Postsynaptic Currents Prior to Onset of Epileptiform Activity in Rat Microgyria

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Abstract

Structural malformations of the cortex, arising as a result of genetic mutation or injury during development are associated with dyslexia, epilepsy, and other neurological deficits. We have used a rat model of a microgyral malformation to examine mechanisms of epileptogenesis. Our previous studies shown that the frequency of miniature excitatory postsynaptic currents (mEPSCs) recorded in neocortical layer V pyramidal neurons is increased in malformed cortex at a time when field potential epileptiform events can be evoked. Here we show that the increase occurs at an age before onset of cortical epileptiform activity and at a time when the frequency of mEPSCs in control layer V pyramidal neurons is stable. An increase in the frequency of spontaneous (s) EPSCs in layer V pyramidal neurons of malformed cortex occurs earlier than that for mEPSCs, suggesting that there may additionally be alterations in intrinsic properties that increase the excitability of the cortical afferents. The frequency of EPSC bursts and late evoked activity was also increased in malformed cortex. These results suggest that a hyperinnervation of layer V pyramidal neurons by excitatory afferents occurs as an active process likely contributing to subsequent development of field epileptiform events.
Introduction

Polymicrogyria is a developmental cortical malformation associated with dyslexia (Taylor et al. 2001; Clark et al. 2000; Clark and Plante 1998; Galaburda and Eidelberg 1982) and epilepsy (Kim et al. 2006; Crino 2004; Segawa et al. 1979) that has a varied etiology, including genetic and acquired causes (Chang et al. 2006; Cantagrel et al. 2006; Piao et al. 2005; Mitchell et al. 2003; Iannetti et al. 1998; Barkovich et al. 1995; Barkovich and Lindan 1994; Richman et al. 1974). Despite identification of genes contributing to some forms of polymicrogyria, including GPR56, PAX6, and AH11 (Guerrini and Marini 2006; Piao et al. 2005; Gleeson et al. 2004; Mitchell et al. 2003), the connection between the structural abnormality and neurological dysfunction is ill-understood. These genes play a role in neuronal migration and differentiation (Fukumitsu et al. 2006; Shashidhar et al. 2005; Tzoulaki et al. 2005; Little et al. 2004). Changes in migration end point and differentiation can elicit subsequent abnormalities in connectivity (Garbossa and Vercelli 2003; Chevassus-Au-Louis et al. 1998; Prince et al. 1998; Jones et al. 1981). The mechanisms that produce the epileptiform activity are likely to occur subsequent to the formation of the initial structural abnormality. In fact there often is a ‘waiting period’ prior to the onset of seizures in patients with epilepsy having a developmental cause (Park et al. 2006; Widdess-Walsh et al. 2005; Kobayashi et al. 2001; Bartolomei et al. 1999).

One goal of research with animal malformation models is to identify the mechanisms that initiate a propensity for hyperexcitability. The freeze lesion model of microgyria mimics all aspects of the histopathology of human four-layered microgyria (Jacobs et al. 1996; Dvorak et al. 1978; Dvorak and Feit 1977). This type of microgyria involves the focal loss of deep layers IV-VIa, resulting in folded superficial layers above a glial scar and layer VIb. Interictal-like epileptiform activity can readily be evoked in vitro in slices containing the microgyral malformation (Jacobs et al. 1999a). Although overt spontaneous seizures have not been observed in freeze-lesioned animals, seizure susceptibility is increased in these animals (Scantlebury et al. 2004). Lack of seizures is not a limitation since our goal is to study the onset of epileptiform activity. In fact, seizures and hyperexcitability may induce additional alterations in the same biological properties that initiated the excitability changes (Parent et al. 2006; Teskey et al. 2006; Sutula 2002; Jankowsky and Patterson 2001; Parent et al. 1999; Vezzani and Hoyer 1999; Motte et al. 1998; Pazman et al. 1997; Sutula et al. 1992). Similar to the clinical waiting period, field potential epileptiform activity is not observed in this rat model until
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postnatal day (P) 12 (Jacobs et al. 1999a), despite the completion of microgyral structure by P7-8 (Rosen et al. 1992; Dvorak et al. 1978). One approach to distinguishing mechanisms that generate the hyperexcitability from processes altered by it is to examine the model prior to onset of the hyperexcitability.

We believe that a major factor contributing to hyperexcitability in the microgyral rat is hyperinnervation of the epileptogenic area surrounding the malformation by excitatory afferents (Jacobs et al. 1999b). We first hypothesized that excitatory afferents might be redirected to this paramicrogyral region (PMG) since these afferents have lost many of their targets within the malformation. For instance, some thalamocortical afferents have lost their layer IV targets (both layer IV neurons and the dendrites of deeper-lying pyramidal neurons). Neurons with the appropriate molecular cues are present within layer IV of the surrounding PMG. The microgyral area from a single lesion typically extends ~1 mm, while single thalamocortical afferents have projection diameters of ~0.5 – 1.5 mm (Arnold et al. 2001). Thus layer IV neurons of the PMG would be found within the projection zone of most thalamocortical afferents. This may aid in redirecting the collaterals away from the malformed region and toward the PMG. Supporting this idea is our finding that within the PMG, mEPSC frequency was significantly increased for layer V pyramidal neurons (Jacobs and Prince 2005), suggesting that they are hyperinnervated by excitatory afferents. In addition, anatomical studies have shown that few thalamocortical afferents invade the malformed region yet a dense projection occurs in the adjacent PMG (Rosen et al. 2000; Jacobs et al. 1999b). Hyperinnervation of the PMG by excitatory afferents would be expected to be pro-epileptogenic. Thus if these changes begin prior to the onset of epileptiform activity, they may be an important contributing factor to the hyperexcitability associated with this malformation. Here we have used whole cell patch clamp recordings to examine spontaneous (s) and evoked (e) EPSCs just prior to onset (P7-11) of epileptiform activity in the rat freeze lesion model of microgyria.

Methods

Focal freeze lesions were made in Sprague Dawley rat pups on P1, as previously described (Jacobs et al. 1996). Pups were anesthetized with hypothermia, the skull was exposed and a rectangular (2 X 5 mm) freezing probe maintained at ~−55°C was placed on the skull over the
somatosensory cortex for 5 seconds. The scalp then was sutured and the pup warmed up and returned to the dam.

Rats aged P7-11 were anesthetized with pentobarbital (55 mg/kg) and decapitated. Brains were removed and placed in a cold sucrose slicing solution (containing in mM) 2.5 KCl, 1.25 NaH$_2$PO$_4$·H$_2$O, 10.0 MgSO$_4$, 0.5 CaCl$_2$·2H$_2$O, 234 sucrose, 11 glucose, 26 NaHCO$_3$. Slices, 300 µm thick, were cut on vibratome in sucrose slicing solution and incubated in normal Ringer solution (aCSF, containing in mM: 126 NaCl, 5 KCl, 1.25 NaH$_2$PO$_4$·H$_2$O, 2 CaCl$_2$·2H$_2$O, 10 glucose, 26 NaHCO$_3$) at 34°C for 25 min and thereafter at room temperature.

Whole cell patch clamp recordings were made under visual control in a submerged chamber at 32°C using a Multiclamp 700A amplifier (Axon Instruments). Recordings were made from layer V pyramidal neurons (0.3-2.5 mm from the sulcus or in homotopic control cortex) using glass micropipettes (2-5 MΩ, Garner glass Co., Claremont, CA) filled with intracellular recording patch solution (containing in mM: 117 gluconic acid, 117 CsOH, 11 CsCl, 10 Hepes, 11 EGTA, 1 MgCl$_2$·6H$_2$O, 1 CaCl$_2$·2H$_2$O and 0.5% biocytin).

EPSCs were recorded at a holding potential of -60 mV. Only recordings with an access resistance less than 21 mΩ that varied <20% were accepted for analysis. Data were digitized online (20 kHz) using clampex software (Axon Instruments). After the recording of spontaneous EPSCs and IPSCs 1µM tetrodotoxin (TTX, 1:1000, Sigma) was applied to the bath solution, and recordings of miniature (m) EPSCs and IPSCs were begun after 5 minutes of wash-in. Currents were detected and measured using MiniAnalysis Software (by Synaptosoft). Only recordings with more than 50 events were accepted for analysis.

Stimulus parameters were similar to those that evoked epileptiform activities in previously described reports (Jacobs et al. 1999a). Pulses were applied within layer V, ≤450 µm from recorded cell with a glass pipette filled with 1M NaCl. A 20 µsec duration pulse was applied and the current intensity needed for evoking a detectable PSC was considered threshold. A series of stimuli was then applied by increasing the pulse duration to 40, 80, 160, and 320 µsec (2X, 4X, 8X and 16X threshold).

After recordings were completed, slices were immediately fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Slices were maintained in this fixative or in placed in 0.1 M phosphate buffer (pH 7.4) after 24 hours until sectioning or reaction for biocytin. In some cases slices were cryoprotected by immersion in 30% sucrose (in 0.1 M
phosphate buffer) until they sank, and then were resected on a freezing microtome at 40µm. Slices were either stained for Nissl with cresyl violet or reacted for biocytin, using standard procedures (Vector abc kit and DAB) in order to verify pyramidal cell morphology and the laminar position of the recorded cell. Measurements are reported as mean ± SEM. Student’s t-tests (Microsoft Excel) and ANOVAs (SigmaStat or SPSS) were used to test for significance.

Results

Spontaneous, miniature, and evoked excitatory and inhibitory postsynaptic currents (EPSCs, IPSCs) were recorded from layer V pyramidal neurons of either PMG or homotopic control cortex. Recordings were made in vitro, in slices from 52 freeze-lesioned rats and 37 naïve rats aged P7-11.

Spontaneous Excitatory Postsynaptic Currents

The frequency of sEPSCs varied greatly between cells, from 0.20 to 8.57 Hz in 65 control cells, and from 0.15 to 16.43 Hz in 55 PMG cells. Mean sEPSC frequency was significantly greater in the PMG cell group relative to the control cells (Fig. 1A, C, 3.0±0.5 vs 1.6±0.2 Hz, respectively, t-test, p<0.005). Peak amplitude, rise time, and decay time of sEPSCs were not significantly different between PMG and control cells (see Table I). The sEPSCs in both control and PMG cells typically lasted ~11 msec (see decay time in Table I). Thus events occurring over intervals shorter than this are likely to sum, creating a larger postsynaptic response. To examine this, we analyzed the incidence of ‘bursts’ of sEPSCs (two or more events occurring with an inter-event interval of less than 10 msec). PMG cells had significantly more bursts per minute than control cells (Fig 1D, 7.3±2.4 vs 2.2±0.5 bursts/min, respectively, t-test, p<0.05). In addition, a significantly larger proportion of PMG cells contained bursts compared to control cells (89% versus 70%, respectively, z-test, p<0.05).

There was little change in the frequency of events after TTX was applied for either control or PMG cells. Indicative of this was the percent decrease in event frequency calculated in individual cells (-5.1±15.7% in 21 control cells and 15.5±7.5% in 17 PMG cells, NS). Similar to the result for sEPSCs, the mEPSCs were significantly more frequent in PMG cells than in control cells (Fig 1B,C, 2.3±0.6 vs 1.1±0.2 Hz for 32 PMG and 32 control cells respectively, t-test, p<0.05). The peak amplitude, rise time, and decay time of the mEPSCs were not significantly different between PMG and control cells (see Table I). To examine inter-event intervals for
mEPSC recordings, we have used the coefficient of variation (CV, standard deviation/mean) for inter-event intervals. This measure was significantly greater in PMG cells compared to controls (Fig 1E, 1.3±0.1 vs 1.1±0.0, respectively, t-test, p<0.05). This further suggests a change has occurred in a presynaptic measure, including number of terminals, probability of release or a redistribution of terminals.

**EPSC Frequency is Independent of Location within PMG Cortex**

We selected layer V pyramidal neurons within the PMG region previously shown to be epileptogenic at later ages (after P12) (Jacobs et al. 1999a). To determine whether there was a bias within this region, we examined the distribution of EPSC frequency versus either location from the sulcus or depth from the pia (Fig. 2). There was no correlation between frequency and distance from the sulcus for either sEPSCs or mEPSCs (Fig. 2A, Pearson Product Moment, R = 0.00 and -0.07 for sEPSCs and mEPSCs respectively, both N.S.).

For both control and PMG cortex, Nissl stains showed that layer V was consistently located 45-80% of the depth from pia to white matter. For control cells there was a significant correlation between cell depth relative to the pia, and EPSC frequency. For control sEPSCs the correlation between event frequency and cell depth increased when data from P7 aged rats was excluded (R = 0.37, Pearson Product Moment, p<0.01 for all control sEPSCs, and R=0.52, p<0.001 for data from animals aged P8-11). The correlation between event frequency and depth from pia was even greater for mEPSCs (fig 2C, R = 0.45 for all control mEPSCs, and R = 0.50 for data from animals aged P8-11, p<0.02 for both). In contrast, for PMG cells, there was no correlation between EPSC frequency and depth from the pia (R = 0.05 for sEPSCs and 0.09 for mEPSCs). Excluding data from P7 aged freeze-lesioned animals did not significantly change the correlation.

**Evoked Excitatory Postsynaptic Currents**

Stimulation within layer V nearby the recorded cell produced short latency EPSCs whose peak and area increased with increasing stimulus intensity. In some cases the EPSC came to a single peak, followed by a smoothly decaying current (see example Fig A2). In other cells multi-peaked short latency EPSCs were observed (example Fig A1). Significantly more PMG than control cells had multipeaked eEPSCs (60% of 15 control and 96% of 26 PMG cells, z-test, p<0.05). The peak amplitude and area of the eEPSC was significantly larger in PMG compared to control cells (Fig. 3B-D, 2-way repeated measures ANOVAs, p<0.01). The interaction
between stimulus intensity and group (PMG vs control) was significant for the eEPSC area (p<0.01), but not for peak amplitude (p=0.06).

In some cases excitatory currents occurred at long latencies following the stimulus (examples in Fig. 3A, see gray arrows). Similar to field potential epileptiform activity, these events were all or none, variable in form and latency from one stimulus presentation to the next. These events were also reminiscent of those described for evoked inhibitory currents in PMG cells from older animals, that were used to distinguish two PMG cell subgroups (Jacobs and Prince 2005; Jacobs et al. 1999a). This type of late activity was observed in only 2 of 15 control cells, one of which is shown in Fig. 3A2. In contrast, late activity was observed in 69% of 26 PMG cells (significantly more than controls, z-test, p<0.005). In some cells the late activity was clearly evoked since it occurred with a similar latency on multiple stimulus presentations.

**Effect of Age on Excitatory Postsynaptic Currents**

We initially hypothesized that during the normal period of synaptogenesis, numbers of excitatory inputs to PMG cells would be increased over control levels. To determine if the number of functional synapses was changing during the ages examined (P7-11), we separated the results by survival age. Surprisingly, for control cells, neither sEPSCs nor mEPSCs increased in frequency with age during this period (Fig. 4A, 1-way ANOVAs, N.S.). The sEPSC frequency showed a relatively stable mean from 1.5±0.4 at P7 to 2.1±0.6 Hz at P11. In contrast for PMG cells, the frequency of sEPSCs significantly increased with age from 0.9±0.1 at P7 to 4.8±1.1 Hz at P11 (Fig. 4A, 1-way ANOVA, p<0.05).

The sEPSC frequency for control and PMG groups (experimental condition) were compared over this age range with a 2-way ANOVA. Age and experimental condition showed significant differences (p<0.01), but without an interaction between these two. The sEPSC amplitude was stable over these ages for both control and PMG groups (Fig. 4C). Two-way ANOVAs applied for sEPSC amplitude, area, and rise time showed no differences for either age, experimental condition or interaction.

For mEPSCs, a one-way ANOVA showed a significant increase in frequency with age for PMG cells but not for control cells (Fig. 4A). Control and PMG (experimental condition) mEPSC frequencies across survival age were compared with a two-way ANOVA, that showed a significant difference for age, experimental condition, and interaction between these two (p<0.001, <0.005, and <0.02, respectively). The mEPSC frequency difference between control
and PMG cells was largest at P10 and 11 (Fig. 4A). Two-way ANOVAs applied for mEPSC amplitude, area, and rise time showed no differences for either age, experimental condition or interaction.

The number of EPSC bursts per minute significantly increased with age for PMG but not control sEPSCs (Fig. 4B, p<0.005). A two-way ANOVA on sEPSC bursts per minute showed significant differences for age, experimental condition, and interaction (p<0.05 for all). For mEPSCs we examined the CV of inter-event interval versus age. Controls showed no significant difference in this measure over the P7-11 age range (1-way ANOVA, N.S.). In contrast, in PMG cells, there was a significant decrease in CV with increasing age (1-way ANOVA, p<0.05). These results show that variability in mEPSC inter-event interval for PMG cells is highest, and most different from control at (P7-9) ages when the mean frequency is not significantly different between control and PMG cells. In addition, variability in this measure is near control levels at P9-10 ages, when the mEPSC frequency is significantly higher. These results further suggest a division in neuronal mechanisms for P7-9 ages compared to P10-11.

To examine the distribution of individual mEPSC events, cumulative probability plots for inter-event interval were constructed (Fig. 5). For this analysis, the first 200 events were selected for the 4 cells closest to the mean, yielding 800 events for each group. The distribution was similar for control and PMG cells at ages P7-9, but showed a significant divergence at ages P10 and 11, with a greater number of small inter-event intervals for PMG cells (K-S tests, N.S. for P7-9, p<0.001 for P10,11). For PMG cells at P11, 61% of the events had an inter-event interval of 1 sec or smaller, while for control cells at P11, this was true for only 19% of events (Fig. 6).

**Discussion**

This study examined excitatory currents in layer V pyramidal neurons of the epileptogenic surround of an induced microgyrus specifically examined during the ‘waiting period’ before onset of epileptiform activity. We found an increase in both sEPSC and mEPSC frequencies and bursting rate compared to recordings from control animals of the same ages. Consistent with this was an increase in the evoked EPSC amplitude and an increase in incidence of evoked late burst activity. These results extend our previous study showing that these effects are maintained after the onset of epileptiform activity (Jacobs and Prince 2005). The fact that there appears to be an
increased excitatory synaptic input prior to the inception of epileptiform activity suggests that this mechanism may contribute to the onset.

If presynaptic release probability for afferents to these layer V neurons is unchanged in PMG cortex, then the increase in mEPSC frequency should be due to hyperinnervation of layer V pyramidal neurons by excitatory afferents. Interestingly, the increase in mEPSC frequency in the PMG occurs at a time when functional level of synaptic input is stable in controls (Fig. 4A, Control mEPSCs). This is somewhat surprising since the density of dendritic spines and complexity of basal dendrites both continue to increase between P7 and P14 (Wise et al. 1979; Eayrs and Goodhead 1959). Additionally, the increase in mEPSC frequency seems to occur suddenly at P10. A number of studies have previously shown a maintenance of an immature state in microgyral animals, including retention of radial glial neurons (Rosen et al. 1994), normally eliminated pathways (Innocenti and Berbel 1991), and a slower action potential for microgyral neurons (Luhmann et al. 1998). This does not appear to be the case for mEPSC frequency, since at P7, for instance, it is even lower in PMG than in controls. The increased variability (as measured by CV) in inter-event interval at young ages suggests that some alterations in presynaptic mechanisms are underway, but not yet stabilized at P7-9. At a time when the mEPSC frequency is significantly larger for PMG cells, the variability in mEPSC inter-event interval has become stable (see Fig 4).

Sprouting of connections commonly occurs in the CNS of both developing and adult animals when target sites become available, through loss of normal input (Serfaty et al. 2005; Carmichael 2003; Uryu et al. 2001; Villablanca and Hovda 2000; van Praag et al. 1996; Salin et al. 1995; Stroemer et al. 1995; Steward 1992). The missing neurons from the malformed region would have normally sent some axonal collaterals horizontally to the adjacent cortex (Burkhalter 1989; Jones 1984). This may account for some vacated target space. An additional source of new target space may come from the increase in basal dendritic length that occurs in the PMG (Di Rocco et al. 2002). It is also possible, that synaptic density is increased on individual layer V neurons. This has not yet been studied.

Pathways that have lost targets within the eliminated middle and deep layers of the malformed region, include thalamocortical, intracortical, and callosal afferents. We have presumed that it is more likely that these afferents will synapse on cells in an abnormal position (medial-lateral or anterior-posterior) of the correct layer rather than on cells of the incorrect layer
but initially specified location. This presumption comes in part from co-culturing studies that show that thalamic axons find neocortical layer IV independent of the position of the two pieces of tissue (Bolz et al. 1992; Yamamoto et al. 1992; Bolz et al. 1990; Yamamoto et al. 1989). Such studies have also shown that regional specificity is not maintained (Molnar and Blakemore 1991). This is in contrast to what is likely to occur when no targets of the correct layer are present. Using a teratogen to kill dividing neuroblasts expected to become layer IV neurons, a microcephalic cortex can be created (Garbossa and Vercelli 2003; Ciaroni et al. 1989; Spatz and Laqueur 1968). In this case where no layer IV is present, thalamocortical axons will synapse on surviving layer III neurons (Jones et al. 1981). In addition, large cortical lesions that also eliminate the subplate often cause degeneration of thalamocortical neurons (Kolb and Cioe 2003; Loopuijt et al. 1995). We expect that with our transcranial lesions, the subplate likely survives (Dvorak et al. 1978; Dvorak and Feit 1977). However, there is still likely some thalamic degeneration. Hermann et al (1997) showed that neonatal transcranial freeze lesions similar to ours, but over occipital cortex caused a significant decrease in volume and cell number within the lateral geniculate nucleus (Herman et al. 1997). This group has also observed gender differences in cell size distribution within the medial geniculate nucleus, with only freeze-lesioned males being altered from controls (Herman et al. 1997). These thalamic changes are unlikely to be related to epileptogenicity, since both males and females consistently show epileptiform activity (Jacobs et al. 1999a).

Interestingly, there appears to be a gradual change in sEPSC frequency over the age range examined, but a sudden change in these measures at P10 for mEPSCs. An increase in sEPSC frequency without a simultaneous increase in mEPSC frequency could be due to increased firing of cortical pyramidal neurons. Intrinsic properties of pyramidal neurons within the PMG have not yet been fully investigated, although changes have been observed for neurons inside the four-layered region (Luhmann et al. 1998). Changes observed there would not be likely to increase intrinsic excitability, as a decrease in action potential amplitude, and decrease in the firing frequency per current (F/I slope) were observed (Luhmann et al. 1998). A change in intrinsic properties of cortical neurons has been demonstrated in other malformation models. In the telencephalic internal structural heterotopia (TISH) model, neurons within the heterotopia rest at more depolarized levels (Trotter et al. 2006). In the methyl azoxymethanol acetate (MAM) model of microcephaly and focal cortical dysplasia, neurons of the heterotopic region lack the
Kv4.2 potassium channel, making them more likely to burst (Castro et al. 2001). In addition, deafferentation in adult cortex can increase the percent of neurons identified as intrinsically-bursting (Topolnik et al. 2003). It is still unknown if there is an increase in the number of intrinsically-bursting neurons in PMG. It is however likely that additional cellular anomalies exist beyond the hyperinnervation of pyramidal neurons by excitatory afferents. We believe this is particularly true since on P10 there is a significant increase in mEPSC frequency, yet field epileptiform activity is not observed at this age (Jacobs et al. 1999a).

The polymicrogyral region in human epilepsy patients has been shown to be functional during visual, motor and language tasks (Araujo et al. 2006; Staudt et al. 2004; Janszky et al. 2003; Zesiger et al. 2002; Innocenti et al. 2001). Despite this, white matter and tract abnormalities identified with MRI suggest that there may still be abnormal connectivity (Bonilha et al. 2007; Trivedi et al. 2006; Munakata et al. 2006; Staudt et al. 2004). Development of mirror movement abnormalities in patients with polymicrogyria also suggests abnormal connectivity in cortical tracts (RamachandranNair et al. 2006; Sahin et al. 2006). Magnetoencephalography studies further suggest abnormalities in organization and a high degree of variation between polymicrogyria patients (Ishitobi et al. 2005). Focal regions of abnormal connectivity, particularly in the spatial location of inputs could explain these results as well as those associated with various forms of dyslexia (Galaburda and Eidelberg 1982).

Currently identified polymicrogyria genes GPR56 and PAX6 are suggested to have a role in migration and cortical patterning (Jansen and Andermann 2005; Piao et al. 2004; Mitchell et al. 2003; Jimenez et al. 2002; Fukuda et al. 2000; Chapouton et al. 1999). Pax6 is also involved in determining the proportion of specific neuronal cell types (Stoykova et al. 2003; Caric et al. 1997). How these genes affect cortical connectivity is currently unknown, but it loss or abnormal positioning of specific cortical cell types is likely to affect molecular cues necessary for afferent targeting. An example of this comes from MAM rats, in which the heterotopic neurons within the hippocampus have characteristics of superficial layer neocortical neurons (Castro et al. 2002; Chevassus-Au-Louis et al. 1998). Hippocampal heterotopia show abnormal connectivity to the overlying neocortex (Tschuluun et al. 2005; Jacobs et al. 1999b; Chevassus-Au-Louis et al. 1998; Colacitti et al. 1998). Additional connectional abnormalities are found in both hippocampus and neocortex, due to both loss of normal targets and abnormal positioning (Chevassus-Au-Louis et al. 1999; Jones et al. 1981).
It is also possible that the polymicrogyria genes are important for functions beyond the time of development, as suggested in two genetic mutants that show spontaneous seizures. An autosomal recessive mutation causes the telencephalic structural heterotopia (TISH) rat that mimics human heterotopia associated with epilepsy (Lee et al. 1997). The seizures are delayed until approximately P30, suggesting that the affected gene may play a role in maintenance of GABAergic populations (Trotter et al. 2006). Inactivation of the urokinase plasminogen activator receptor (uPAR) gene in mice produces a structurally normal cortex, but with a substantial decrease in the numbers of GABAergic parvalbumin-immunopositive cells (Powell et al. 2003; Powell et al. 2001). Both the decrease in interneurons and the onset of seizures is delayed beyond the time of cortical development (Bae et al. 2006; Bae et al. 2005), again suggesting that both development and maintenance of GABAergic neurons are modulated by uPAR. Seizures are often delayed in humans with polymicrogyria (Kobayashi et al. 2001; Bartolomei et al. 1999), as they are in the freeze lesion model, again suggesting that multiple factors, including hyperinnervation by excitatory cortical afferents, are at play to cause seizure onset.

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Reference List


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Figure Legends

Figure 1. Spontaneous and miniature EPSCs recorded in control and PMG cortex. **A1.** Continuous recording from a control layer V pyramidal neuron. **A2,3.** Recordings from a PMG layer V pyramidal neuron, showing typical higher frequency and bursting behavior. Bursts such as that shown in A3 were rarely observed in control cells. **B.** Continuous recording during TTX application in a control (1) and PMG (2) neuron. **C.** Mean frequency of sEPSCs and mEPSCs for control (gray) and PMG (black) cells. **D.** Number of bursts per minute in sEPSC recordings from control (gray) and PMG (black) cells. Burst was defined as two or more events occurring with an inter-event interval of less than 10 msec. **E.** Coefficient of variation (CV) for inter-event intervals in mEPSC recordings from control (gray) and PMG (black) cells. Data in C and D for sEPSCs from 65 control and 55 PMG cells, for mEPSCs from 32 control and 32 PMG cells. ***= t-test, p<0.005, *= t-test, p<0.05.

Figure 2. Correlations between EPSC frequency and location of recorded cell. **A.** EPSC frequency versus distance from sulcus for PMG cells, measures of sEPSCs (black diamonds) and mEPSCs (gray squares). Regression lines shown for sEPSCs (black, R = 0.00) and mEPSCs (gray, R = -0.07). **B.** sEPSC frequency versus percent depth from pia for control (black circles) and PMG (gray triangles) cells. Regression lines shown for control (black, R = 0.37) and PMG (gray, R = 0.05) data. **C.** Data for mEPSCs displayed as in B. Regression lines shown for control (black, R = 0.45) and PMG (gray, R = 0.09) data. Correlation significant for control but not PMG cells in B and C (Pearson Product Moment).

Figure 3. EPSCs evoked in response to nearby extracellular stimulation. **A.** Result of single stimulus presentations in control cells (**A1,2**) and PMG cells (**A3,4**). **A1.** Typical multipeaked short latency response. **A2.** Smoothly decaying short latency response followed by longer latency EPSCs, that may be evoked or spontaneous. **A3.** Long latency response could be repeatedly evoked with varying form and latency in response to low intensity stimuli. **A4.** Multipeaked short latency response followed by long latency EPSCs riding on a ‘wave’ of inward current that is not typically observed without extracellular stimulation. **B.** Responses to increasing stimulus intensities (1,2,4,8, and 16 X threshold), for control (**B1**) and PMG (**B2**) cells. Traces shown are averages of 5 stimulus presentations. **C.** Mean peak amplitude of
eEPSCs versus stimulus intensity for control (gray) and PMG (black) cells. **D.** Mean eEPSC area versus stimulus intensity for control (gray) and PMG (black) cells. For C and D, data shown is for 15 control and 26 PMG cells. * = significant difference between groups, 2-way repeated measures ANOVA, p<0.01.

Figure 4. Effect of Age on EPSC measures of Frequency (**A**), bursts per minute and coefficient of variation (CV) for inter-event intervals, (**B**), and amplitude (**C**). The sEPSC graphs are shown in the left column and mEPSC graphs are shown in the right column over age (P7:white; P8:light gray; P9:medium gray; P10:dark gray; P11:black). For all sEPSC measures, the following number of cells were analyzed for P7-11 respectively: Control 13, 13, 11, 14, 14; and PMG 9, 10, 12, 9, 14. For mEPSC measures, the following number of cells were analyzed for P7-11 respectively: Control 4, 5, 7, 9, 7 and PMG 6, 5, 8, 5, 6. Arrows with * indicate significant changes with age, based on a 1-way ANOVA for PMG cells. For results on 2-way ANOVAs, see Results section.

Figure 5. Cumulative probability plots for mEPSC inter-event intervals (**A-E**) and mEPSC amplitudes (**F**) for control (gray circles) and PMG cells (black triangles). Survival age shown in upper left corner of plot. Distributions for control and PMG cells were similar at P7-9 (**A-C**), but diverged at P10 & 11 (**D, E**). * = significant difference in distributions, based on K-S test, p<0.001. Despite inter-event interval differences, amplitude distributions for control and PMG were similar at P11 (**F**).

Figure 6. Percent of mEPSCs with an inter-event interval equal to or smaller than 1 second. Values were calculated by generating exponential growth fits of cumulative probability plots shown in Fig. 5 (Origin software). For PMG cells from P11 aged rats, the majority (61%) of events have short inter-event intervals, with values smaller than 1 second. In contrast, for P11 control cells, only 19% of events have inter-event intervals this short.
Table 1. Spontaneous and miniature EPSC measures.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PMG</th>
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<tbody>
<tr>
<td><strong>sEPSCs: 65 control, 55 PMG</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sEPSC Frequency (Hz)</td>
<td>1.6 ±0.2</td>
<td>3.0 ±0.5 **</td>
</tr>
<tr>
<td>sEPSCs Amplitude (pA)</td>
<td>23.8 ±1.9</td>
<td>20.1 ±0.8 NS</td>
</tr>
<tr>
<td>sEPSC Rise Time (msec)</td>
<td>1.6 ±0.1</td>
<td>1.6 ±0.1 NS</td>
</tr>
<tr>
<td>sEPSC Decay Time (msec)</td>
<td>11.3 ±0.4</td>
<td>10.9 ±0.4 NS</td>
</tr>
<tr>
<td><strong>mEPSCs: 32 control, 32 PMG</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mEPSC Frequency (Hz)</td>
<td>1.1 ±0.2</td>
<td>2.3 ±0.6 **</td>
</tr>
<tr>
<td>mEPSCs Amplitude (pA)</td>
<td>15.6 ±1.2</td>
<td>14.5 ±0.8 NS</td>
</tr>
<tr>
<td>mEPSC Rise Time (msec)</td>
<td>1.8 ±0.1</td>
<td>1.9 ±0.1 NS</td>
</tr>
<tr>
<td>mEPSC Decay Time (msec)</td>
<td>9.5 ±0.5</td>
<td>9.3 ±0.5 NS</td>
</tr>
</tbody>
</table>

Values are means ±SEM. ** = p<0.
Figure 1. Spontaneous and miniature EPSCs recorded in control and PMG cortex. A1. Continuous recording from a control layer V pyramidal neuron. A2,3. Recordings from a PMG layer V pyramidal neuron, showing typical higher frequency and bursting behavior. Bursts such as that shown in A3 were rarely observed in control cells. B. Continuous recording during TTX application in a control (1) and PMG (2) neuron. C. Mean frequency of sEPSCs and mEPSCs for control (gray) and PMG (black) cells. D. Number of bursts per minute in sEPSC recordings from control (gray) and PMG (black) cells. Burst was defined as two or more events occurring with an inter-event interval of less than 10 msec. E. Coefficient of variation (CV) for inter-event intervals in mEPSC recordings from control (gray) and PMG (black) cells. Data in C and D for sEPSCs from 65 control and 55 PMG cells, for mEPSCs from 32 control and 32 PMG cells. *** = t-test, p<0.005, * = t-test, p<0.05.
Figure 2. Correlations between EPSC frequency and location of recorded cell. A. EPSC frequency versus distance from sulcus for PMG cells, measures of sEPSCs (black diamonds) and mEPSCs (gray squares). Regression lines shown for sEPSCs (black, R = 0.00) and mEPSCs (gray, R = -0.07). B. sEPSC frequency versus percent depth from pia for control (black circles) and PMG (gray triangles) cells. Regression lines shown for control (black, R = 0.37) and PMG (gray, R = 0.05) data. C. Data for mEPSCs displayed as in B. Regression lines shown for control (black, R = 0.45) and PMG (gray, R = 0.09) data. Correlation significant for control but not PMG cells in B and C (Pearson Product Moment).
Figure 3. EPSCs evoked in response to nearby extracellular stimulation. A. Result of single stimulus presentations in control cells (A1,2) and PMG cells (A3,4). A1. Typical multipeaked short latency response. A2. Smoothly decaying short latency response followed by longer latency EPSCs, that may be evoked or spontaneous. A3. Long latency response could be repeatedly evoked with varying form and latency in response to low intensity stimuli. A4. Multipeaked short latency response followed by long latency EPSCs riding on a 'wave' of inward current that is not typically observed without extracellular stimulation. B. Responses to increasing stimulus intensities (1,2,4,8, and 16 X threshold), for control (B1) and PMG (B2) cells. Traces shown are averages of 5 stimulus presentations. C. Mean peak amplitude of eEPSCs versus stimulus intensity for control (gray) and PMG (black) cells. D. Mean eEPSC area versus stimulus intensity for control (gray) and PMG (black) cells. For C and D, data shown is for 15 control and 26 PMG cells. * = significant difference between groups, 2-way repeated measures ANOVA, p<0.01.
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