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Altered Intrinsic Properties of Neuronal Subtypes in Malformed Epileptogenic Cortex

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Abstract

Neuronal intrinsic properties control action potential firing rates and serve to define particular neuronal subtypes. Changes in intrinsic properties have previously been shown to contribute to hyperexcitability in a number of epilepsy models. Here we examined whether a developmental insult producing the cortical malformation of microgyria altered the identity or firing properties of layer V pyramidal neurons and two interneuron subtypes. Trains of action potentials were elicited with a series of current injection steps during whole cell patch clamp recordings. Cells in malformed cortex identified as having an apical dendrite had firing patterns similar to control pyramidal neurons. The duration of the second action potential in the train was increased in paramicrogyral (PMG) pyramidal cells, suggesting that these cells may be in an immature state, as was previously found for layer II/III pyramidal neurons. Based on stereotypical firing patterns and other intrinsic properties, fast-spiking (FS) and low threshold-spiking (LTS) interneuron subpopulations were clearly identified in both control and malformed cortex. Most intrinsic properties measured in malformed cortex were unchanged, suggesting that subtype identity is maintained. However, LTS interneurons in lesioned cortex had increased maximum firing frequency, decreased initial afterhyperpolarization duration, and increased total adaptation ratio compared to control LTS cells. FS interneurons demonstrated decreased maximum firing frequencies in malformed cortex compared to control FS cells. These changes may increase the efficacy of LTS while decreasing the effectiveness of FS interneurons. These data indicate that differential alterations of individual neuronal subpopulations may endow them with specific characteristics that promote epileptogenesis.

Keywords

epilepsy; freeze-lesion; GABAergic; neocortex; parvalbumin; somatostatin

1. Introduction

Neuronal output is shaped by intrinsic properties that influence the reliability and pattern of action potential firing in response to synaptic input ([Berger et al., 2003], [Kim et al., 2005], [Beck et al., 2008]). These properties are secondary to the complement of ion channels located in the neuronal membrane, as well as the currents that pass through them ([Jung et

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al., 2001], [Yue et al., 2004], [Beck et al., 2008]). Increasingly, central nervous system disorders such as epilepsy are being linked to aberrant ion channel expression and the corresponding current alterations ([Castro et al., 2001], [Bernard et al., 2004], [Jung et al., 2007], [Steinlein et al., 2009], [Estacion et al., 2010], [Graef et al., 2010]). In particular, this has been demonstrated in pediatric epilepsy related to cortical dysplasia ([Mathern et al., 2000], [Cepeda et al., 2003], [Gil-Nagel, 2003]). Although the mechanisms that underlie seizures are not fully known, it is likely that changes to both neuronal intrinsic properties and synaptic connectivity play a role ([Prince et al., 1993], [Bush et al., 1999], [Bender et al., 2008]). In fact, when modeling epilepsy in a paradigm that does not include disinhibition, alteration of intrinsic properties is necessary for the development of epileptiform activity (Bush et al., 1999). Specifically, the creation or enhancement of a neuron's capacity to generate bursts of action potentials promotes the development of hyperexcitability ([Prince, 1985], [Chagnac-Amitai et al., 1989], [Sanabria et al., 2001], [Wellmer et al., 2002]).

Alterations in intrinsic cellular properties that appear to play a role in the pathology of hyperexcitability have been demonstrated in various animal models, including those of developmental malformations. For example, in the telencephalic internal structural heterotopias (TISH) rat that mimics subcortical band heterotopia, pyramidal cells identified morphologically and by firing pattern had resting potentials that were more depolarized than those in control cells (Trotter et al., 2006). In a model of post-traumatic cortical hyperexcitability, pyramidal cells demonstrate higher input resistance, a longer time constant and a steeper f-I slope than control (Prince et al., 1993). Also, in the methylazoxymethanol (MAM) model of cortical dysplasia and heterotopia, some pyramidal cells exhibited smaller peak amplitudes, higher input resistance, longer action potential duration, less adaptation and higher firing frequency than control (Castro et al., 2001). Abnormal potassium channel expression in hippocampus (Castro et al., 2001) and excessive bursting behavior in neocortex (Sancini et al., 1998) were also observed in this model. In a previous study of this model, pyramidal cells in layer II/III were shown to have altered intrinsic properties, including decreased: spike amplitude; maximum rate of spike rise; dV/dt ratio; and primary f-I slope (Luhmann et al., 1998). These characteristics may reflect delayed maturation, which has emerged as a theme in this model ([Rosen et al., 1992], [Super et al., 1997], [Luhmann et al., 1998], [Hablitz et al., 2000]) and is also characteristic of human cortical dysplasia (Cepeda et al., 2006). For the purpose of comparison with the layer II/III results, but also because epileptiform activity likely originates in layer V (Telfeian et al., 1998), the work presented here was focused on layer V. In the freeze-lesioned cortex, excitatory synaptic input to layer V pyramidal cells gradually increases during the second postnatal week (Zsombok et al., 2007), but network epileptiform activity cannot be evoked until postnatal day (P)12 (Jacobs et al., 1999a). It is possible that until P12, concurrent developmental processes in the inhibitory cell population are sufficient to dampen the effect of increased excitation. Normally, by the end of the second postnatal week, a number of properties of both inhibitory and excitatory neurons approach mature values ([McCormick et al., 1987], [Alcantara et al., 1993], [Massengill et al., 1997], [Franceschetti et al., 1998], [Rivera et al., 1999]). If maturation of intrinsic properties is delayed or otherwise altered in either population, it may be sufficient to "unmask" the synaptic changes. Therefore, we have chosen to investigate the intrinsic properties not only of pyramidal cells, but also of interneurons in layer V of malformed cortex.

Interneurons exhibit a multiplicity of properties that have been used to categorize them into a number of distinct groups ([Kawaguchi et al., 1993], [Kawaguchi, 1995], [Cauli et al., 1997], [Cauli et al., 2000], [Hernandez-Miranda et al., 2010]). The morphological variety of inhibitory cell types suggests that there is normally an anatomical division of labor among the interneurons of the cortex (Markram et al., 2004). Previous epilepsy studies suggest that

interneuron subtypes can be selectively affected in both animal model and human patient tissue ([DeFelipe et al., 1993], [Buckmaster et al., 1997], [Rosen et al., 1998], [Powell et al., 2003], [Trotter et al., 2006]). For this reason, it is necessary to compare not only excitatory and inhibitory cell types, but also interneuron subtypes individually. In particular, fast-spiking (FS) and low-threshold-spiking (LTS) interneurons have been well-described and differ significantly on a number of intrinsic characteristics ([Kawaguchi et al., 1993], [Kawaguchi, 1993], [Gibson et al., 1999], [Beierlein et al., 2003]). FS interneurons are immune-positive for parvalbumin (PV, [Kawaguchi, 1995], [Cauli et al., 1997]), have basket cell or chandelier cell morphology ([Kawaguchi, 1995], [Kawaguchi et al., 2002]), receive powerful thalamocortical input and synaptically target cell bodies and thick dendrites (Kawaguchi et al., 1997). In FS cells, action potential half-width is typically less than 1 ms with little to no adaptation in the characteristically high action potential firing frequency ([Kawaguchi et al., 1993], [Cauli et al., 1997]). In contrast, LTS interneurons have an action potential half-width slightly greater than 1 ms and an action potential firing pattern that shows clear adaptation (Kawaguchi et al., 1993). LTS interneurons are often immunopositive for somatostatin (SS) ([Kawaguchi, 1995], [Cauli et al., 1997]), have bipolar or bitufted morphology ([Kawaguchi, 1995], [Kawaguchi et al., 2002]), receive little input from thalamus, and synaptically target thin dendrites distant from the soma (Kawaguchi et al., 1997).

Here we have examined the intrinsic properties of layer V pyramidal cells and two subtypes of interneurons to determine: (1) whether subtype identity is maintained in malformed cortex; (2) if the focal loss of deep layers during the last stages of neuroblast migration into the cortical plate can alter intrinsic properties; and (3) whether neuronal subtypes are differentially affected by this developmental insult.

2. Results

Classification of Neuronal Subtypes

Whole cell recordings were made in current clamp from neurons in layer V of control and paramicrogyral (PMG) cortex. Cells were depolarized with a series of currents (400 ms duration step pulse), eliciting low and high frequency trains of action potentials (Fig. 1). Within PMG cortex, we observed a range of neuronal action potential firing patterns that was similar to what was seen in control cortex. A number of previously published studies have used these characteristic patterns to identify interneuron subtypes ([Kawaguchi, 1993], [Kawaguchi, 1995], [Gibson et al., 1999], [Beierlein et al., 2000], [Beierlein et al., 2003], [Bacci et al., 2003], [Bacci et al., 2004], [Xiang et al., 2006]). Based on firing patterns, the recorded cells were placed into one of four groups; pyramidal neurons (Fig. 1A,B), FS interneurons (Fig. 1C), LTS interneurons (Fig. 1D), or unknown subtype. Some neurons recorded in this study showed firing patterns similar to our recordings of pyramidal cells from other studies, and thus we have called these suspected pyramidal cells (Fig. 1B). To help classify these cells, the firing patterns from pyramidal cells identified in a separate study were used here for comparison (Fig. 1A).

A quantitative confirmation of this pattern-based group assignment is shown in figure 2, where total adaptation is plotted against the amplitude of the first AHP minus the amplitude of the last AHP for individual cells (AHP difference). Adaptation of action potential firing frequency is commonly used to distinguish LTS from FS interneurons ([Kawaguchi, 1993], [Beierlein et al., 2003]). The AHP difference has also been shown to reliably distinguish pyramidal cells from LTS interneurons (Beierlein et al., 2003). In order to confirm that this measure could be used in our data to separate LTS cells from the pyramidal cells mistakenly recorded while looking for interneurons, we included the previously identified pyramidal cells in this plot as well. This graph shows a clear demarcation between three neuronal cell

types (pyramidal, FS, LTS) for both control *and* PMG populations (Fig. 2). Using this methodology, several cells were not classified as FS, LTS, or pyramidal cells. The AHP difference indicated that these cells were unlikely to be pyramidal, but their adaptation ratio did not clearly align them with one of the two interneuron subpopulations. It is likely that these cells represent one or more additional subtypes of interneurons that are beyond the scope of this study. Additionally, the “suspected pyramidal cells” clearly segregated with the group of known pyramidal cells (Fig. 2).

Intrinsic Properties of Cortical Neuronal Subtypes

A number of intrinsic properties have been useful in differentiating interneuron subtypes ([Kawaguchi, 1993], [Cauli et al., 1997], [Cauli et al., 2000], [Kawaguchi et al., 2002]). Here we have made the same measurements as Cauli et al (Cauli et al., 1997, 2000), since they had the most extensive list that clearly separated cell types. This analysis allows us to determine if the intrinsic properties that differentiate interneuron subtypes are altered in PMG cortex. It also confirms whether each action potential firing pattern is consistent with a specific array of intrinsic properties in PMG cortex as it is in control. For control cells, our values were very similar to those reported by Cauli et al (2000) in most cases, including first and second action potential duration, percent increase in action potential duration, first and second afterhyperpolarization amplitudes, and relative values for early adaptation ratio (Table 1). Our LTS cells were similar to their RSNP-SS interneurons. There were some dissimilarities between our values and Cauli's for accommodative hump, minimal firing frequency and time of minimal firing frequency. All of these discrepancies are likely due to the difference in the length of our depolarizing step (400 msec) compared to theirs (800 msec). In addition, we did not restrict our calculation of minimal frequency to sweeps that began with frequencies of 100 Hz or more. However, even with these differences, minimal firing frequency showed the same relative differences between FS and LTS cell populations in our data (LTS significantly lower than FS, Table 1) as FS and RSNP-SS did in Cauli's. The other measures that distinguished FS and LTS interneurons within the control group were the first and second action potential duration, percent increase in action potential duration, AHP peak, early and late adaptation ratios, and accommodative hump (Table 1, Fig. 3).

Pyramidal cell intrinsic properties

A previous study of layer II/III pyramidal neurons in freeze-lesioned microgyral cortex found that cells in lesioned cortex exhibited slower action potentials compared to control, suggesting delayed maturation of this intrinsic property (Luhmann et al., 1998). Similarly, we found that in a train of action potentials, the half-width of the second action potential was wider for layer V pyramidal neurons in PMG cortex compared to control (Fig 3). For other measures, including maximum rate of rise or decay, or primary frequency vs current (F/I) slope, we found no differences between control and PMG layer V pyramidal neurons (Table 2), in contrast to previous findings in layer II/III.

Interneuron Subtype Identity is Maintained in Malformed Cortex

For a number of intrinsic properties, there was no difference between control and PMG interneurons, including the first and second action potential duration, percent increase in this duration, first and second AHP duration, early and late adaptation ratios, accommodative hump, minimal frequency and time for minimal frequency (Table 1, Fig. 3). The measures that distinguished FS and LTS interneuron subtypes in control cortex also distinguished these subtypes in PMG cortex (Table 1). Some of these parameters, including action potential half width and AHP amplitude have repeatedly been used to distinguish these two subtypes in normal neocortex ([Porter et al., 1983], [Kawaguchi, 1993], [Cauli et al., 1997], [Cauli et al., 2000]).

The intracellular content of interneurons provides an additional basis for subtype identification. Molecular markers PV, SS, calbindin (CB), calretinin (CR) and VIP are each found within selective subpopulations of interneurons ([Kawaguchi et al., 1993], [Kubota et al., 1994], [Kawaguchi et al., 1996]). These identifiers are often correlated with particular morphologies and electrophysiological characteristics ([Kawaguchi et al., 1993], [Cauli et al., 2000]). One helpful feature of these markers for distinguishing interneuron subtypes is that some of them are present in non-overlapping populations of interneurons (PV, SS, VIP, [Kawaguchi et al., 1997]). These markers are present early in development (Hof et al., 1999) and therefore may be a useful tool to assess whether the path of neuronal differentiation is altered during the formation of microgyria. Thus we addressed the specific question of whether these markers were also found in non-overlapping populations in PMG cortex. The quantitative assessment of the numbers of neurons has previously been done for both PV (Rosen et al., 1998) and SS (Patrick et al., 2006) and is beyond the scope of this analysis, since we are concerned here with interneuron identity rather than number. High power z-stack images show that no cells contained more than one of the immunohistochemical labels in either control or PMG cortex (Fig. 4H,L,P,T). This finding was confirmed by examining twelve separate fields in each of two control and two freeze-lesioned brains. In no case was an overlap of any of the three markers present within a single cell. High power images in control cortex show that PV-stained cells had a rounded somata and multipolar morphology typical of basket cells (Fig 4E,I). In PMG cortex, the morphology of PV cells is similar, although individual neurons are less heavily stained, with less clear dendritic arbors. In both control and PMG cortex, SS-stained neurons had ovoid somata typical of bipolar neurons (Fig. 4F,J,N,R). VIP neurons were less prevalent, but no differences in morphology were evident between control and PMG cortex.

Dendritic and axonal morphology can also be used to distinguish interneuron subtypes. While not all cells recorded and filled with biocytin can be recovered in subsequent processing, in this study dendritic and/or axonal morphology was clear in 40 control and 43 PMG neurons from coronal slices. Quantitative examination of this morphology will be performed in a separate study. Here we have made the following qualitative classification of cell morphologies: (1) pyramidal neurons having a clear apical dendrite (large, single dendrite directed toward the pia); (2) bipolar neurons having multiple dendrites and/or axons that project vertically, typically both towards the pia and towards the white matter, and typically crossing into other laminae; and (3) basket neurons that have a round field of dendritic projection (approximately equal vertical and horizontal distance of dendritic projection) within layer V. Clear examples of all of these cell types were found in PMG cortex (e.g., Fig. 5). After classification of the morphological type, this was compared to the electrophysiological type. In the majority of cases for both control and PMG cortex, there was a match. In control cortex, 77% of electrophysiologically-identified pyramidal neurons had a clear apical dendrite. The same was true for 89% of PMG pyramidal neurons. In control cortex, 92% of LTS neurons had a clear bipolar morphology, while this was true for 69% of PMG neurons. In control cortex, 60% of FS cells had clear basket cell morphology, and this was true for 64% of PMG FS neurons. There were 2 cells in each of control and PMG cortex whose morphology could not be clearly characterized.

Interneuron firing rate is altered in PMG cortex

Although many intrinsic properties were unchanged in PMG interneurons compared to control, several characteristics were altered that may affect inhibitory tone in malformed cortex. Interestingly, these characteristics were differentially altered for LTS and FS interneurons. In PMG cortex, the maximum firing frequency was decreased for FS neurons compared to control FS cells (Fig. 6A,C). In contrast, PMG LTS interneurons had an increased maximum frequency compared to control LTS neurons (Fig. 6B,C). These

respective changes result in maximum firing frequencies for LTS and FS interneurons that are not different from each other in PMG cortex, while this measure is significantly different for the two interneurons subtypes in control cortex. The increase in firing frequency for LTS interneurons may be due, in part, to the decreased duration of the first AHP (Fig. 6D,F). In particular, the early action potentials fired at a faster rate in PMG LTS cells than in control. Despite the faster initial firing, adaptation still occurred in PMG LTS cells. The shorter AHP and higher frequency at the beginning of the train contribute to the increase in total adaptation observed in PMG compared to control LTS interneurons (Fig 6E,G). LTS interneurons also had a slightly less negative resting potential in PMG compared to control (Table 2). FS interneurons in PMG cortex fired action potentials with slightly larger amplitudes than FS cells in control cortex (Table 2).

3. Discussion

Based on intrinsic properties, pyramidal, FS and LTS neuronal subtypes can clearly be distinguished in control cortex ([Gibson et al., 1999], [Cauli et al., 2000], [Bacci et al., 2003]). Here we have found that this holds true in malformed, epileptogenic cortex (Fig 2B). Thus the *identity* of these neuronal subtypes is not altered by a developmental insult in the late stages of cortical migration. While unique cell types can still be distinguished, some intrinsic properties are altered in the PMG. This includes a slowing of the second action potential in a train for pyramidal cells, suggesting that this property is in an immature state. The changes also include a decrease in the maximum firing frequency for FS, but an increase in this rate for LTS interneurons. Both of these changes are unlikely to be explained by the same alteration in, for instance, potassium channels. These results demonstrate that neuronal subtypes are in fact differentially altered during the formation of a microgyrus and the adjacent hyperexcitable region of neocortex. In addition, the changes in maximum firing rate suggest that the ratio of synaptic output from FS versus LTS interneurons could be altered, with that from LTS increasing while that from FS decreases. Whether this occurs in individual neurons and how it might contribute to epileptiform activity requires further exploration.

Maintenance of interneuron subtypes in PMG suggests that differentiation proceeds normally after the lesion is made, or that most mechanisms defining cell type are complete in layer V prior to the lesion and are unaffected by it. This is consistent with our current understanding of interneuron subtype determination. First, the cells that will eventually populate the PMG cortex are already born when the lesion is induced (Rosen et al., 1996). Secondly, interneurons likely receive their subtype-instructive information at the place of origin, rather than from signals during migration (Wonders et al., 2008). The sources of cortical interneurons in rodents include the medial (MGE), lateral and caudal ganglionic eminences ([Anderson et al., 2001], [Wichterle et al., 2001], [Nery et al., 2002]), with the MGE serving as the major point of origin for cells expressing SS and PV (Xu et al., 2004). Parallel electrophysiological findings indicate that the two predominant mature interneuron types formed from the MGE exhibit firing characteristics of FS and LTS cells (Butt et al., 2005). In the MGE, prenatally active transcription factors are important for determining the eventual interneuron fate, identified by the expression of PV and SS ([Sussel et al., 1999], [Anderson et al., 2001], [Du et al., 2008]).

It is clear from our data that selective intrinsic properties may be altered, leaving others intact. Here we have specifically shown that the lack of co-localization of PV, SS and VIP in control tissue is maintained in the PMG (Fig. 4), further suggesting that this aspect of interneuron subtype identity is preserved. This is an essential precursor to further studies of, for instance, synaptic input to interneuron subtypes. It is necessary to establish that the same subtypes exist before they can be compared between control and PMG.

In addition to the expression of calcium-binding proteins (Hof et al., 1999), dendritic outgrowth (Ulyings, 1990) and some complex firing properties of interneurons (Long et al., 2005) have reached near-adult levels by the end of the second postnatal week. Firing properties and action potential characteristics of pyramidal cells also approach mature values by the second and third postnatal weeks ([McCormick et al., 1987], [Franceschetti et al., 1998]). Alterations in one or more of these during development may contribute to the abnormal activity seen in epileptogenic cortex (Beck et al., 2008). This appears to be true in human dysplastic cortex, where the presence of dysmorphic, immature cells indicate disruption of maturational processes ([Cepeda et al., 2003], [Cepeda et al., 2006]). For example, cytomegalic neurons demonstrate abnormal membrane properties (Cepeda et al., 2003) and the ability to generate calcium spikes with little provocation, possibly suggesting a role in epileptiform activity (Cepeda et al., 2005). It is often difficult to discern whether intrinsic property alterations cause the observed pathologies or occur in response to it, but neuronal alterations in disease states tend to resemble non-homeostatic changes, rather than compensatory mechanisms (Beck et al., 2008). Often these changes include mis-expression of channels or subunits that are important for excitability or repolarization. For example, increased neuronal activity (modeled with short term synaptic plasticity induction or glutamate application) can cause altered expression of voltage-gated potassium channels ([Misonou et al., 2004], [Kim et al., 2007]), directly affecting cellular excitability (Misonou et al., 2004). A lack of functional Kv2.4 channels in heterotopic neurons of the MAM model (Castro et al., 2001), as well as an upregulation of intrinsic bursting behavior following pilocarpine-induction of status epilepticus (Wellmer et al., 2002) both create a tendency for hyperexcitability. Interestingly, the type of alteration that occurs for a given channel or type of current appears to depend both on the experimental model and the developmental state of the animal. In immature rats (P10–11), hyperthermia-induced seizures resulted in an enhanced I_h (Chen et al., 2001) that contributed to hyperexcitability, while kainate seizure induction in more mature animals (five to six weeks) caused a decrease in I_h and the responsible hyperpolarization activated cyclic-nucleotide gated channel subunits (Shah et al., 2004). Channel alterations likely underlie changes in firing mode that have also been shown to promote epileptiform activity, including the development of intrinsic bursting in layer V (Sanabria et al., 2001), increased bursting in CA1 following *in utero* treatment with MAM (Baraban et al., 1995), and a transient suppression of AHP and increased firing frequency in the kindling model (Behr et al., 2000). Selke et al. (Selke et al., 2006) have also demonstrated the dual loss of one AHP component and adaptive firing behavior in hippocampal granule cells, causing transient hyperexcitability in this population.

Pyramidal cell intrinsic property delayed maturation

A number of findings have contributed to the theme of delayed maturation associated with malformation models (Jacobs et al., 1999b). In the freeze lesion/PMG model, this includes delayed expression of mature GABA subunits ([Defazio et al., 1999], [Hablitz et al., 2000]) and chloride transporters (Shimizu-Okabe et al., 2007), as well as persistent radial glia (Rosen et al., 1992) and Cajal-Retzius cells (Super et al., 1997). There is previous evidence of an immature state in pyramidal cell intrinsic properties within layer II/III (Luhmann et al., 1998), and we find at least some similarities with an increased duration of the action potential in the layer V pyramidal neurons recorded here (Fig. 3). Typically, action potentials become shorter in duration and larger in amplitude by the end of the second postnatal week ([McCormick et al., 1987], [Franceschetti et al., 1998]). Accompanying this change is a larger action potential peak and a faster rate of rise and fall (McCormick et al., 1987). Although we did not observe as many changes in the pyramidal cell intrinsic properties, this may reflect the older age of the animals at the time of recording in the previous study or a true difference between laminae. In any case, both layers show an increased duration of the action potential under some conditions. Prolonging the action

potential could limit high frequency firing or bursting, however, and may actually have an anti-epileptogenic effect in PMG (Bikson et al., 2002).

Differential alterations in interneuron subtypes

While the FS and LTS subtypes have maintained their identity in PMG cortex, specific changes in their firing frequencies may represent altered efficacy for each cell type, with interesting implications for interneuron function, as well as potential underlying subcellular mechanisms. In particular, PMG LTS interneurons have an increased maximum firing frequency (Fig. 6). One possible mechanism for increased frequency is the elevated expression of voltage-gated potassium channels that permit rapid re-polarization responsible for high-frequency firing. These channels normally have a differential expression pattern in neuron subpopulations for both neocortex ([Toledo-Rodriguez et al., 2004], [Chang et al., 2007]) and hippocampus (Bourdeau et al., 2007). The rapid action potential firing capabilities of FS cells may rely, at least in part, on the expression of a specific potassium channel, Kv3.1 ([Chow et al., 1999], [Toledo-Rodriguez et al., 2004]). Aberrant expression of channels like this could endow LTS cells with increased firing capability. A decreased expression of this channel could contribute to retarding the speed with which FS interneurons fire in the PMG. However, adaptive behavior typical of LTS cells in normal cortex still occurs in PMG, and is actually increased due to a higher initial firing frequency (Fig. 6).

Taken together, these data suggest that the differentiation of neuronal subtypes proceeds relatively normally during reorganization of the cortex induced by focal loss of deep layer neurons during the time of cortical migration

4. Methods and Materials

Freeze lesion surgery

Freeze lesions were made as described previously (Zsombok et al., 2007). Rat pups aged postnatal day (P)1 were anesthetized with hypothermia by placing them in ice for 4–6 minutes, and an anterior-posterior incision was then made in the scalp. The freeze probe, consisting of a copper bar with a 2×5 mm rectangular tip cooled to -50°C , was applied to the exposed skull over the somatosensory cortex of the left hemisphere for 5 seconds. The skin was sutured, the pup warmed and returned to the dam.

In vitro slice preparation and electrophysiology

Lesioned animals and unoperated control rats aged P12–17 were anesthetized with isoflurane and decapitated. The brain was quickly removed and immediately placed into cold (4°C) sucrose slicing solution containing (in mM): 2.5 KCl, 1.25 NaH_2PO_4 , 10 MgCl_2 , 0.5 CaCl_2 , 26 NaHCO_3 , 11 glucose and 234 sucrose. For interneuron recordings, coronal sections 300 μm thick were made and transferred to a holding chamber filled with artificial cerebrospinal fluid (aCSF) containing (in mM): 126 NaCl, 3 KCl, 1.25 NaH_2PO_4 , 2 MgCl_2 , 2 CaCl_2 , 26 NaHCO_3 , and 10 glucose. In attempts to select and record from various interneuron subtypes, the inclusion of some pyramidal neurons in the group of recorded cells is inevitable. As an aid to determine which cells were pyramidal, we have included here, for comparison, the intrinsic properties of pyramidal neurons recorded in a separate study. The focus in that separate study was to isolate layer V pyramidal neurons from other laminar influences, and therefore, the slices were cut horizontally (300 μm) through layer V. Neurons in the control horizontal slices had intrinsic properties similar to those previously reported for pyramidal neurons in coronal slices (Luhmann et al., 1998). After sectioning, slices were transferred to a holding chamber filled with artificial cerebrospinal fluid (aCSF) containing (in mM): 126 NaCl, 5 KCl, 1.25 NaH_2PO_4 , 2 MgCl_2 , 2 CaCl_2 , 26 NaHCO_3 , and

10 glucose. For these cells, recordings were made in normal aCSF containing dinitroquinoxaline [(6,7)],2,3(1H,4H)-dione (DNQX, 20 μ M) and D,L-2-amino-5-phosphonopentanoic acid (APV, 100 μ M) to block AMPA and NMDA receptors, respectively. Sections were maintained at 34°C for 45 minutes and at room temperature thereafter. All solutions were infused with 95% O₂/5% CO₂, maintaining pH at 7.4. Recordings were made in aCSF at 30–32°C, osmolarity was adjusted to ~290 mOsm.

The whole-cell patch clamp technique was used to record from layer V interneurons and pyramidal cells in somatosensory cortex from both control and malformed cortex. Prior to recording, access resistance (Ra) was measured, and only those cells with Ra less than 23 M Ω were used for analysis. Recordings were terminated if access resistance increased more than 20% or if there was any evidence of declining cell health. The cortical area used for recording was 0.5–1.5 mm on either side of the lesion (paramicrogyral region) and from homotopic cortex in controls. Interneurons were selected based on morphological criteria identified under DIC optics: absence of the apical and basal dendrites and smaller soma than the typical layer V pyramidal cell. These recordings specifically targeted LTS and FS cells. LTS cells were identified by their spindle-shaped appearance and large processes extending towards both the pial surface and subcortical white matter. FS cells had smaller somata with thinner processes in a multipolar distribution. Pyramidal cells were identified by their relatively larger somata and prominent apical dendrites. The intracellular solution used for interneuron recordings contained (in mM): 130 K-gluconate, 10 Hepes, 11 EGTA, 2 MgCl₂, 2 CaCl₂, 4 Na-ATP, and 0.2 Na-GTP. Patch solution for pyramidal cell recordings in horizontal slices contained (in mM): 70 K-gluconate, 70 KCl, 10 Hepes, 4 EGTA, 2 NaCl, 4 Na-ATP, 0.3 Na-GTP. Osmolarity and pH of the intracellular solution was adjusted to 275–285 mOsm and pH 7.3. In some cases biocytin (0.05–0.2%) was included in the pipette in order to identify neuronal morphology with subsequent staining. Biocytin-filled cells were visualized using fluorescein-conjugated streptavidin (10 μ g/mL, Molecular Probes).

Recordings in current clamp were made using a Multiclamp 700A (Axon Instruments) and digitized with a Digidata 1320A and pClamp software (Axon Instruments, at 20 kHz). The resulting files were analyzed using Clampfit (Axon Instruments) and Minianalysis (Synaptosoft) software.

Intrinsic property measurements

Each cell was injected with a series of hyperpolarizing and depolarizing square pulse currents, 400 ms in duration. Current was delivered at an initial level of –40 pA, then increased by steps of 20, 30, or 40 pA to the point where the cell fired at a maximal rate. From these recordings, a number of intrinsic properties were calculated for both pyramidal cells and interneurons. Adaptation was calculated three ways. Total adaptation was calculated by dividing the frequency of the last two action potentials by the frequency of the first two. Early adaptation was calculated as the ratio of the frequency of action potential firing at 200 msec to the frequency of the first pair of action potentials. Late adaptation was calculated as the ratio of the frequency of the last pair of action potentials and the fourth pair. A ratio of 1 represents no adaptation, while increased adaptation is reflected by a smaller number. Other measures included action potential half-width, percent increase in half-width from the first to second action potential, peak of first and second afterhyperpolarizations (AHP, amplitude measured relative to the threshold for action potential firing), time to peak for the first AHP (relative to the first action potential peak), accommodative hump (the difference between the peak of the smallest action potential and the peak of the subsequent largest action potential), minimal discharge frequency and time of minimal discharge. Action potential amplitude, maximum rates of rise and decay, dV/dt ratio and primary F/I slope were also calculated as described previously (Luhmann et al., 1998). Briefly, the amplitude and the max rise and decay rates were determined for the

initial action potential in the first sweep with a suprathreshold current. Primary F/I slope was determined from a plot of the frequency of the first two action potentials (Hz) versus the magnitude of injected current (nA). Student's *t*-tests with Bonferroni corrections were used to compare control vs. PMG for each group, *p* <0.05 was considered to be significant.

Immunohistochemistry

Rats aged P13–14 were anesthetized with isoflurane and transcardially perfused with 4% paraformaldehyde. The rats were decapitated, each brain removed and stored in paraformaldehyde at 4°C overnight. Parasagittal sections 80 µm thick were made from the left hemisphere of both lesioned animals and un-operated controls. Simultaneous triple immunohistochemical staining was performed for SS (rabbit anti-SS, 1:1000, Peninsula Labs; AlexaFluor 568 goat anti-rabbit, 1:100), PV (mouse anti-PV, 1:5000, Sigma; AlexaFluor 405 goat anti-mouse, 1:100) and vasoactive intestinal peptide (VIP, guinea pig anti-VIP, 1:1000, Peninsula Labs; Fluorescein goat anti-guinea pig, 1:200). Sections were incubated in primary antibody solution for SS, PV and VIP on the shaker overnight at room temperature (10%NGS, 2%BSA, 0.5%TritonX-100/TBS). Sections were washed in TBS, incubated in secondary antibody solution for three hours on the shaker at room temperature (10%NGS, 2%BSA, 0.5%TritonX-100). Sections were mounted on unsubbed slides and coverslipped with Prolong Gold anti-fade mounting medium. Images were obtained using a Leica TCS-SP2 AOBS confocal laser scanning microscope at the Virginia Commonwealth University Department of Anatomy and Neurobiology Microscopy Facility.

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Figure 1.

Example traces of neuronal responses to a series of current steps. Firing patterns recorded in control (1,3) and PMG (2,4) cortex in response to depolarizing current. Traces are shown for low frequency responses to small current steps (3,4) and high frequency responses to larger steps in the same cells (1,2). These traces demonstrate patterns typical for: **A.** Pyramidal cells identified in horizontal slices; **B.** Suspected pyramidal cells recorded in coronal slices; **C.** FS; and **D.** LTS interneurons recorded in coronal slices.

Figure 2.

Identification of neuronal subpopulations based on action potential firing properties. The difference between first and last AHP amplitudes is plotted against total adaptation ratio, which is the frequency of the first two action potentials divided by the frequency of the last two action potentials. Each symbol shows the result for a single cell. **A.** Control cells. **B.** PMG cells. Five cell types are represented: 1) FS = fast-spiking; 2) LTS = low threshold-spiking; 3) pyramidal; 4) Suspected pyramidal (initially expected to be interneurons but characterized as pyramidal cells based on action potential characteristics); 5) unknown cell type (non-pyramidal, unclassified interneurons).

Figure 3.

Comparison of action potential half-width in pyramidal, FS and LTS cells. **A.** Example traces from pyramidal cells in control (1) and PMG (2). **B.** Enlarged versions of the first (1) and second (2) action potentials from the traces shown above in A, for control (gray, thicker line) and PMG (black, thinner line). **C.** Mean second action potential half-width in control (gray) and PMG (black) for 29 and 22 pyramidal cells, 34 and 30 FS cells, and 33 and 23 LTS cells, respectively. * = *t*-test, $p < 0.05$.

Figure 4.

Confocal images of parasagittal sections at low power (**A–D**) and high power (**E–T**) showing immunohistochemistry to visualize PV (blue), SS (red), and VIP (green). To prevent cross-talk between channels, a sequential scanning procedure was used. Panel **A**, overlay of all three fluorophores in control cortex. Panels **B–D**, overlay of all three fluorophores in PMG cortex at locations within, near, and distant from the microgyrus, respectively. Panels **E–H**, images of each fluorophore separately followed by overlay of the three (same visual field) taken from layers II/III in control cortex. Panels **I–L**, images taken from layer V in control cortex. Panels **M–P**, images taken from layer II/III in PMG cortex. Panels **Q–T**, images taken from layer V in PMG cortex. For **E–T**, images are z-stacks with a 1.5 μm slice. Scale bar in **D** for **A–D** = 200 μm , scale bar in **T** for **E–T** = 20 μm .

Figure 5. Confocal images of individual cells that were recorded and later visualized with fluorescein-conjugated biocytin. All cells are from PMG cortex, oriented with pia up and are examples of **A.** bipolar, **B.** basket and **C.** pyramidal cell morphology. Scale bar = 10 μ m.

Figure 6.

Altered action potential firing properties in neuronal subpopulations. **A.** Example traces from FS cells in control (1) and PMG (2) demonstrating decreased maximum firing frequency in PMG. **B.** Example traces from LTS cells in control (1) and PMG (2) demonstrating increased maximum firing frequency in PMG. **C.** Maximum firing frequency in pyramidal, FS and LTS cells. **D.** Example traces of initial action potential firing from typical control (1) and PMG (2) LTS cells, showing decreased AHP duration in PMG. **E.** Full trains of action potentials showing adaptation in control (1) and PMG (2) LTS cells, increased in PMG. **F.** Duration of AHP. **G.** Average total adaptation. Control (gray) and PMG (black) for 29 and 22 pyramidal cells, 34 and 30 FS cells and 33 and 23 LTS cells, respectively. * = *t*-test, $p < 0.05$.

Table 1

Intrinsic properties of layer V interneurons and pyramidal cells.

Electrophysiological Parameter	FS Control (n=34) PMG (n=30)	LTS Control (n=33) PMG (n=23)	Pyr Control (n=29) PMG (n=22)
1 st spike duration, ms	0.77 ±0.04 §	1.08 ±0.05	1.86 ±0.08
	0.81 ±0.06 §	1.03 ±0.05	2.03 ±0.12
2 nd spike duration, ms	0.79 ±0.04 §	1.23 ±0.06	2.35 ±0.13
	0.84 ±0.07 §	1.17 ±0.06	2.84 ±0.22**
Duration increase, %	2.8 ±0.6 §	13.4 ±1.4	25.8 ±4.4
	2.7 ±1.0 §	12.6 ±1.7	39.6 ±6.9
1 st spike AHP, mV	-11.8 ±0.6 §	-8.6 ±0.6	-3.9 ±0.7
	-11.5 ±0.6 §	-7.2 ±0.9	-3.3 ±0.6
2 nd spike AHP, mV	-14.9 ±0.6 §	-11.2 ±0.6	-14.0 ±0.8
	-14.6 ±0.6 §	-10.0 ±0.9	-14.8 ±0.8
Early adaptation ratio	0.08 ±0.02	0.11 ±0.02	0.22 ±0.02
	0.09 ±0.02	0.07 ±0.01	0.16 ±0.03
Late adaptation ratio	0.86 ±0.02 §	0.55 ±0.03	0.77 ±0.02
	0.83 ±0.01 §	0.55 ±0.04	0.76 ±0.01
Accommodative hump, mV	4.8 ±0.6 §	1.5 ±0.2	3.4 ±1.1
	4.3 ±0.6 §	2.2 ±0.6	3.1 ±1.3
Minimal frequency, Hz	32.4 ±3.6 §	9.2 ±0.6	6.3 ±0.3
	25.5 ±3.3 §	11.6 ±2.3	6.7 ±0.5
Time for min. freq., ms	188 ±21	201 ±14	175 ±9
	171 ±17	170 ±16	176 ±12

Values are means ±SEM. n, number of cells; FS, fast-spiking cells; LTS, low-threshold spiking cells; Pyr, pyramidal cells; Student's t-test between control and PMG for each group,

** indicates p <0.05., Student's t-test between FS and LTS in control and PMG populations,

§. indicates p <0.05.

Table 2

Intrinsic properties of layer V interneurons and pyramidal cells.

Electrophysiological Parameter	FS Control PMG	LTS Control PMG	Pyr Control PMG
Resting membrane potential, mV	-62.7 ±6.3 (31)	-55.4 ±4.2 (31)	-56.2 ±5.3 (28)
	-63.0±7.3 (24)	-58.7±5.2 (19) **	-57.3±8.2 (22)
Input resistance, MΩ	120 ±7 (32)	191 ±16 (32)	121 ±16 (29)
	123 ±7 (25)	173 ±22 (21)	140 ±18 (21)
Spike amplitude, mV	81.8 ±1.1 (34)	99.2 ±1.4 (33)	103.6 ±0.8 (28)
	85.3±1.3 (30) **	96.3 ±1.7 (23)	102.4 ±1.0 (16)
Max rate of rise, V/s	256 ±12 (34)	244 ±13 (33)	287 ±11 (28)
	253 ±13 (30)	239 ±11 (23)	272 ±13 (16)
Max rate of decay, V/s	117 ±7 (34)	85 ±4 (33)	42 ±2 (28)
	115 ±9 (30)	84 ±5 (23)	39 ±2 (16)
dV/dt ratio	2.3 ±0.1 (34)	2.9 ±0.1 (33)	6.9 ±0.2 (28)
	2.4 ±0.1 (30)	2.9 ±0.1 (23)	7.1 ±0.3 (16)
Primary F/I slope (Hz/nA)	407 ±25 (34)	471 ±33 (33)	87 ±18 (28)
	400 ±23 (30)	481 ±41 (23)	97 ±20 (16)

All data are expressed as means ±SE (n cells). Student's t-test was used to compare control and PMG values within each group,

** indicates $p < 0.05$.