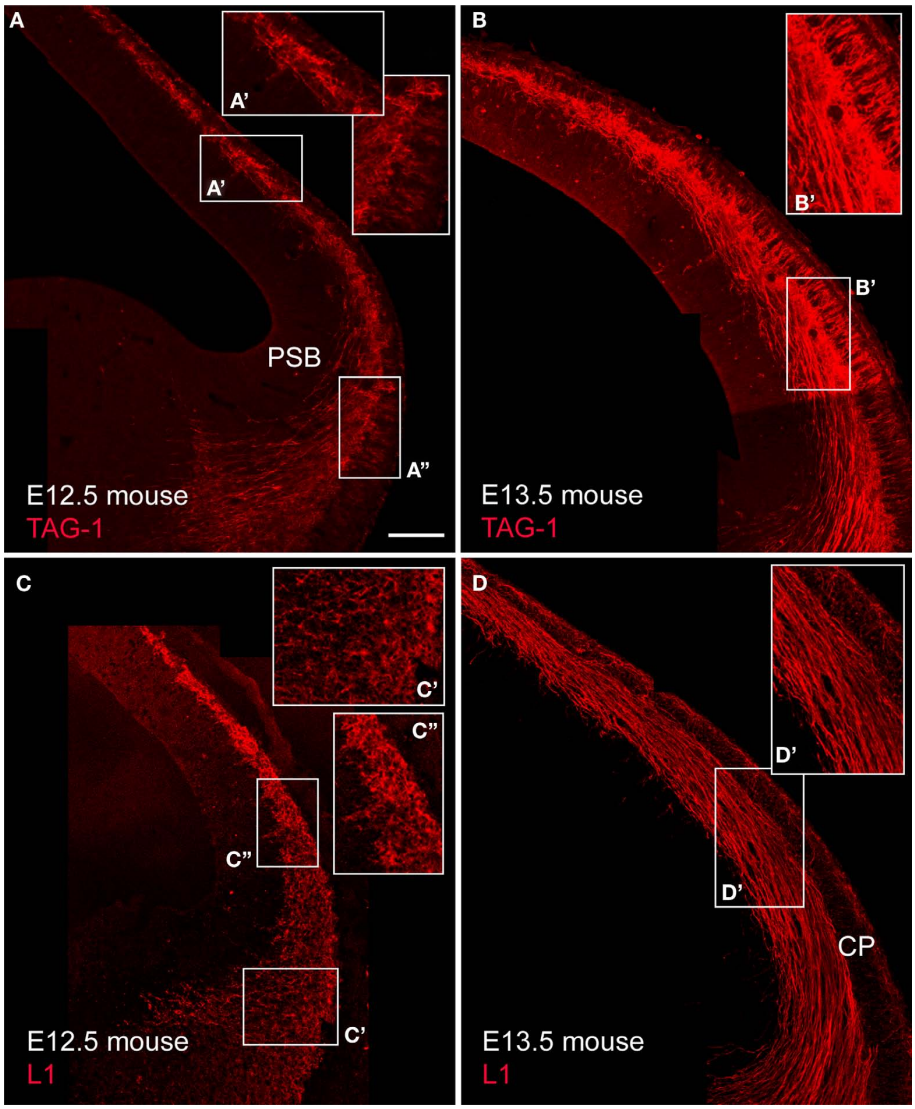

FIGURE 4 | TAG-1$^+$ and L1$^+$ cells in the mouse cortical primordium and their axonal projections. (A,B) show TAG-1 expression. Enlarged boxes show **(A′)** the dorsal cortex and **(A″)** the LP/VP territory at E12.5, and **(B′)** the dorsal cortex at E13.5, with TAG-1$^+$ cells in the upper cortical plate. **(C)** and **(D)** show L1 expression. **(C′)** shows the LP/VP mantle and **(C″)** the dorsal cortex at E12.5, and **(D′)** the dorsal cortex at E13.5. Single confocal sections. Calibration bar: 100 μm.

neurons, other types of glutamatergic neurons and GABAergic interneurons in their migration into the cortical plate (reviewed in Hevner and Zecevic, 2006). As we have shown, additional cohorts of early-differentiated neuron populations co-exist with subplate neurons in the developing cortex. In particular, we have focused our attention on one such cohort that we named non-subplate pioneer neurons. This name summarizes the fact that these neurons never associate with the cortical subplate along the course of their development.

Our definition of non-subplate pioneer neurons implies their transient settlement in the preplate and, as development progresses, their incorporation into the MZ. In the case of the TAG-1$^+$ neurons, incorporation is into the upper tier of the cortical plate. Regrettably, non-subplate projection neurons have received far less attention than subplate neurons in recent literature. The intent of our present work has been to call attention to the important contribution of these non-subplate neurons to a network of neuron connections in the developing cortex, and to the need for further functional analyses. Additionally, we tried to set the bases for a rigorous comparative approach to evaluate early-differentiated cortical neurons across mammals.

We have identified two subtypes of non-subplate pioneer neurons in rats: the cells of the first subtype co-express CB, CR and L1 and are situated in the upper part of the preplate before its partition. Cells of the second subtype are characterized by the expression of TAG-1 and are located slightly deeper than the previous population in the preplate. After the preplate partition, the neurons co-expressing CB, CR and L1 remain in the marginal zone whereas those expressing TAG-1 become localized in the upper cortical plate. In mice, we identified two subtypes

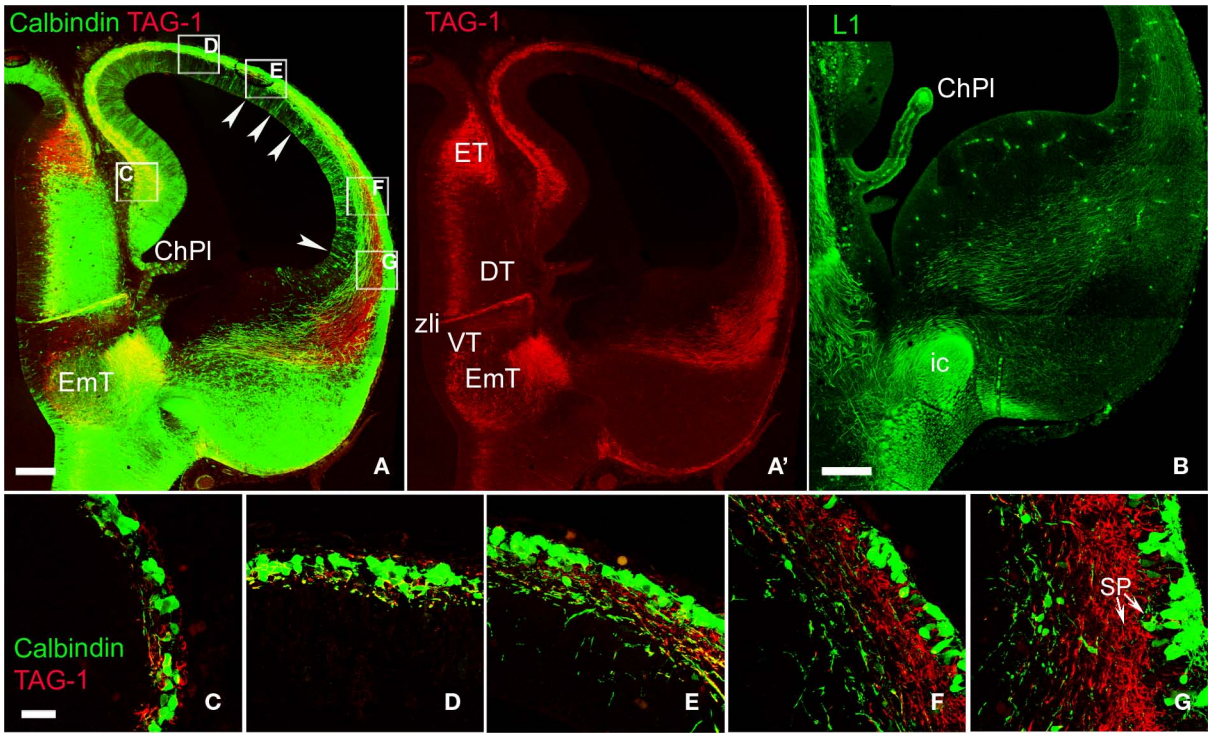

FIGURE 5 | Comparison of the axonal projections of CB⁺ and TAG-1⁺ neurons in rats. (A) Shows a fluorescence overlay of both markers and **(A')** shows TAG-1 immunoreactivity separately. Note that the territory of distribution of TAG-1⁺ axons overlaps only partially with that of CB⁺ axons. Arrowheads in **(A)** point to CB⁺ cells in the ventricular zone. **(B)** Distribution of L1⁺ corticofugal axons avoiding the posterior limb of the internal capsule. **(C–G)** show high magnification fluorescence overlays of the boxed areas in **(A)**. Note the lateral-to-medial development gradient, where TAG-1⁺ or CB⁺ cells accumulated in the ventrolateral extreme of the subplate. CB and TAG-1 localized in independent tissue elements. Note in **(G)** that subplate contained both TAG-1⁺ cells and the deepest CB⁺ cells (arrows). ChPl, choroid plexus; DT, dorsal thalamus; EmT, *Eminentia thalami*; ET, epithalamus; ic, posterior limb of the internal capsule; SP, subplate. VT, ventral thalamus; VZ, ventricular zone; zli, zona limitans intrathalamica. Rat, E15.5. Confocal Z-stacks. Calibration bars: **(A,A')** 100 µm; **(B)** 200 µm; **(C–G)** 30 µm.

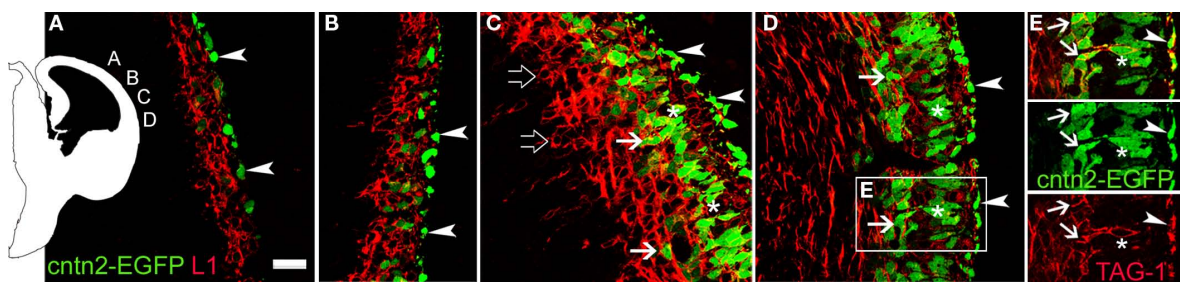

FIGURE 6 | Complementary distributions of L1⁺ neurons and cntn2-EGFP⁺ neurons in the cortical primordium of E12.5 mice. The scheme on the left indicates the places were photomicrographs were taken. **(A)** cntn2-EGFP⁺ subpial cells [arrowheads, also in **(B–E)**], interpreted as Cajal-Retzius cells, and deeper L1⁺ cells in the preplate. **(B)** The preplate was filled with L1⁺ cells, and a few cntn2-EGFP⁺ cells interspersed among them. **(C)** L1⁺ cells concentrated in the MZ below cntn2-EGFP⁺ Cajal-Retzius cells, and in the subplate (open arrows). cntn2-EGFP⁺ cells located in the incipient cortical plate (asterisks), and a few were in the subplate (arrows). **(D)** At this lateral level, cntn2-EGFP⁺ cells were in the subplate (arrows), and inside the upper cortical plate (asterisks). **(E)** From the boxed area in **(D)**: cntn2-GFP⁺ cells in the upper cortical plate (asterisks) were surrounded by TAG-1 immunoreactivity. The latero-medial gradient of maturation suggests that cntn2-EGFP⁺/TAG-1⁺ cells in the subplate (arrows) appeared later than in the upper cortical plate. E12.5 mouse. Single confocal sections. Calibration bar: 30 µm.

of non-subplate pioneer neurons that express either L1 or cntn2 (TAG-1). We propose that these two subtypes of mouse cells are homologues of the two subtypes of non-subplate pioneer neurons of the rat, shown to respectively co-express CB, CR and L1, or TAG-1. The anatomical distribution of these neuron populations is identical in rat and mouse. Additionally, non-subplate pioneer neurons in both rats and mice are not GABAergic and in both species express the T-box transcription factor Tbr1, so that they are putatively glutamatergic (Hevner et al., 2001). **Table 1** and the scheme in **Figure 10** summarize the major aspects of the anatomical distribution of these cells and the markers that are shared by these cells in rats and mice.

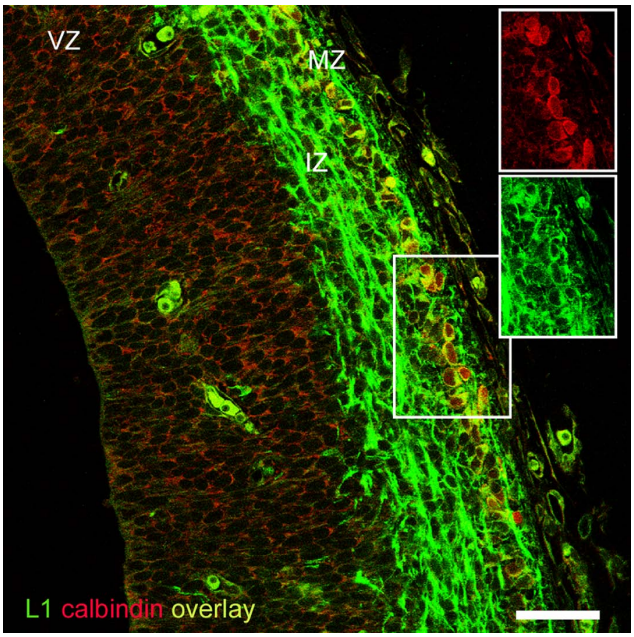

FIGURE 7 | CB⁺ pioneer cells in the rat preplate co-expressed L1. Note the axonal projection of L1⁺ cells along the intermediate zone. Although adequate to revealing CB⁺ cell bodies, Zenon immunostaining was not sensitive enough to detect CB⁺ axons. Boxes show the separation of the fluorescence channels. Rat E15.5. Single confocal section. IZ, intermediate zone; MZ, marginal zone; VZ, ventricular zone. Calibration bar: 50 μm.

The different populations of non-subplate pioneer neurons differ in their axonal projections (**Figure 11**). Non-subplate pioneer neurons of the L1 subtype project to the ganglionic eminences and the anterior preoptic area, but avoid following the internal capsule into the dorsal thalamus. The projections of the neurons of the TAG-1 subtype end up at the lateral parts of the ganglionic eminences. A corticothalamic TAG-1⁺ projection becomes apparent much later, in postnatal mice (Wolfer et al., 1994).

Apparent differences in anatomy and tempo of deployment of early axonal projections of subplate vs. non-subplate neurons may result from technical factors; that is, the different advantages and pitfalls of the axonal tract-tracing methods utilized in different studies (e.g., McConnell et al., 1989; de Carlos and O'Leary, 1992; Clascá et al., 1995; Molnár et al., 1998; Molnár and Cordery, 1999; Jacobs et al., 2007). Here, we decided to trace axonal projections by taking advantage of the chemical properties of the early neurons, as detected by immunohistochemistry. In fortunate cases, this approach produced selective staining of different neuronal populations together with high resolution anatomical detail of their projecting axons.

A major discrepancy among the various studies of the early corticofugal connections concerns whether or not subplate axons are considered as reaching the dorsal thalamus (McConnell et al., 1989; de Carlos and O'Leary, 1992), or, according to more recent studies, these subplate axons (and axons deriving from the marginal zone) are considered as entering only the part of the internal capsule that traverses the ganglionic eminences (Meyer et al., 1998; Molnár et al., 1998; Molnár and Cordery, 1999). However, subplate axons seem not

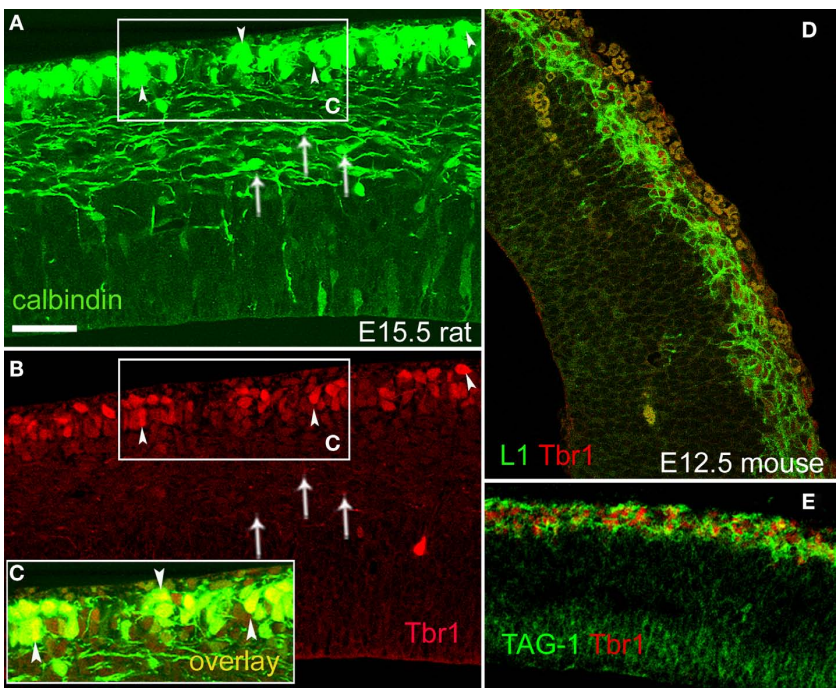

FIGURE 8 | Pioneer neurons in the preplate co-expressed Tbr1 in rats and mice. (A–C) CB⁺ preplate cells co-expressed Tbr1; E15.5 rat. Arrowheads signal co-labeled cells. Overlay is shown in the boxed area **(C)**. CB⁺ cells in the intermediate zone (arrows), possibly migrating interneurons, were Tbr1-negative. CB⁺ cells in the ventricular zone (in **A**) were also Tbr1-negative. **(D)** L1⁺/Tbr1⁺ cells in the E12.5 mouse preplate. **(E)** TAG-1⁺/Tbr1⁺ cells in the E12.5 mouse preplate. Single confocal sections. Calibration bar, 50 μm, in **(A)**.

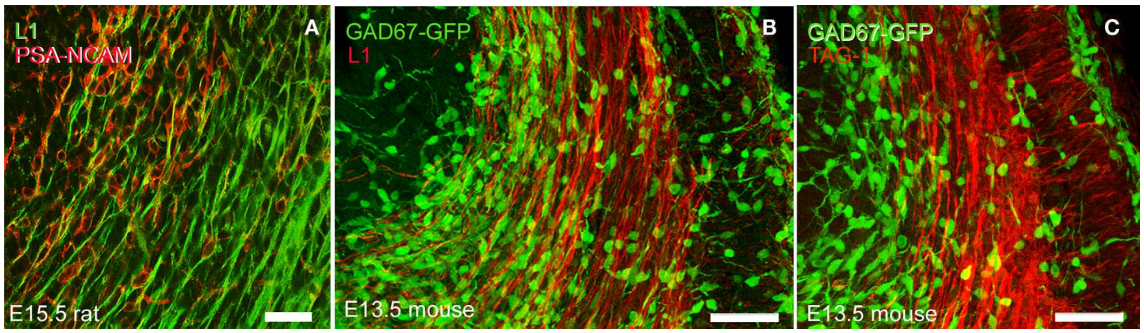

FIGURE 9 | The microanatomical proximity of migratory interneurons with L1⁺ and TAG-1⁺ axons. (A) PSA-NCAM⁺ migratory interneurons associated with L1⁺ axons in an E15.5 rat. **(B)** GAD67-GFP⁺ migratory interneurons in E13.5 mice in contact with L1⁺ axons. In both species, the bulk of migratory GABAergic interneurons exiting from the ganglionic eminences occupied a medial territory, where they overlapped with L1⁺ axons. A less dense population of migrating interneurons located more laterally also overlapped with L1⁺ axons. **(C)** GAD67-GFP⁺ migratory interneurons in E13.5 mice in contact with TAG-1⁺ axons. The sparse population of interneurons located at the lateral tier of the migratory path could associate with TAG-1⁺ axons, which accumulated below the subplate. Vertically oriented TAG-1⁺ axons and cell bodies in the cortical plate could interact with interneurons entering this compartment. Calibration bar: **(A)** 30 μm. **(B,C)** 50 μm.

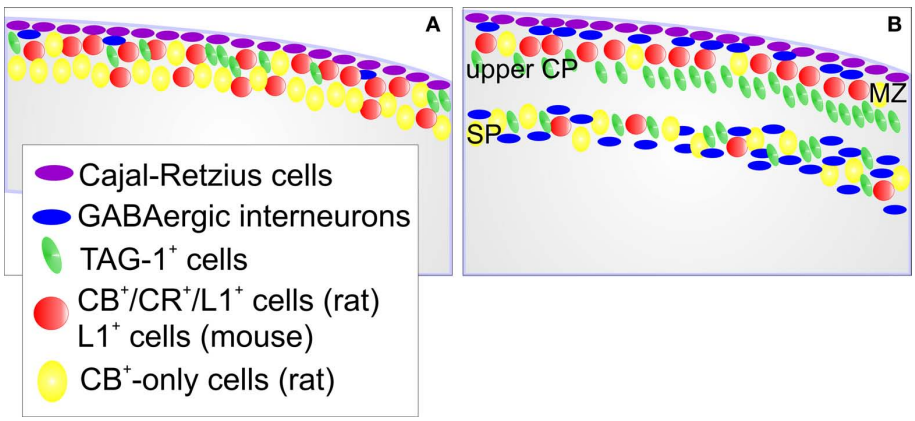

FIGURE 10 | Scheme of the cellular composition of the preplate and its derivatives in rodents. (A) Preplate stage. Cajal-Retzius cells, and sparse GABAergic interneurons are in a subpial sublayer of the preplate. Cells that express CB, CR and L1 in rats, or the homologue L1⁺ cells in mice, locate in the upper preplate. TAG-1⁺ cells are also in the upper preplate in both species. In rats, cells that express CB but not CR (CB⁺-only cells) tend to localize deeper in the preplate. **(B)** This scheme reflects the situation immediately after preplate partition. Cajal-Retzius cells remain in their subpial position. GABAergic interneurons are now apparent in the marginal zone and the subplate. Most of the CB⁺/CR⁺/L1⁺ cells (rats) or L1⁺ cells (mice) end up in the marginal zone. TAG-1⁺ cells end up in the upper cortical plate and in the subplate. In the subplate, the packing density of TAG-1⁺ cells increases from medial to lateral (not shown). Most of the CB⁺-only cells become subplate neurons. MZ, marginal zone; upper CP, upper tier of the cortical plate; SP, subplate.

to project to the dorsal thalamus at the initial steps of corticogenesis (Clascá et al., 1995; Molnár et al., 1998; Molnár and Cordery, 1999; Jacobs et al., 2007). If this is so, the subcortical distributions of subplate axons are similar to that of the non-subplate axons described here.

One caveat: both adhesion molecules TAG-1/cntn2 and L1 are simultaneously expressed by different neurons of the marginal zone and the subplate. This makes it hard to distinguish which descending corticofugal projections originate from neurons located in each one of these two transitory cortical compartments, or from neurons residing in both places. Unfortunately, this seems to somewhat limit the usefulness of most neurochemical markers of the marginal zone and the subplate described so far. For example, the recent study by Osheroff and Hatten (2009) described diverse populations of neurons in the preplate, marginal zone and subplate expressing phosphodiesterase 1c (Pde1c). In particular, preplate Reelin-negative Pde1c-EGFP neurons below Cajal-Retzius cells may share some characteristics with the L1 and TAG-1 neuron subtypes presented here in the same location, but these Pde1c-EGFP cells may distribute in the marginal zone and the subplate after preplate partition. No doubt further refinements of the identification of molecular markers of early cortical neurons will help to sort out the marginal zone and subplate components of the pioneer cortical axonal projections.

The diversified group of early cortical neurons with their diversified axonal projections that we have considered here has not yet been identified in species other than rodents. However, further study is highly relevant to advancing our understanding of cerebral cortex development. Not only would this support the development of functional murine experimental models, but there is the promise of extending these observations to non-human primates and humans. To emphasize the importance of such a comparative

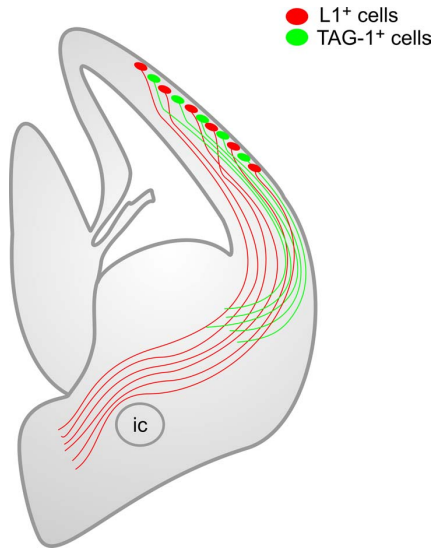

FIGURE 11 | Scheme of the descending corticofugal axonal projections of the two subtypes of non-subplate pioneer neurons at the preplate stage. This scheme highlights the fact that both sets of axons are segregated, although they overlap in a common territory. Note that L1+ axons avoid entering the internal capsule towards the dorsal thalamus. ic, posterior limb of the internal capsule.

approach, it may suffice to consider two examples; namely, the *pioneer plate* described in humans by Meyer et al. (2000) and the *predecessor neurons* described in the human preplate by Bystron et al. (2008).

The *pioneer plate* (Meyer et al., 2000) designates a transient structure in the human preplate formed by CR+ pioneer neurons that are split into marginal zone and subplate neuron populations by the arrival of the neurons that form the cortical plate. In our present analysis of the rat cortex, we have seemingly described the same transient arrangement of early-differentiated neurons, which in our case include both CB+ and CR+ cells, of the preplate and its derivatives. Since the expression of calcium binding proteins may vary widely among species, other markers for human preplate neurons will eventually facilitate comparisons.

It is of historical interest to compare the human pioneer plate model with the preplate partition model first suggested by Marin-Padilla (1971), where the primordial plexiform layer or preplate divides into an upper layer that contains Cajal-Retzius cells and a lower layer containing projection neurons. In the model of Meyer et al. (2000), the partition of the preplate occurs by separating early projection neurons of the preplate. From our point of view, the difference only reflects the fact that the early neuron populations of the cortical primordium are now known to be more diverse than conceived initially. Our results also show that the rodent preplate contains more diversified populations of neurons than those found by Meyer et al. (2000) in their pioneer plate. A case in point is the TAG-1+ neurons that first invade the preplate but, immediately after preplate partition, end up in the upper tier of the cortical plate. Thus, a simple model in which a uniform population of preplate cells divides into marginal zone and subplate neuronal populations is insufficient to account for the rodent data that we have presented here.

The *predecessor neurons* described in the human preplate by Bystron et al. (2008) have not been described as projection neurons. A comparative context may still be useful to explore to what extent these cells are unique to humans or could have homologues in non-primates. Obviously, further advances on the identification of distinct chemical markers in predecessor cells and in other early cortical neurons will facilitate such comparisons between species.

We have described here a close anatomical relationship of pioneer L1+ axons with migrating GABA interneurons during their exit from the ganglionic eminences to the pallium, along the intermediate zone of the cortex. Other interneurons migrate in a compartment located more laterally, where they acquire spatial relationships with L1+ and TAG-1+ axons. TAG-1+ axons also accumulate below the subplate, and are accompanied by migrating interneurons in this territory. TAG-1+ axons in the cortical plate could serve as a hypothetical guide for interneurons from the marginal zone or the subplate to the cortical plate (Denaxa et al., 2001). Our observations strongly suggest that L1+ and TAG1+ corticofugal axons could be a substrate for migration of interneurons along the cortical intermediate zone (see also, McManus et al., 2004), even though a role for L1 in the migration of cortical interneurons has been excluded in *in vitro* perturbation experiments (Denaxa et al., 2001). The role of TAG-1 in interneuron migration is controversial (Denaxa et al., 2001, 2005; Morante-Oria et al., 2003; Tanaka et al., 2003), although this molecule is active in the control of tangential migration of other neuron cohorts (Kyriakopoulou et al., 2002; Denaxa et al., 2005). The obvious anatomical relationships of migrating interneurons with the different sets of early corticofugal axons described here suggest, however, that this issue merits further analyses.

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REFERENCES

Anderson, S. A., Eisenstat, D. D., Shi, L., and Rubenstein, J. L. (1997). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* 278, 474–476.

Bulfone, A., Smiga, S. M., Shimamura, K., Peterson, A., Puelles, L., and Rubenstein, J. L. (1995). T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. *Neuron* 15, 63–78.

Bystron, I., Rakic, P., Molnár, Z., and Blakemore, C. (2006). The first neurons of the human cerebral cortex. *Nat. Neurosci.* 9, 880–886.

Clascá, F., Angelucci, A., and Sur, M. (1995). Layer-specific programs of development in neocortical projection neurons. *Proc. Natl. Acad. Sci. U.S.A.* 92, 11145–11149.

de Carlos, J. A., and O'Leary, D. D. (1992). Growth and targeting of subplate axons and establishment of major cortical pathways. *J. Neurosci.* 12, 1194–1211.

Denaxa, M., Chan, C. H., Schachner, M., Parnavelas, J. G., and Karagogeos, D. (2001). The adhesion molecule TAG-1 mediates the migration of cortical interneurons from the ganglionic eminence along the corticofugal fiber system. *Development* 128, 4635–4644.

Denaxa, M., Kyriakopoulou, K., Theodorakis, K., Trichas, G., Vidaki, M., Takeda, Y., Watanabe, K., and Karagogeos, D. (2005). The adhesion molecule TAG-1 is required for proper migration of the superficial migratory stream in the medulla but not of cortical interneurons. *Dev. Biol.* 288, 87–99.

Dodd, J., Morton, S. B., Karagogeos, D., Yamamoto, M., and Jessell, T. M. (1988). Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons. *Neuron* 1, 105–116.

Fukuda, T., Kawano, H., Ohyama, K., Li, H. P., Takeda, Y., Oohira, A., and Kawamura, K. (1997). Immunohistochemical localization of neurocan and L1 in the formation of thalamocortical pathway of developing rats. *J. Comp. Neurol.* 382, 141–152.

García-Moreno, F., López-Mascaraque, L., and de Carlos, J. A. (2007). Origins and migratory routes of murine Cajal-Retzius cells. *J. Comp. Neurol.* 500, 419–432.

Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E., and Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425, 917–925.

Hevner, R. F., Neogi, T., Englund, C., Daza, R. A., and Fink, A. (2003). Cajal-Retzius cells in the mouse: transcription factors, neurotransmitters, and birthdays suggest a pallial origin. *Dev. Brain Res.* 141, 39–53.

Hevner, R. F., Shi, L., Justice, N., Hsueh, Y., Sheng, M., Smiga, S., Bulfone, A., Goffinet, A. M., Campagnoni, A. T., and Rubenstein J. L. (2001). Tbr1 regulates differentiation of the preplate and layer 6. *Neuron* 29, 353–366.

Hevner, R. F., and Zecevic, N. (2006). Pioneer neurons and interneurons in the developing subplate: molecular markers, cell birthdays, and neurotransmitters. In Development and Plasticity in Sensory Thalamus and Cortex, R. S. Erzurumlu, W. Guido and Z. Molnár, eds (New York, Springer), pp. 1–17.

Hoerder-Suabedissen, A., Wang, W. Z., Lee, S., Davies, K. E., Goffinet, A. M., Rakić, S., Parnavelas, J., Reim, K., Nicolić, M., Paulsen, O., and Molnár, Z. (2009). Novel markers reveal subpopulations of subplate neurons in the murine cerebral cortex. *Cereb. Cortex* 19, 1738–1750.

Jacobs, E. C., Campagnoni, C., Kampf, K., Reyes, S. D., Kalra, V., Handley, V., Xie, Y. Y., Hong-Hu, Y., Spreur, V., Fisher, R. S., and Campagnoni, A. T. (2007). Visualization of corticofugal projections during early cortical development in a tau-GFP-transgenic mouse. *Eur. J. Neurosci.* 25, 17–30.

Kyriakopoulou, K., de Diego, I., Wassef, M., and Karagogeos, D. (2002). A combination of chain and neurophilic migration involving the adhesion molecule TAG-1 in the caudal medulla. *Development* 129, 287–296.

Liu, F.-C., and Graybiel, A. M. (1992). Transient calbindin-D28K-positive system in the telencephalon: ganglionic eminence, developing striatum and cerebral cortex. *J. Neurosci.* 12, 647–690.

López-Bendito, G., Shigemoto, R., Fairén, A., and Luján, R. (2002a). Differential distribution of group I metabotropic glutamate receptors during rat cortical development. *Cereb. Cortex* 12, 625–638.

López-Bendito, G., Shigemoto, R., Kulik, A., Paulsen, O., Fairén, A., and Luján, R. (2002b). Expression and distribution of metabotropic GABA receptor subtypes $GABA_B$R1 and $GABA_B$R2 during rat neocortical development. *Eur. J. Neurosci.* 15, 1766–1778.

Marin-Padilla, M. (1971). Early prenatal ontogenesis of the cerebral cortex (neocortex) of the cat (*Felis domestica*). A Golgi study. I. The primordial neocortical organization. *Z. Anat. Entwicklungsgesch.* 134, 117–145.

McConnell, S. K., Ghosh, A., and Shatz C. J. (1989). Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science* 245, 978–982.

McManus, M. F., Nasrallah, I. M., Gopal, P. P., Baek, W. S., and Golden, J. A. (2004). Axon mediated interneuron migration. *J. Neuropathol. Exp. Neurol.* 63, 932–941.

Meyer G., Goffinet A. M., and Fairén A. (1999). What is a Cajal-Retzius cell? A reassessment of a classical cell type based on recent observations in the developing neocortex. *Cereb. Cortex* 9, 765–775.

Meyer, G., Schaaps, J. P., Moreau, L., and Goffinet, A. M. (2000). Embryonic and early fetal development of the human neocortex. *J. Neurosci.* 20, 1858–1868.

Meyer, G., Soria, J. M., Martínez-Galán, J. R., Martín-Clemente, B., and Fairén, A. (1998). Different origins and developmental histories of transient neurons in the marginal zone of the fetal and neonatal rat cortex. *J. Comp. Neurol.* 397, 493–518.

Molnár, Z., Adams, R., and Blakemore, C. (1998). Mechanisms underlying the early establishment of thalamocortical connections in the rat. *J. Neurosci.* 18, 5723–5745.

Molnár, Z., and Cordery, P. (1999). Connections between cells of the internal capsule, thalamus, and cerebral cortex in embryonic rat. *J. Comp. Neurol.* 413, 1–25.

Morante-Oria, J., Carleton, A., Ortino, B., Kremer, E. J., Fairén, A., and Lledo, P. M. (2003). Subpallial origin of a population of projecting pioneer neurons during corticogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 100, 12468–12473.

Osheroff, H., and Hatten, M. E. (2009). Gene expression profiling of preplate neurons destined for the subplate: genes involved in transcription, axon extension, neurotransmitter regulation, steroid hormone signaling, and neuronal survival. *Cereb. Cortex* 19(Suppl. 1), i126–i134.

Puelles, L., Kuwana, E., Puelles, E., Bulfone, A., Shimamura, K., Keleher, J., Smiga, S., and Rubenstein, J. L. (2000). Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. *J. Comp. Neurol.* 424, 409–438.

Soria, J. M., and Fairén, A. (2000). Cellular mosaics in the rat marginal zone define an early neocortical territorialization. *Cereb. Cortex.* 10, 400–412.

Soria, J. M., Martínez-Galán, J. R., Luján, R., Valdeolmillos, M., and Fairén, A. (1999). Functional NMDA and $GABA_A$ receptors in pioneer neurons of the cortical marginal zone. *Eur. J. Neurosci.* 11, 3351–3354.

Tamamaki, N., Yanagawa, Y., Tomioka, R., Miyazaki., J, Obata, K., and Kaneko, T. (2003). Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. *J. Comp. Neurol.* 467, 60–79.

Tanaka, D., Nakaya, Y., Yanagawa, Y., Obata, K., and Murakami, F. (2003). Multimodal tangential migration of neocortical GABAergic neurons independent of GPI-anchored proteins. *Development* 130, 5803–5813.

Weisenhorn, D. M., Prieto, E. W., and Celio, M. R. (1994). Localization of calretinin in cells of layer I (Cajal-Retzius cells) of the developing cortex of the rat. *Dev. Brain Res.* 82, 293–297.

Wolfer, D. P., Henehan-Beatty, A., Stoeckli, E. T., Sonderegger, P., and Lipp H. P. (1994). Distribution of TAG-1/axonin-1 in fibre tracts and migratory streams of the developing mouse nervous system. *J. Comp. Neurol.* 345, 1–32.

Yamazaki, H., Sekiguchi, M., Takamatsu, M., Tanabe, Y., and Nakanishi, S. (2004). Distinct ontogenic and regional expressions of newly identified Cajal-Retzius cell-specific genes during neocorticogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 101, 14509–14514.

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Doublecortin-expressing cells persist in the associative cerebral cortex and amygdala in aged nonhuman primates

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A novel population of cells that express typical immature neuronal markers including doublecortin (DCX+) has been recently identified throughout the adult cerebral cortex of relatively large mammals (guinea pig, rabbit, cat, monkey and human). These cells are more common in the associative relative to primary cortical areas and appear to develop into interneurons including type II nitrinergic neurons. Here we further describe these cells in the cerebral cortex and amygdala, in comparison with DCX+ cells in the hippocampal dentate gyrus, in three age groups of rhesus monkeys: young adult (12.3 ± 0.2 years, $n = 3$), mid-age (21.2 ± 1.9 years, $n = 3$) and aged (31.3 ± 1.8 years, $n = 4$). DCX+ cells with a heterogeneous morphology persisted in layers II/III primarily over the associative cortex and amygdala in all groups (including in two old animals with cerebral amyloid pathology), showing a parallel decline in cell density with age across regions. In contrast to the cortex and amygdala, DCX+ cells in the subgranular zone diminished in the mid-age and aged groups. DCX+ cortical cells might arrange as long tangential migratory chains in the mid-age and aged animals, with apparently distorted cell clusters seen in the aged group. Cortical DCX+ cells colocalized commonly with polysialylated neural cell adhesion molecule and partially with neuron-specific nuclear protein and γ-aminobutyric acid, suggesting a potential differentiation of these cells into interneuron phenotype. These data suggest a life-long role for immature interneuron-like cells in the associative cerebral cortex and amygdala in nonhuman primates.

Keywords: neuroplasticity, interneurons, neurogenesis, aging, neuropsychiatric disorders

INTRODUCTION

One of the most fundamental features of the brain is its plasticity, a constant interplay between neural structure, function and experience. The scope and complexity of neuroplasticity appear to increase during evolution in parallel with the enlargement of brain through encephalization, corticalization and gyrification (Finlay and Darlington, 1995). Thus, plasticity is likely the foundation of the so-called "higher brain functions" (e.g., learning, memory, decision-making, or intelligence) that are mostly sophisticated in humans (Azmitia, 2007). In general, the associative cortical areas together with the amygdala and hippocampal formation, which are greatly expanded during late mammalian evolution, are the anatomic niches for many high-level cognitive functions (Krubitzer, 2009; Pierani and Wassef, 2009). Greater neuroplasticity is likely inherent with higher vulnerability to environmental and internal insults. In line with this notion, cellular deficits and structural changes in the associative cortical and limbic areas are associated with some neurological or neuropsychiatric disorders (e.g., mode disorders, schizophrenia, epilepsy, Alzheimer's disease) in the adolescent, adult and aged human populations (Arendt, 2005; Di Cristo, 2007; Brambilla et al., 2008; Pavuluri and Passarotti, 2008; Siebzehnrubl and Blumcke, 2008).

Neuroplasticity is a complex process involving structural modulations at synaptic, neuronal and circuitry/pathway levels (Bruel-Jungerman et al., 2007). Of particular interest, formation of new neurons in the adult brain has been recently recognized as a key substrate for neuroplasticity and cognition (Lledo et al., 2006; Aimone et al., 2009). For instance, adult neurogenesis in the forebrain subventricular and subgranular zones (SVZ, SGZ) appears to be essential for olfaction and hippocampus-dependent learning and memory in rodents (Shors et al., 2001; Magavi et al., 2005; Hernández-Rabaza et al., 2009).

Newly-generated neurons in the SVZ and SGZ express a set of immature neuronal markers including doublecortin (DCX+) and polysialylated neural cell adhesion molecule (PSA-NCAM, Magavi et al., 2000; Gritti et al., 2002; Couillard-Despres et al., 2005). Concurrent with morphological development, these new neurons differentiate through a correlated process of downregulation of the above-mentioned immature markers and upregulation of common (e.g., neuron-specific nuclear protein, NeuN) or specific terminal neuronal markers, thus eventually become fully integrated into functional neuronal circuitries in the adult brain (Brown et al., 2003).

Besides the SVZ/SGZ, a novel population of DCX+ cells coexpressing other immature neuronal markers has been recently characterized in the cerebral cortex of guinea pigs, rabbits, cats and primates from young to mid-age adulthood (Liu et al., 2008; Xiong et al., 2008; Cai et al., 2009; Luzzati et al., 2009). These DCX+ cells are more common in the associative relative to primary cortical areas, and appear to develop into interneuron subgroups in guinea pigs and cats (Xiong et al., 2008; Cai et al., 2009). We show here in rhesus monkeys that DCX+ cells persist into advanced ages in the associative frontal and temporal lobe cortex and amygdala, even in very old animals with substantial cerebral amyloid pathology. In contrast, DCX+ cells are rare in the hippocampus by mid-age. DCX+ cells in the monkey cortex also appear to develop into GABAergic phenotype. These data point to a life-long role for novel immature interneuron-like cells in nonhuman primate cerebral structures that are crucial for cognitive functions.

MATERIALS AND METHODS

ANIMAL AND TISSUE PREPARATION

Brain sections from 10 male ($n = 6$) and female ($n = 4$) normal rhesus monkeys (*Macaca mulatta*) were used in the present study (Chu and Kordower, 2007). The animals were divided into young adult (12.1, 12.4 and 12.5 years, mean = 21.2 ± 1.9 years), mid-age (19, 22 and 22.5 years, mean = 12.3 ± 0.2 years), and aged (30, 30.3, 31 and 34 years, mean = 31.3 ± 1.8 years) groups. According to the 1:3 monkey verse human age converting ratio, the mean ages of these groups were approximately 37, 64 and 94 years old relative to human age. DCX immunolabeling pattern from the young adult group has been described in our recent report (Cai et al., 2009). Therefore, we only include densitometric data from this group in the current study. All monkeys were housed individually on a 12-h on/12-h off lighting schedule with ad libitum access to food and water. Animals were sedated with Ketamine (20 mg/kg, i.m.) and then were deeply anesthetized with sodium pentobarbital (25 mg/kg, i.v.). Prior to perfusion, monkeys were injected with 1 ml of heparin (20,000 IU) into the left ventricle of the heart. Animals were then perfused with normal saline followed by fixation with 4% paraformaldehyde. The brains were then removed and cryoprotected in 30% sucrose in 0.1 M sodium phosphate buffer at 4°C. Serial sections throughout the cerebral hemisphere were cut frozen (40 um) at the frontal plane on a sliding microtome and stored at −20°C in cryoprotectant before they were analyzed in the present study.

Animal use was in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and was approved by Institutional Animal Care and Use Committee of Rush University.

IMMUNOHISTOCHEMISTRY

Sections were first treated with 1% H_2O_2 in PBS for 30 min, and pre-incubated in 5% normal rabbit serum in PBS with 0.3% Triton X-100 for 1 h at room temperature, followed by incubation with goat anti-DCX antibody (Santa Cruz Biotechnology, sc-8066, 1:2000) overnight at 4°C. Sections were further reacted with biotinylated rabbit anti-goat IgG at 1:400 for 2 h, and subsequently with ABC reagents (1:400) (Vector Laboratories, Burlingame, CA, USA) for 1 h. Immunoreaction product was visualized using 0.003% hydrogen peroxide and 0.05% diaminobenzidine. Three 10-min washes with PBS were used between incubations. Sections were mounted on slides, allowed to air-dry, and then coverslipped. Some immunostained sections were lightly counterstained with cresyl violet to verify the laminar distribution of the labeled cells. Some sections from the aged monkey group were stained for amyloid deposition using mouse anti-amyloid peptide amino acids 1–16, clone 6E10 (Signet, #39320, 1:4000).

For double immunofluorescence, sections were incubated overnight at 4°C in PBS containing 5% normal donkey serum, 0.3% Triton X-100 and goat anti-DCX (1:2000) together with one of the following antibodies: mouse anti-PSA-NCAM (Chemicon, MAB5324, 1:2000), mouse anti-NeuN (Chemicon, MAB377, 1:4000), mouse anti-GABA (Sigma-Aldrich, A0310, 1:10000) and 6E10. Sections were then reacted for 2 h with Alexa-Fluor® 488 conjugated donkey anti-goat and Alexa-Fluor® 594 conjugated donkey anti-mouse IgGs (1:200, Invitrogen, Carlsbad, CA, USA). Sections were finally counter-stained with bisbenzimide (Hoechst 33342, 1:50000), washed and coverslipped with anti-fading medium (Vector Laboratories).

IMAGING, DENSITOMETRY AND DATA ANALYSIS

Peroxidase-DAB stained sections were examined using an Olympus (BX60) fluorescent microscope equipped with a digital camera and imaging system (Optronics, Goleta, CA, USA). In each brain five equally-spaced sections (1 mm apart) around the middle levels of the amygdala were used to quantify DCX+ cells in the amygdala, entorhinal cortex (Ent) and inferior temporal gyrus (ITG). Similarly, five sections (1 mm apart) across the mid-hippocampus and anterior to the genu of corpus callosum were used to count DCX+ cells in the dentate gyrus and the medial orbital gyrus (MOG), respectively. Because the labeled somata were distributed as cellular bands in these analyzed forebrain regions, the lengths of overlying white matter border, cortical pial surface and granular cell layer (GCL) were used as internal references to calculate the relative abundance of cells in the amygdala, various cortical areas and dentate gyrus, respectively. Cell numbers were counted over montages of images taken at 10X using Image-J software as descried in detail in Xiong et al. (2008). For the purpose of cross-age and cross-region comparisons, averaged relative densities of DCX+ cells in a given brain region were normalized to the mean density of the same region from the young adult group. Mean densities were analyzed statistically using one- and two-way ANOVA, together with Bonferroni posttests between multiple pairs of mean values, whenever applicable (Prism GraphPad 4.1, San Diego, CA, USA). The minimal significance level was set at $p < 0.05$.

A Zeiss fluorescent microscope (Axio Imager, equipped with Apo Tom analysis system) was used to study colocalization of immunolabelings. Immunofluorescence emitted from the top ~4 μ of the tissue was captured. Image overlapping was done with the Optronics imaging software, and illustrations were prepared with Photoshop 7.1.

RESULTS

OVERALL DISTRIBUTION OF DCX+ CELLS IN THE MONKEY FOREBRAIN

DCX+ cells in the SGZ of the dentate gyrus were considerably common in the young adult group (as shown in Figure 8E in Cai et al., 2009). In the mid-age group a few labeled cells occurred in the

DG deep to the GCL (**Figure 1**). These immature granule cells were small and bipolar, had limited dendritic branches, and were mostly oriented with the long somal diameter oblique or parallel to the GCL. In contrast, DCX+ granule cells were very rare or essentially absent in the dentate gyrus in all aged monkeys examined in the present study (images not shown).

Two previous studies demonstrated putative immature neurons in the amygdala of young adult nonhuman primates (Bernier et al., 2002; Tonchev et al., 2003). We noted DCX labeling in this population of amygdalar cells in all age groups of rhesus monkeys. These DCX+ cells were located along the junction of the amygdale to the whiter matter of the Ent, appearing as a cellular band (**Figures 2A,B**). The ventral end of this amygdalar band appeared to somewhat continue with the DCX+ cell band in layers II/III of the Ent (**Figures 2C,D and 3A–C**).

DCX+ cortical cells in the young adult monkeys were present virtually across the cerebral hemisphere, and appeared as a cellular band in layers II/III with an overall ventrodorsal high to low gradient at a given frontal plane (also see Figure 8 in Cai et al., 2009). For instance, in the temporal lobe sections a distinct cellular band continued from the Ent, across the temporal gyri, and to the insular (Ins) cortex. In the mid-age animals, the cellular band was seen over the Ent and ITG, but became less evident in the medial temporal gyrus (MTG) and more dorsally-located cortical areas (**Figure 2**). In the aged monkeys, this cellular band became largely restricted to the junction of the Ent and ITG, although labeled cells were still seen in small groups or clusters in the MTG, STG and Ins cortex (**Figure 3**).

DCX+ cells were occasionally detected in the primary motor and sensory cortical areas as isolated cells or small groups of cells in layer II, without a consistent or noticeable region-specific pattern in the mid-age monkeys. However, labeled perikarya were fairly common in the dorsal lateral and ventral prefrontal cortical areas (**Figures 2M–O and 3G**).

We illustrated the above-mentioned age-related changes in relative abundance of DCX+ cells over the temporoparietal cortical areas using hemispheric maps created based on visual scoring of labeled profiles in multiple sections in each brain (**Figure 4**). The low temporal lobe regions exhibited most abundant DCX labeling in each age group. Also, considerable amount of labeled profiles persisted in these regions to the aged group (**Figure 4I–L**). Thus, more detailed quantitative and morphological studies of DCX+ cells were carried out in these regions (the amygdala, Ent and ITG), together with the MOG of the prefrontal cortex and DG (see below).

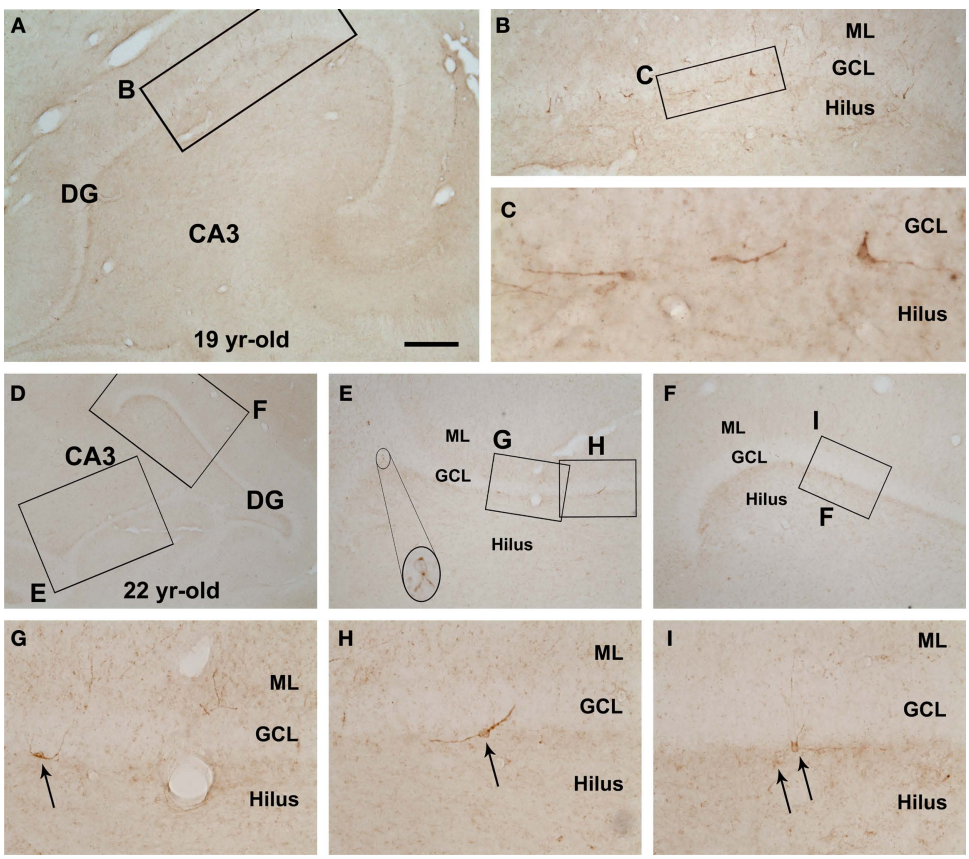

FIGURE 1 | Rare doublecortin-immunoreactive (DBX+) cells in the hippocampal dentate gyrus (DG) of rhesus monkeys aged around 20 years old (mid-age group). Shown are low magnification views of the DG and CA3 sector in a 19-year-old **(A,B)** and a 22-year-old **(D–I)** animals, with enlarged areas depicting examples of apparently dysmorphic DCX+ cells at the border between the granular cell layer (GCL) and the hilus. DCX+ cells are small, mostly bipolar, and often arranged with their long somal diameter parallel to the GCL. Some cells (arrows in **G–I**) have a few dendritic processes extending across the GCL into the molecular layer (ML). Scale bar = 1 mm in **(A)** applying to **(E,F)**, equivalent to 2 mm for **(D)**, 100 μm for **(B)** and 50 μm for **(G–I)**, and 25 μm for **(C)**.

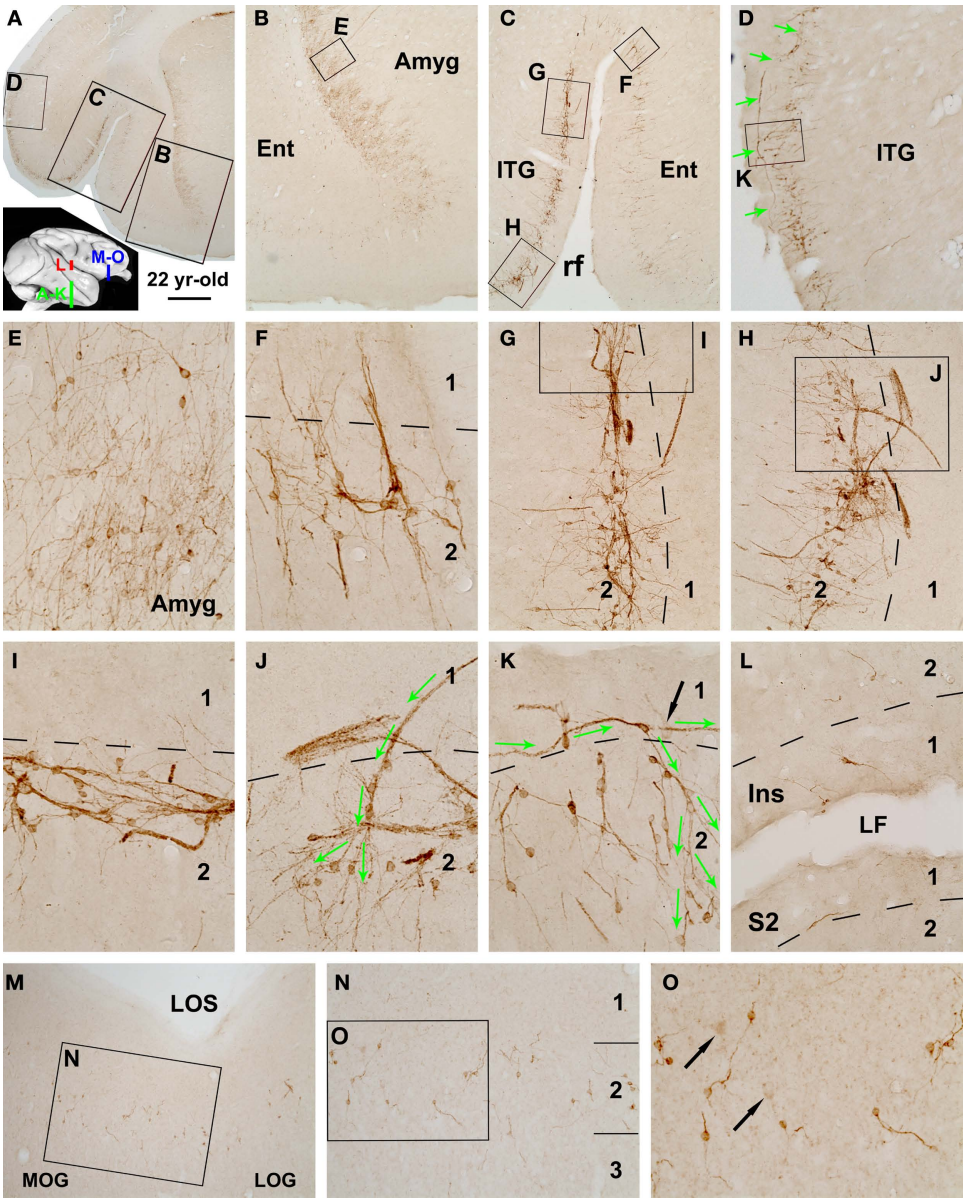

FIGURE 2 | Distribution of DCX+ cells in the temporal lobe cortical areas surrounding the amygdala (A–K), in the insular (Ins) and secondary somatosensory (S2) cortex (L), and in the ventral prefrontal cortex including the medial and lateral orbital gyri (MOG, LOG) (M–O) in a 22-year-old rhesus monkey. Framed areas in lower magnification panels are enlarged sequentially as indicated. DCX+ cells in the amygdala (Amyg) are located near the white matter deep to the entorhinal cortex (Ent) as a cellular band, which continues into the adjoining Ent and further dorsally into the inferior temporal gyrus (ITG) around the border of layers I and II **(A–C)**. Labeled cells are mostly small and bipolar, often arrange as clusters and chains with cells seemingly migrate outside-in intracortically **(C,F)**. However, in the ITG a large number of cells are associated with tangentially arranged migratory chains that can extend very long **(D,G–K)**. Some of these chains appear to extend from layer I and then enter the cortical plate, with cells dispatching or being dispersed around the end of the chains (**D,J,K**, green arrows). Note that labeled cells are also fairly common in the MOG and LOG **(M,N)**, but fewer in the Ins cortex and S2 **(L)**. A few relatively large cells with reduced DCX reactivity are present in layers II/III (indicated with black arrows in **K,N,O**). rf, rhinal fissure; LF, lateral fissure. Arab numbers indicate cortical layers. Scale bar = 3 mm in **(A)** applying to **(E,F)**, equivalent to 1 mm for **(B,C)**, 500 μm for **(D)**, 100 μm for **(G,H,L–N)** and 50 μm for **(E,F,I–K,O)**.

DECLINE OF DCX+ CELLS WITH AGE IN MONKEY FOREBRAIN REGIONS

Overall, the numeric densities of DCX+ cells were reduced significantly in all analyzed forebrain areas in the mid-age and aged relative to young adult groups (**Figure 5**). DCX+ cells in the amygdala dropped to 41 ± 13% (mean ± SD, same below) in the mid-age and 24 ± 10% in the aged relative to the young adult (100 ± 15%) groups ($p = 0.003$, $F = 33$, df = 2, one-way ANOVA). In the Ent and ITG, labeled cells reduced to 32 ± 22 and 34 ± 8% in the mid-age and 13 ± 8 and 11 ± 6% in the aged groups compared to the young adults (100 ± 20 and 100 ± 15%) ($p < 0.0001$, $F = 41$ and 72, df = 2,

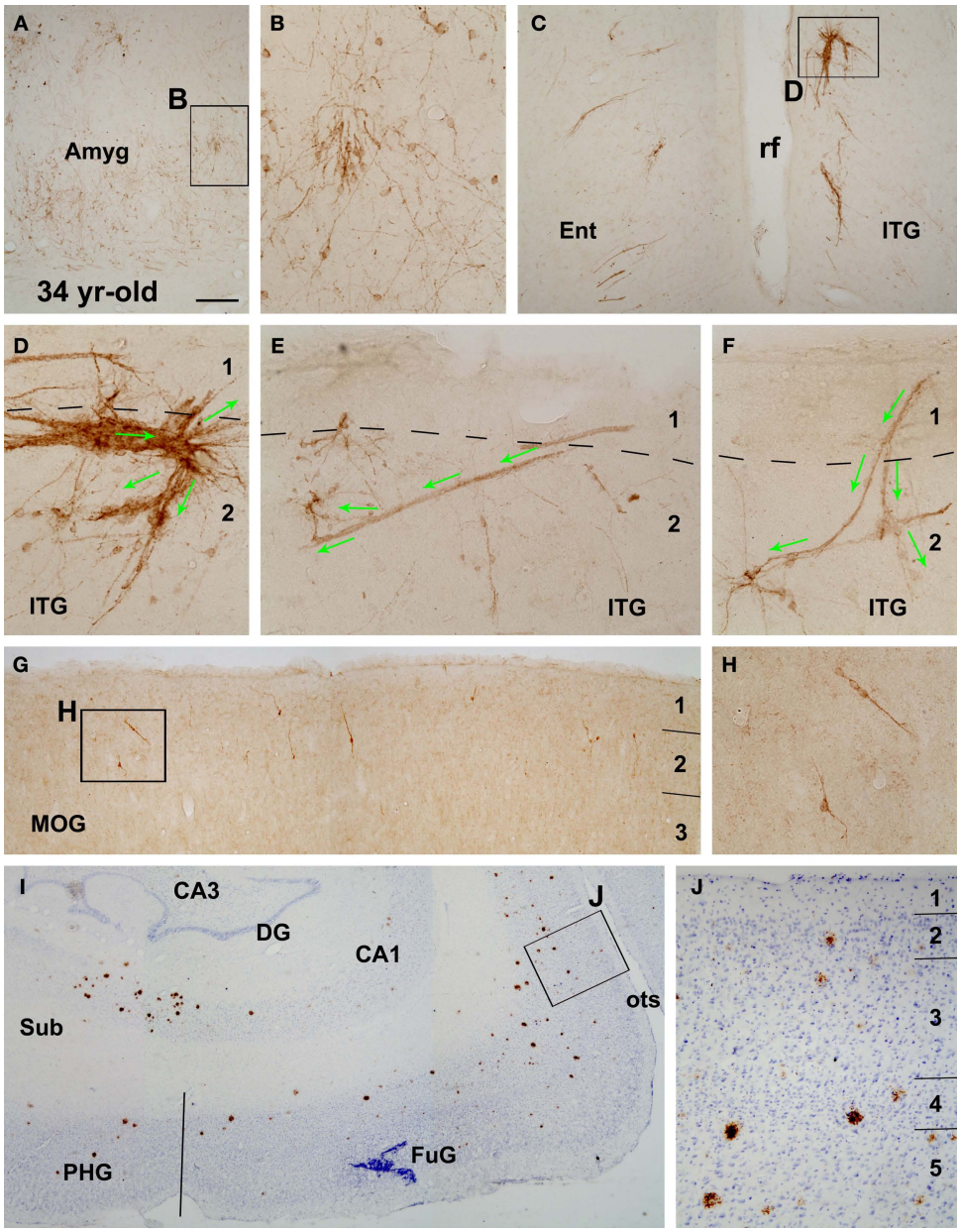

FIGURE 3 | Presence of DCX+ cells (A–H) in the amygdala (A,B) and the cortex (C–H) of a 34-year-old monkey with cerebral amyloid plaques (I,J), as indicated. A decreased amount of DCX labeling is noticeable as compared to **Figure 2** in corresponding cerebral areas. Labeled profiles in layers I–III, especially their clusters, appear disorganized **(C–F)**. An expanded bizarre-looking migratory cell cluster is illustrated in **(D)**. Long tangential and oblique migratory chains occur around layers I and II **(E,F)**. The distribution of amyloid plaques around the hippocampal formation and temporal lobe structures is shown with 6E10 immunolabeling with Nissl counterstain **(I,J)**. Some plaques occur in layers II and III wherein DCX+ cells exist **(J)**. Sub, subiculum; PHG, parahippocampal gyrus, FuG, fusiform gyrus; opts, occipitotemporal sulcus. Other abbreviations are the same as in **Figure 2**. Scale bar = 250 μm in **(A)**, equivalent to 500 μm for **(I)**, 150 μm for **(C,G)**, 100 μm for **(J)** and 50 μm for **(B,D–F,H)**.

one-way ANOVA). DCX+ cells in the MOG reduced to 27 ± 13% in the mid-age and 13 ± 7% in the aged relative to the young adult (100 ± 19%) groups ($p < 0.0001$, $F = 45$, df = 2, one-way ANOVA). DCX+ cell density in the DG was 9 ± 3 and 1.2 ± 1% in the mid-age and the aged compared to the young adult (100 ± 14%) groups ($p < 0.0001$, $F = 170$, df = 2, one-way ANOVA). There existed a significant difference in the extent of decline of normalized mean densities across the analyzed regions in both the mid-age and aged groups ($p < 0.0005$, $F = 12$, df = 3, 20, two-way ANOVA). Bonferroni posttests indicated that this region-related difference was due to a more dramatic reduction of DCX+ cells in the DG relative to the amygdala and all cortical areas ($p < 0.05$ to $p < 0.01$).

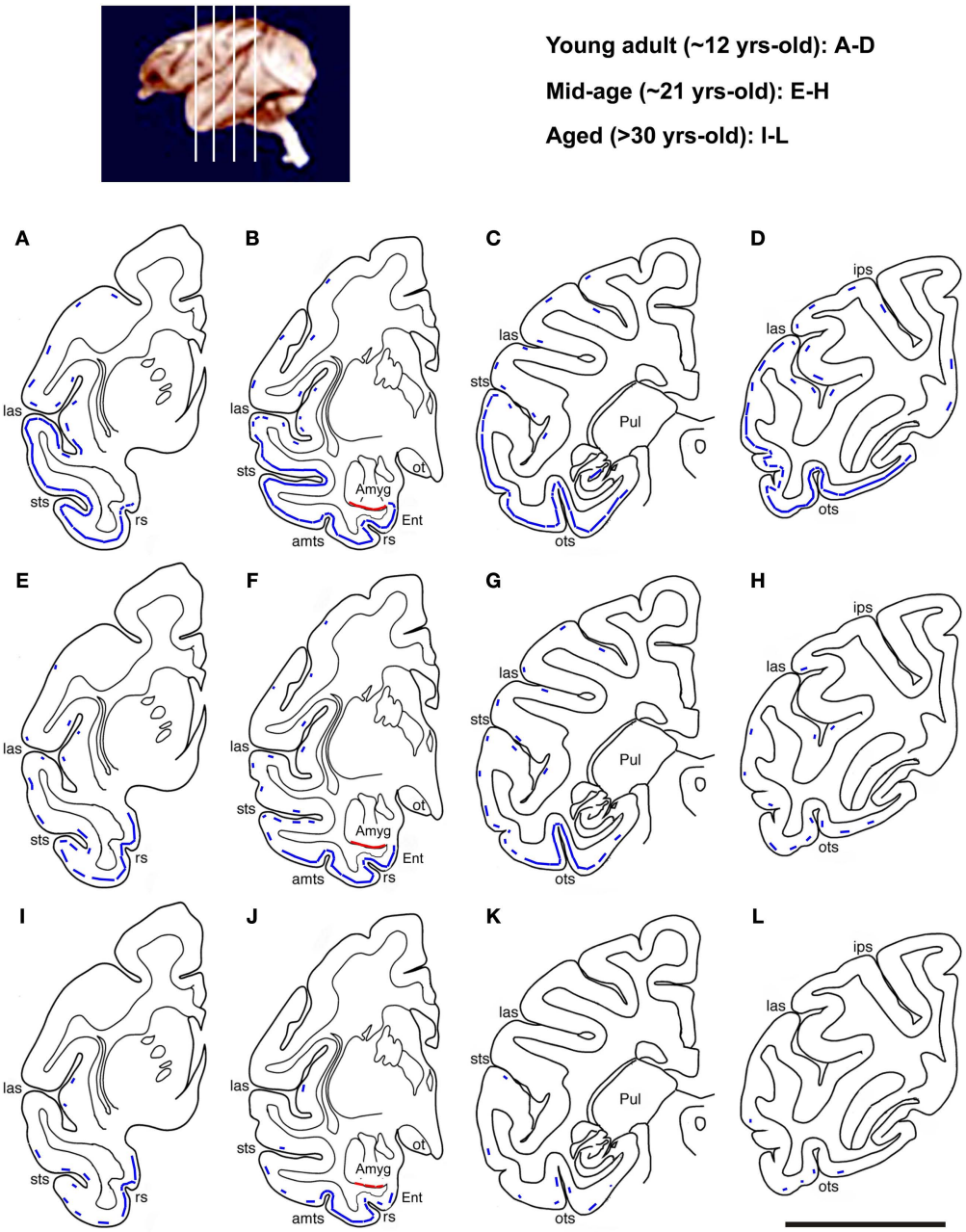

FIGURE 4 | Schematic drawings illustrating an overview of age-related change in DCX+ cell distribution in the temporoparietal cortical areas at four representative anterioposterior frontal levels as indicated in the top-left map. In the young adult group **(A–D)**, DCX+ cortical cells occur in layers II/III as a continuous cellular band (blue line) extending ventrally from the entorhinal cortex (Ent) to laterally all of the temporal neocortical areas. There follows a parallel decline in the abundance of labeled cells along this band with age **(E–L)**. Thus, the cellular band "retracts" dorsoventrally to become impressive only in the ventral temporal areas, especially around the joining portion between the Ent and inferior temporal gyrus, in the aged group **(I–L)**. A small number of labeled cells exists in the insular and parietal cortical areas in young and mid-age animals (broken line), and they disappears in the aged animals. A cellular band at the border of the amygdala and the cortical white matter is seen in all groups (red line): sts, superior temporal sulcus; amts, anteriomedial temporal sulcus; ots, occipitotemporal sulcus; Pul, putamen; ips, interparietal sulcus; ot, optic tract; rs, rhinal sulcus. Scale bar = 1 cm.

CHAIN MIGRATION AND CLUSTERIZATION OF DCX+ CELLS IN MID-AGE AND AGED MONKEY CORTEX

We recently described chain migratory structures of DCX+ cells in the young adult cat and monkey cortex arranged largely perpendicular to the pial surface and with labeled cells seemingly migrating from layer II to deeper layers (Cai et al., 2009). In the current study somewhat different intracortical migratory pattern and clusterization of the labeled cells were noticed in mid-age and aged monkeys relative to young adults. Thus, besides the inwardly arranged chains (**Figure 2F**), considerably long migratory chains of DCX+ cells and

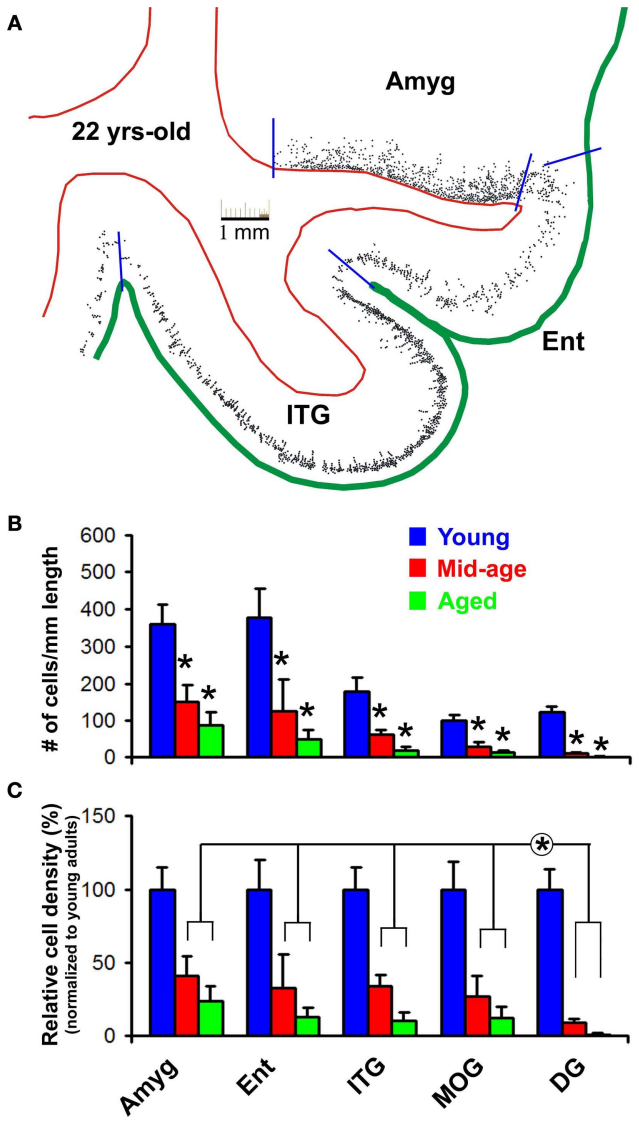

FIGURE 5 | Age-related decline of DCX+ cells in the monkey forebrain based on densitometry over representative areas including the amygdala (Amyg), entorhinal cortex (Ent), inferior temporal gyrus (ITG), medial orbital gyrus (MOG) and dentate gyrus (DG). (A) shows an example of cell map reproduced from montaged 10× microscopic images over a mid-amygdala section from the 22-year-old animal. Red lines mark the borders between the white matter and gray matter of the cortex or between the amygdala and cortical white matter, and green line defines the pial surface. Blue bars indicate the locations used to divide the cortical areas, and to define the length of corresponding cellular bands in the amygdala (along the white matter border) and cortex (along the pial surface). Bar graph **(B)** shows cell densities (mean ± SD) in different areas in the three age groups. Bar graph **(C)** represents averaged densities normalized to the mean densities of corresponding regions in the young adult group (i.e., defined as 100%). Statistic analyses indicate a significant (*) reduction of DCX+ cells with age in all analyzed areas **(B)**, and a significantly greater decline of cells in the DG relative to the amygdala and cortical areas **(C)**.

processes occurred tangentially around the border of layers I and II in mid-age and aged monkeys, mostly prominent in the ITG. Closer examination indicated that some of these chains appeared to derive from layer I then invaded the cortical plate (**Figures 2G-J, 3E,F and 6D–H**). DCX+ cells appeared to migrate along these chains, then leave en route or radially at the end of a given chain to disperse in layers II/III (**Figures 2J,K, 3D–F and 6D,H**). Of note, in the aged group irregular or bizarre-shaped cell aggregations or clusters occurred around the border of layers I and II. These clusters might be in small to fairly large sizes (up to 0.5 mm), with some associated cells appearing to migrate away "aberrantly" – toward the cortical plate or even the pia (**Figures 3C,D**).

Compared to the cortex, DCX+ cells in the amygdala did not exhibit obvious chain-like arrangement and clusterization, although the density of labeled cells decreased sharply from the white matter border toward the center of the amygdala (**Figures 2B,E and 3A,B**).

It should be mentioned that the 34-year-old animal in the aged group exhibited considerable amyloid plaque pathology in the hippocampus and across the cerebral cortex (**Figures 3I,J and 7L–O**). The 30-year-old animal also showed amyloid deposition to a lesser extent in these same brain areas, whereas the forebrains of the remaining two aged animals were essentially plaque-free (data not shown).

IMMUNOFLUORESCENT CHARACTERIZATION OF DCX+ CELLS IN MONKEY CEREBRAL CORTEX

In freshly-processed cat cerebral cortex, NeuN and γ-aminobutyric acid (GABA) are co-expressed in relatively large mature-looking cells with peak and with reduced levels of DCX expression. In contrast, glutamic acid decarboxylase, calbindin, parvalbumin, neuronal nitric oxide synthase are only detectable in mature-looking cells with attenuated DCX expression (Cai et al., 2009). In the monkey materials we examined the latter subgroup of DCX+ cells occurred infrequently (as shown in **Figures 2K,O**), potentially due to the long time tissue storage (3–4 years) that might have caused some loss of DCX antigenicity. Therefore, double immunofluorescence was carried out only for potential colocalization of DCX with PSA-NCAM, NeuN and GABA to evaluate the presumed immature and developing neuronal phenotype as well as GABAergic fate of the DCX+ cells.

There existed a virtually complete colocalization of DCX and PSA-NCAM among individually-distributed small or larger cells, as well as among small cells associated with long migratory chains in all monkeys (**Figures 6A–D**). As expected, we also detected colocalization of NeuN or GABA in a small number of cortical DCX+ cells with relatively large somal size and low levels of DCX reactivity (**Figures 6E–K and 7A–F**).

Certain antidepressants that modulate monoamine neurotransmission may influence the expression of DCX or PSA-NCAM in the hippocampus and prefrontal cortex (Sairanen et al., 2007; Varea et al., 2007; Perera et al., 2008). Also, the superficial cortical layers including layer I are innervated by monoaminergic terminals from subcortical structures, including catecholaminergic projection neurons (Campbell et al., 1987; Lewis et al., 1987). Therefore, it was of interest to explore whether DCX+ cells in the primate cerebral cortex might be in a position to be influenced by catecholaminergic inputs. Indeed, DCX+ cells and processes around the border of layers I/II were found to be surrounded by or in close proximity to axon terminals immunolabeled for tyrosine hydroxylase (TH).

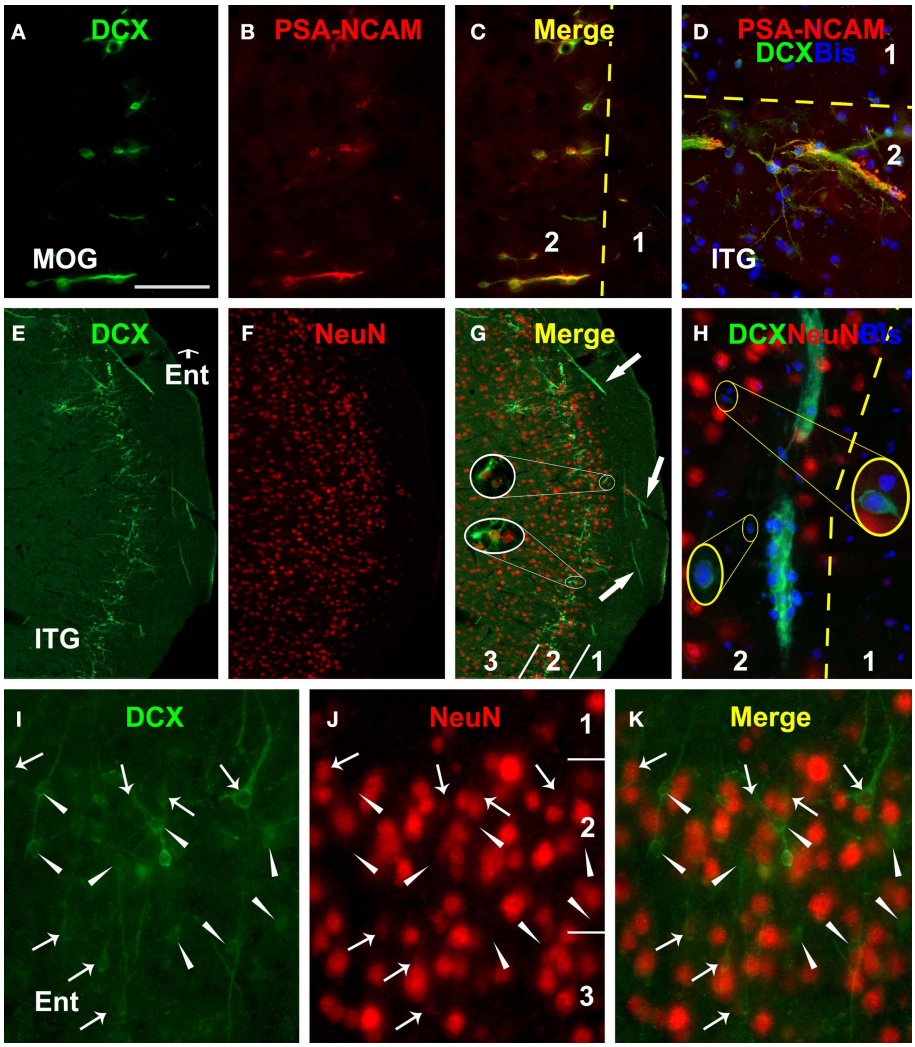

FIGURE 6 | Immunofluorescent images from the 22-year-old monkey illustrating colocalization of polysialylated neural cell adhesion molecule (PSA-NCAM) and neuron-specific nuclear protein (NeuN) in DCX+ cells (including bisbenzimide nuclear stain in D and H) in the prefrontal (MOG, A–C) and temporal lobe cortex (D–K). PSA-NCAM and DCX are colocalized in individual cells as well as cells arranged in chains perpendicular **(A–C)** or tangential **(D)** to the cortical surface. NeuN is detectable in a small number of DCX+ cells with relatively larger size and lighter DCX reactivity (arrows in **I–J**, and enlarged circles in **G**) than those lack colocalization (arrowheads in **I–K**). Note DCX+ tracts and migratory chains in layers I **(E–G)** and II **(H)**. Abbreviations are the same as in **Figure 2**. Scale bar = 100 μm in **(A–C)**, equivalent to 200 μm for **(E–G)**, 50 μm for **(D,I–K)**.

Moreover, some TH+ axonal plexuses appeared to extend alongside or coil around the chain-like migratory elements of DCX+ cells oriented inwardly or tangentially in the superficial cortical layers (**Figures 7G–K**).

Following an initial check for extracellular amyloid deposition in all aged animals, we carried out DCX and 6E10 double immunofluorescence in the cortex from two aged monkeys with cerebral plaques in an effort to explore if there was direct and apparent impact between DCX+ cells and plaque pathology. DCX+ cells were not found to preferentially occur around 6E10 labeled amyloid plaques. Also, in layers II and III the distribution or number of DCX+ cells did not appear to be apparently altered around areas with plaques relative to plaque-free areas in the sections we examined (**Figures 7L–O**).

DISCUSSION

DCX+ CORTICAL CELLS IN COMPARISON WITH ADULT-BORN DENTATE GRANULAR CELLS

Recent studies by our group and others have gathered substantial information on cortical DCX+ cells or alike in various mammalian species, which now allows a comparison between these novel cortical cells and the conventional adult-born neuronal populations in SVZ and SGZ (Gómez-Climent et al., 2008; Liu et al., 2008; Xiong et al., 2008; Luzzati et al., 2009). Taking the newly-generated SGZ immature neuronal population as an example, we attempted to update relevant features of cortical and hippocampal DCX+ cells (see **Table 1**). Similar properties of these two populations include that both groups: (1) express the very same set of immature neuronal markers including DCX, PSA-NCAM, neuron-specific β-tubulin-III

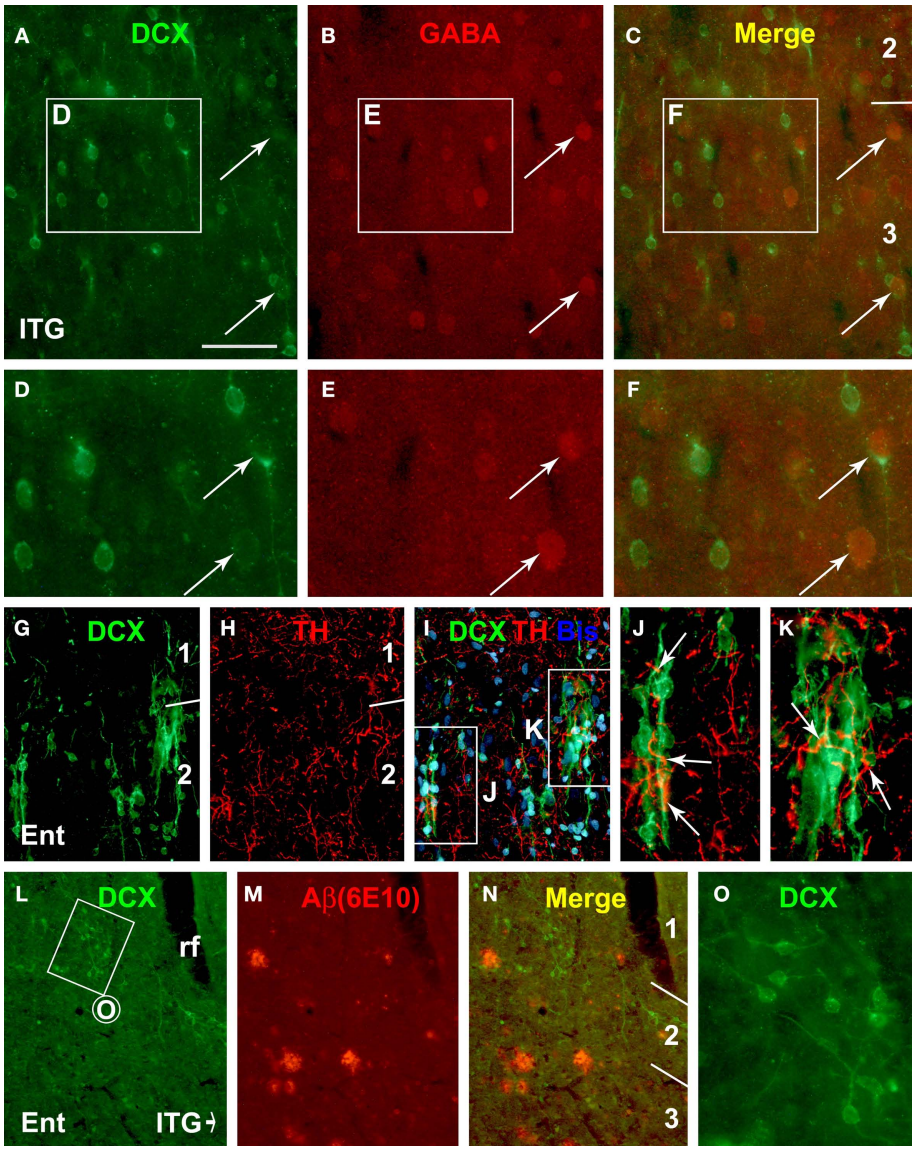

FIGURE 7 | Representative immunofluorescent images from the 19-year-old (A–K) and the 34-year-old (L–O) monkeys depicting double labeling of DCX with γ-aminobutyric acid (GABA), tyrosine hydroxylase (TH) and β-amyloid peptide (Aβ, 6E10 staining) in the cortical areas as indicated. GABA immunoreactivity is detectable in a small subset of relatively large DCX+ cells (arrows, **A–F**). TH immunoreactive axonal plexuses (arrows) occur around DCX+ cells and chains in layer II **(G–K)**. DCX+ cells do not appear to preferentially occur around amyloid plaques, nor do they apparently disappear from areas with plaques **(L–N)**. Scale bar = 50 μm in **(A–C)** applying to **(J,K)**, equivalent to 100 μm for **(G–I,L–N)**, and 25 μm for **(D–F,O)**.

(TuJ1) and Hu; (2) exhibit a diverse variation in somal size and shape, nuclear appearance in bisbenzimide stain and complexity of neuritic processes, all suggesting cell growth and morphological maturation; (3) display a pattern of transient expression of immature neuronal markers that overlap partially with the emergence of mature neuronal markers but correlate with morphological development (Brown et al., 2003; Cai et al., 2009); (4) may arrange as tightly-apposed cell clusters and/or as migratory chains, suggestive of certain neuroblast-like behavior or property (Gritti et al., 2002; Seki et al., 2007; Xiong et al., 2008; Cai et al., 2009); (5) reduce in number with age in all studied mammals; (6) might be modulated by some antidepressants at least according to rodent studies (Sairanen et al., 2007).

However, to date the most confusing (yet compelling) issue regarding cortical DCX+ cells is whether they are generated prenatally, postnatally or even throughout adult life. Several groups report a low rate of BrdU incorporation into DCX+ cells in the piriform or temporal lobe cortex in small laboratory rodents and primates (Bernier et al., 2002; Tonchev et al., 2003; Pekcec et al., 2006; Shapiro et al., 2007, 2008). In contrast, others suggest that this population of cortical cells is produced prenatally (Gómez-Climent et al., 2008; Luzzati et al., 2009). With this regard, the current finding of persistent occurrence of DCX+ cells in the cortex and amygdala virtually to the end of life in nonhuman primates is very puzzling. For instance, it appears difficult to explain how prenatally-born

Table 1 | Comparison of hippocampal with cortical DCX+ cells.

Features or observations	SGZ	Layers II/III
Transient expression of immature neuronal markers[1]	Yes	Yes
Partial co-expression of mature neuronal markers[2]	Yes	Yes
Heterogeneous developing neuronal morphology	Yes	Yes
Response to antidepressants	Yes	Likely
Time of cell presence during postnatal life	To mid-age	Life long
Migration	Across the GCL	Inwardly
Definitive or putative destiny	Granule cells	Interneurons
BrdU incorporation	Yes	Inconsistent
Retrovirus incorporation	Yes	No report

[1]*DCX, PSA-NCAM, neuron-specific* β*-tubulin-III (TuJ1) and Hu.*
[2]*NeuN and some terminal phenotype markers of interneurons.*

neuronal precursors form seemingly more extended migratory chains and overtly expanded or bizarre-looking cell clusters in aged rather than younger adult animals.

POTENTIAL EFFECT OF AGING ON DCX+ CELL MIGRATION IN THE CORTEX

As aforementioned, the arrangement of cortical DCX+ cells as extended tangential migratory chains and expanded/distorted migratory apparatus in layers I/II in mid-age and aged primates is of interest. This pattern seems to fit with our earlier speculation that the lamination and clusterization of DCX+ cells at this location might be somehow related to layer I or the marginal zone, which is an embryonic and potentially postnatal neurogenic site (Marin-Padilla, 1978; Letinic et al., 2002; Costa et al., 2007; Xiong et al., 2008). As shown earlier (Cai et al., 2009), cortical DCX+ cells in young adult cats and monkeys appear to mostly migrate from layer II to deeper layers. Thus, the increased tendency of tangential cell migration in older animals might implicate certain type of age-related alteration in the distribution of cortical DCX+ cells.

Previous studies suggest that the radial migration of newly-generated hippocampal neurons might be impaired in aged rodents, dogs and monkeys (Siwak-Tapp et al., 2007; Ribak and Shapiro, 2008; Hwang et al., 2009). Thus, DCX+ cells in aged dentate gyrus tend to retain at the SGZ with their long somal diameter parallel to the GCL. In contract, dentate DCX+ cells in younger animals migrate across the GCL during their morphological maturation (Shapiro et al., 2005). Thus, the extended tangential migration and distorted clusterization of DCX+ cells around layers I and II in aged monkeys could potentially reflect certain deficit of migration or dispersion/descending of these cells in the cortex.

FUNCTIONAL CONSIDERATION FOR LIFE-LONG PRESENCE OF DCX+ CELLS IN PRIMATE CEREBRUM

Little is currently known about the functional implication for a life-long presence of putative immature and developing neurons in mammalian associative cerebral cortex and amygdala. Giving their potential GABAergic fate, it seems plausible that these novel cells might be involved in interneuron plasticity under physiological conditions, and perhaps interneuron dysfunction under certain disease conditions as exampled below.

Recent studies suggest that interneuron deficits might relate to the etiology of certain neuropsychiatric diseases. It appears that bipolar disorder, major depression and schizophrenia are associated with reduced GABAergic interneurons and/or altered inhibitory neurotransmission in the prefrontal cortex (Benes et al., 2000; Beasley et al., 2002; Lewis et al., 2004, 2008; Di Cristo, 2007; Rajkowska et al., 2007; Sanacora and Saricicek, 2007; Lodge et al., 2009). We observe a substantial population of DCX+ cells in the prefrontal cortex of young adult guinea pigs, cats and monkeys, and as shown in the present study, these cells are likely to be in close anatomic relationship with dopaminergic and/or norepinephrinergic projections. Thus, whether the differentiation and maturation of DCX+ cortical cells into putative interneuron subgroups may be impaired under certain conditions and be modulated by antidepressant or anti-psychiatric drugs are of potential medical relevance.

Structural and functional changes in the interneuron systems may relate to the pathophysiology of temporal lobe epilepsy (Ben-Ari, 2006). Two late studies show altered DCX expression in the temporal cortex from epileptic human subjects relative to controls (Liu et al., 2008; Srikandarajah et al., 2009). Other studies suggest changes in GABAergic neurons and inhibitory neuronal circuitry in chronic epileptic human temporal cortex and amygdala (Arellano et al., 2004; Yilmazer-Hanke et al., 2007; Knopp et al., 2008; González-Martínez et al., 2009). Thus, the relevance of cortical DCX+ cells to seizure or epilepsy-related aberrant interneuron plasticity deserves further investigation.

A role for impaired neurogenesis in cognitive decline during aging and in Alzheimer's disease has been lately proposed according to studies of adult-born neuronal populations in the hippocampus and SVZ (Galvan and Bredesen, 2007; Jessberger and Gage, 2008). We observe diminished DCX+ subgranular cells in mid-age and aged monkeys, consistent with other reports that hippocampal neurogenesis is dramatically reduced around mid-age in most mammals (Simon et al., 2005; Leuner et al., 2007; Siwak-Tapp et al., 2007; Hattiangady et al., 2008; Ribak and Shapiro, 2008; Hwang et al., 2009). Our current study however reveals a remarkably intriguing fact that putative immature cortical neurons persist into very old age in non-human primates, in sharp contrast to a great loss of hippocampal neurogenesis well before the onset of senescence. Thus, there exists an extended time window for a potential involvement of cortical and amygdalar DCX+ cells in aging-related changes in neuronal plasticity and perhaps cognitive decline in nonhuman primates. Of particular concern regarding this finding includes whether DCX+ cortical and amygdalar neurons may be altered in Alzheimer's disease. The present study could not establish a clear inductive or destructive effect of amyloid plaques on DCX+ cells in aged monkey cerebrum. Obviously, we could not rule out such a possibility, giving the limited sample size available for this study.

In summary, the present study demonstrates a population of putative developing neurons with a presumable GABAergic fate that persists into advanced age in rhesus monkeys, especially in the associative cerebral cortical areas and amygdala. This finding is in contrast to a great, if not complete, loss of DCX+ cells in the hippocampal dentate gyrus in the aged animals. The data implicate a potential life-long role

for novel immature neurons in cognition-related cerebral structures, as well as a protracted time window for possible interaction or modulation between these cells and aging-related neurobiological or neuropathological factors in primates during their late life.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/neuroanatomy/paper/10.3389/neuro.05/017.2009/

REFERENCES

Aimone, J. B., Wiles, J., and Gage, F. H. (2009). Computational influence of adult neurogenesis on memory encoding. *Neuron* 61, 187–202.

Arellano, J. I., Muñoz, A., Ballesteros-Yáñez, I., Sola, R. G., and DeFelipe, J. (2004). Histopathology and reorganization of chandelier cells in the human epileptic sclerotic hippocampus. *Brain* 127, 45–64.

Arendt, T. (2005). Alzheimer's disease as a disorder of dynamic brain self-organization. *Prog. Brain Res.* 147, 355–378.

Azmitia, E. C. (2007). Cajal and brain plasticity: insights relevant to emerging concepts of mind. *Brain Res. Rev.* 55, 395–405.

Beasley, C. L., Zhang, Z. J., Patten, I., and Reynolds, G. P. (2002). Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Biol. Psychiatry* 52, 708–715.

Ben-Ari, Y. (2006). Seizures beget seizures: the quest for GABA as a key player. *Crit. Rev. Neurobiol.* 18, 135–144.

Benes, F. M., Todtenkopf, M. S., Logiotatos, P., and Williams, M. (2000). Glutamate decarboxylase(65)-immunoreactive terminals in cingulate and prefrontal cortices of schizophrenic and bipolar brain. *J. Chem. Neuroanat.* 20, 259–269.

Bernier, P. J., Bedard, A., Vinet, J., Levesque, M., and Parent, A. (2002). Newly generated neurons in the amygdala and adjoining cortex of adult primates. *Proc. Natl. Acad. Sci. USA* 99, 11464–11469.

Brambilla, P., Hatch, J. P., and Soares, J. C. (2008). Limbic changes identified by imaging in bipolar patients. *Curr. Psychiatry Rep.* 10, 505–509.

Brown, J. P., Couillard-Després, S., Cooper-Kuhn, C. M., Winkler, J., Aigner, L., and Kuhn, H. G. (2003). Transient expression of doublecortin during adult neurogenesis. *J. Comp. Neurol.* 467, 1–10.

Bruel-Jungerman, E., Davis, S., and Laroche, S. (2007). Brain plasticity mechanisms and memory: a party of four. *Neuroscientist* 13, 492–505.

Cai, Y., Xiong, K., Chu, Y., Luo, D. W., Luo, X. G., Yuan, X. Y., Struble, R. G., Clough, R. W., Spencer, D. D., Williamson, A., Kordower, J. H., Patrylo, P. R., and Yan, X. X. (2009). Doublecortin expression in adult cat and primate cerebral cortex relates to immature neurons that develop into GABAergic subgroups. *Exp. Neurol.* 216, 342–356.

Campbell, M. J., Lewis, D. A., Foote, S. L., and Morrison, J. H. (1987). Distribution of choline acetyltransferase-, serotonin-, dopamine-beta-hydroxylase-, tyrosine hydroxylase-immunoreactive fibers in monkey primary auditory cortex. *J. Comp. Neurol.* 261, 209–220.

Chu, Y., and Kordower, J. H. (2007). Age-associated increases of alpha-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: is this the target for Parkinson's disease? *Neurobiol. Dis.* 25, 134–149.

Costa, M. R., Kessaris, N., Richardson, W. D., Götz, M., and Hedin-Pereira, C. (2007). The marginal zone/layer I as a novel niche for neurogenesis and gliogenesis in developing cerebral cortex. *J. Neurosci.* 27, 11376–11388.

Couillard-Despres, S., Winner, B., Schaubeck, S., Aigner, R., Vroemen, M., Weidner, N., Bogdahn, U., Winkler, J., Kuhn, H. G., and Aigner, L. (2005). Doublecortin expression levels in adult brain reflect neurogenesis. *Eur. J. Neurosci.* 21, 1–14.

Di Cristo, G. (2007). Development of cortical GABAergic circuits and its implications for neurodevelopmental disorders. *Clin. Genet.* 72, 1–8.

Finlay, B. L., and Darlington, R. B. (1995). Linked regularities in the development and evolution of mammalian brains. *Science*, 268, 1578–1584.

Galvan, V., and Bredesen, D. E. (2007). Neurogenesis in the adult brain: implications for Alzheimer's disease. *CNS Neurol. Disord. Drug Targets* 6, 303–310.

Gómez-Climent, M. A., Castillo-Gómez, E., Varea, E., Guirado, R., Blasco-Ibáñez, J. M., Crespo, C., Martínez-Guijarro, F. J., and Nácher, J. (2008). A population of prenatally generated cells in the rat paleocortex maintains an immature neuronal phenotype into adulthood. *Cereb. Cortex* 18, 2229–2240.

González-Martínez, J. A., Möddel, G., Ying, Z., Prayson, R. A., Bingaman, W. E., and Najm, I. M. (2009). Neuronal nitric oxide synthase expression in resected epileptic dysplastic neocortex. *J. Neurosurg.* 110, 343–349.

Gritti, A., Bonfanti, L., Doetsch, F., Caille, I., Alvarez-Buylla, A., Lim, D. A., Galli, R., Verdugo, J. M., Herrera, D. G., and Vescovi, A. L. (2002). Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents. *J. Neurosci.* 22, 437–445.

Hattiangady, B., Rao, M. S., and Shetty, A. K. (2008). Plasticity of hippocampal stem/progenitor cells to enhance neurogenesis in response to kainite-induced injury is lost by middle age. *Aging Cell* 7, 207–224.

Hernández-Rabaza, V., Llorens-Martín, M., Velázquez-Sánchez, C., Ferragud, A., Arcusa, A., Gumus, H. G., Gómez-Pinedo, U., Pérez-Villalba, A., Roselló, J., Trejo, J. L., Barcia, J. A., and Canales J. J. (2009). Inhibition of adult hippocampal neurogenesis disrupts contextual learning but spares spatial working memory, long-term conditional rule retention and spatial reversal. *Neuroscience* 159, 59–68.

Hwang, I. K., Yoo, K. Y., Park, O. K., Choi, J. H., Lee, C. H., and Won, M. H. (2009). Comparison of density and morphology of neuroblasts in the dentate gyrus among variously aged dogs, German shepherds. *J. Vet. Med. Sci.* 71, 211–215.

Jessberger, S., and Gage, F. H. (2008). Stem-cell-associated structural and functional plasticity in the aging hippocampus. *Psychol. Aging* 23, 684–691.

Knopp, A., Frahm, C., Fidzinski, P., Witte, O. W., and Behr, J. (2008). Loss of GABAergic neurons in the subiculum and its functional implications in temporal lobe epilepsy. *Brain* 131, 1516–1527.

Krubitzer, L. (2009). In search of a unifying theory of complex brain evolution. *Ann. NY. Acad. Sci.* 1156, 44–67.

Letinic, K., Zoncu, R., and Rakic, P. (2002). Origin of GABAergic neurons in the human neocortex. *Nature* 417, 645–649.

Leuner, B., Kozorovitskiy, Y., Gross, C. G., and Gould, E. (2007). Diminished adult neurogenesis in the marmoset brain precedes old age. *Proc. Natl. Acad. Sci. USA* 104, 17169–17173.

Lewis, D. A., Campbell, M. J., Foote, S. L., Goldstein, M., and Morrison, J. H. (1987). The distribution of tyrosine hydroxylase-immunoreactive fibers in primate neocortex is widespread but regionally specific. *J. Neurosci.* 7, 279–290.

Lewis, D. A., Cruz, D., Eggan, S., and Erickson, S. (2004). Postnatal development of prefrontal inhibitory circuits and the pathophysiology of cognitive dysfunction in schizophrenia. *Ann. NY. Acad. Sci.* 1021, 64–76.

Lewis, D. A., Hashimoto, T., and Morris, H. M. (2008). Cell and receptor type-specific alterations in markers of GABA neurotransmission in the prefrontal cortex of subjects with schizophrenia. *Neurotox. Res.* 14, 237–248.

Liu, Y. W., Curtis, M. A., Gibbons, H. M., Mee, E. W., Bergin, P. S., Teoh, H. H., Connor, B., Dragunow, M., and Faull, R. L. (2008). Doublecortin expression in the normal and epileptic adult human brain. *Eur. J. Neurosci.* 28, 2254–2265.

Lledo, P. M., Alonso, M., and Grubb, M. S. (2006). Adult neurogenesis and functional plasticity in neuronal circuits. *Nat. Rev. Neurosci.* 7, 179–193.

Lodge, D. J., Behrens, M. M., and Grace, A. A. (2009). A loss of parvalbumin-containing interneurons is associated with diminished oscillatory activity in an animal model of schizophrenia. *J. Neurosci.* 29, 2344–2354.

Luzzati, F., Bonfanti, L., Fasolo, A., and Peretto, P. (2009). DCX and PSA-NCAM expression identifies a population of neurons preferentially distributed in associative areas of different pallial derivatives and vertebrate species. *Cereb. Cortex* 19, 1028–1041.

Magavi, S. S., Leavitt, B. R., and Macklis, J. D. (2000). Induction of neurogenesis in the neocortex of adult mice. *Nature* 405, 951–955.

Magavi, S. S., Mitchell, B. D., Szentirmai, O., Carter, B. S., and Macklis, J. D. (2005). Adult-born and preexisting olfactory granule neurons undergo distinct experience-dependent modifications of their olfactory responses in vivo. *J. Neurosci.* 25, 10729–10739.

Marin-Padilla, M. (1978). Dual origin of the mammalian neocortex and evolution of the cortical plate. *Anat. Embryol. (Berl.)* 152, 109–126.

Pavuluri, M. N., and Passarotti, A. (2008). Neural bases of emotional processing in pediatric bipolar disorder. *Expert Rev. Neurother.* 8, 1381–1387.

Pekcec, A., Löscher, W., and Potschka, H. (2006). Neurogenesis in the adult rat piriform cortex. *Neuroreport* 17, 571–574.

Perera, T. D., Park, S., and Nemirovskaya, Y. (2008). Cognitive role of neurogenesis

in depression and antidepressant treatment. *Neuroscientist* 14, 326–338.

Pierani, A., and Wassef, M. (2009). Cerebral cortex development: from progenitors patterning to neocortical size during evolution. *Dev. Growth Differ.* 51, 325–342.

Rajkowska, G., O'Dwyer, G., Teleki, Z., Stockmeier, C. A., and Miguel-Hidalgo, J. J. (2007). GABAergic neurons immunoreactive for calcium binding proteins are reduced in the prefrontal cortex in major depression. *Neuropsychopharmacology* 32, 471–482.

Ribak, C. E., and Shapiro, L. A. (2008). Newly generated neurons in the dentate gyrus of aged epileptic Sprague-Dawley rats. *Soc. Neurosci. Abstr.* 122.25.

Sairanen, M., O'Leary, O. F., Knuuttila, J. E., and Castrén, E. (2007). Chronic antidepressant treatment selectively increases expression of plasticity-related proteins in the hippocampus and medial prefrontal cortex of the rat. *Neuroscience* 144, 368–374.

Sanacora, G., and Saricicek, A. (2007). GABAergic contributions to the pathophysiology of depression and the mechanism of antidepressant action. *CNS Neurol. Disord. Drug Targets* 6, 127–140.

Seki, T., Namba, T., Mochizuki, H., and Onodera, M. (2007). Clustering, migration, and neurite formation of neural precursor cells in the adult rat hippocampus. *J. Comp. Neurol.* 502, 275–290.

Shapiro, L. A., Korn, M. J., Shan, Z., and Ribak, C. E. (2005). GFAP-expressing radial glia-like cell bodies are involved in a one-to-one relationship with doublecortin-immunolabeled newborn neurons in the adult dentate gyrus. *Brain Res.* 1040, 81–91.

Shapiro, L. A., Ng, K., Zhou, Q. Y., and Ribak, C. E. (2008). Subventricular zone-derived, newly generated neurons populate several olfactory and limbic forebrain regions. *Epilepsy Behav.* 14, 74–80.

Shapiro, L. A., Ng, K. L., Kinyamu, R., Whitaker-Azmitia, P., Geisert, E. E., Blurton-Jones, M., Zhou, Q. Y., and Ribak, C. E. (2007). Origin, migration and fate of newly generated neurons in the adult rodent piriform cortex. *Brain Struct. Funct.* 212, 133–148.

Shors, T. J., Miesegaes, G., Beylin, A., Zhao, M., Rydel, T., and Gould, E. (2001). Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 410, 372–376.

Siebzehnrubl, F. A., and Blumcke, I. (2008). Neurogenesis in the human hippocampus and its relevance to temporal lobe epilepsies. *Epilepsia* 49, 55–65.

Simon, M., Czéh, B., and Fuchs, E. (2005). Age-dependent susceptibility of adult hippocampal cell proliferation to chronic psychosocial stress. *Brain Res.* 1049, 244–248.

Siwak-Tapp, C. T., Head, E., Muggenburg, B. A., Milgram, N. W., and Cotman, C. W. (2007). Neurogenesis decreases with age in the canine hippocampus and correlates with cognitive function. *Neurobiol. Learn. Mem.* 88, 249–259.

Srikandarajah, N., Martinian, L., Sisodiya, S. M., Squier, W., Blumcke, I., Aronica, E., and Thom, M. (2009). Doublecortin expression in focal cortical dysplasia in epilepsy. *Epilepsia*. doi: 10.1111/j.1528-1167.2009.02194.x

Tonchev, A. B., Yamashima, T., Zhao, L., Okano, H. J., and Okano, H. (2003). Proliferation of neural and neuronal progenitors after global brain ischemia in young adult macaque monkeys. *Mol. Cell. Neurosci.* 23, 292–301.

Varea, E., Blasco-Ibáñez, J. M., Gómez-Climent, M. A., Castillo-Gómez, E., Crespo, C., Martínez-Guijarro, F. J., and Nácher, J. (2007). Chronic fluoxetine treatment increases the expression of PSA-NCAM in the medial prefrontal cortex. *Neuropsychopharmacology* 32, 803–812.

Xiong, K., Luo, D. W., Patrylo, P. R., Luo, X. G., Struble, R. G., Clough, R. W., and Yan, X. X. (2008). Doublecortin-expressing cells are present in layer II across the adult guinea pig cerebral cortex: partial colocalization with mature interneuron markers. *Exp. Neurol.* 211, 271–282.

Yilmazer-Hanke, D. M., Faber-Zuschratter, H., Blümcke, I., Bickel, M., Becker, A., Mawrin, C., and Schramm, J. (2007). Axo-somatic inhibition of projection neurons in the lateral nucleus of amygdala in human temporal lobe epilepsy: an ultrastructural study. *Exp. Brain Res.* 177, 384–399.

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