Maturation of Layer V Pyramidal Neurons in the Rat Prefrontal Cortex: Intrinsic Properties and Synaptic Function

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Zhang, Zhong-wei. Maturation of layer V pyramidal neurons in the rat prefrontal cortex: intrinsic properties and synaptic function. J Neurophysiol 91: 1171–1182, 2004. First published November 5, 2003; 10.1152/jn.00855.2003. Layer V pyramidal neurons in the rat medial prefrontal cortex (PFC) were examined with whole cell patch-clamp recording in acute slices from postnatal day 1 (P1) to P36. In the first few days after birth, layer V pyramidal neurons had low resting potentials, high-input resistance, and long membrane time constant. During the next 2 wk, the resting potential shifted by −14 mV, while the input resistance and time constant decreased by 15- and 4-fold, respectively. Between P3 and P21, the surface area of the cell body doubled, while the total lengths of apical and basal dendrites increased by 5- and 13-fold, respectively. Action potentials (APs) were observed at all aged tested. The peak amplitude of APs increased by 30 mV during the first 3 wk, while AP rise time and half-maximum duration shortened significantly. Compared with neurons at P21 or older, neurons in the first week required much smaller currents to reach their maximum firing frequencies, but the maximum frequencies were lower than those at older ages. Stimulation of layer II/III induced monosynaptic responses in neurons older than P5. Paired-pulse responses showed a short-term depression at P7, which shifted progressive to facilitation at older ages. These results demonstrate that, similar to other neurons in the brain, layer V pyramidal neurons in the PFC undergo a period of rapid development during the first 3 wk after birth. These findings suggest that the intrinsic properties of neurons and the properties of synaptic inputs develop concomitantly during early life.

INTRODUCTION

The mammalian prefrontal cortex (PFC) makes up a large portion of the frontal lobe and undergoes progressive expansion in higher mammals, reaching its greatest development in humans (Le Gros Clark 1932; Rakic and Goldman-Rakic 1982). The PFC consists of several cortical areas that receive specific innervation from the mediodorsal nucleus of the thalamus (Ongur and Price 2000; Uylings and van Eden 1990). In rats, the prelimbic, infralimbic, and dorsal anterior cingulate areas are the major subdivisions of the PFC (Groenewegen 1988; Krettek and Price 1977).

Functional studies suggest that the PFC, via its integration into the neural network of the basal ganglia, participates in the organization and planning of goal-directed tasks (Fuster 1991; Goldman-Rakic 1995). A key mechanism involved is a form of short-term memory called the working memory, which serves as a workspace for holding momentarily an item of information and using it subsequently to guide correct responses (Fuster 1991; Goldman-Rakic 1990). Excitatory recurrent neural circuits in the PFC are believed to form the cellular basis for the working memory (Fuster and Alexander 1971; Kubota and Niki 1971; Wang 2001). As expected, dysfunction of the PFC has been implicated in several mental illnesses, particularly schizophrenia. Deficiency in the working memory process in the PFC has been associated with the symptoms and cognitive deficits that are prominent of schizophrenia (Goldman-Rakic 1994; Weinberger and Berman 1996). Although the causes for such malfunction may be complex, many studies suggest abnormal neural connections that occur during early postnatal development (Jones 1997; Lewis and Levitt 2002; Raedler et al. 1998).

Electrical activities play important roles in developmental processes including neuronal differentiation, cell migration, formation, and refinement of synaptic connections (Katz and Shatz 1996; Rakic and Komuro 1995; Spitzer 1991). Two broad mechanisms control the activity of any given neuron. The first is intrinsic membrane properties of the neuron, which are determined by its morphology and the level and distribution of various ion channels (Connors 1994). The second type of mechanisms consists of interactions with other cells, in particular synaptic transmission between neurons. Numerous studies conducted in various parts of the brain have shown that both intrinsic properties of neurons and synaptic transmission undergo dramatic changes during early postnatal development and that each immature site may have distinct combinations of intrinsic and synaptic properties (Burgard and Hablitz 1993; Kriegstein et al. 1987; McCormick and Prince 1987; Picken Bahrey and Moody 2003; Ramoa and McCormick 1994a). Although several studies have suggested a late maturation for the PFC (Kolb and Nonneman 1976, 1978), little is known about the development of functional properties of neurons in the PFC. In this study, I examine postnatal changes in intrinsic properties and synaptic transmission in layer V pyramidal neurons in the prelimbic area of the PFC. These neurons provide the principal efferent of the cortex and project extensively to the striatum (Berendse et al. 1992; Jones et al. 1977; Levesque and Parent 1998).

METHODS

Slice preparations

Brain slices were prepared from Sprague-Dawley rats, of either sex, aged P1–P36 (with the day of birth as P0) as described previously (Zhang 2003). All procedures were performed according to the guide-
lines of the Canadian Council on Animal Care and were approved by the Animal Care Committee at Laval University. Briefly, rats were deeply anesthetized with ketamine and xylazine and decapitated. The brain was removed quickly (<60 s) and placed in ice-cold solution containing (in mM) 210 sucrose, 3.0 KCl, 1.0 CaCl₂, 3.0 MgSO₄, 1.0 NaH₂PO₄, 26 NaHCO₃, and 10 glucose, saturated with 95% O₂-5% CO₂ (Aghajanian and Rasmussen 1989; Moyer and Brown 1998). Coronal slices, including the prelimbic area, were cut at 300–350 μm on a vibrating tissue slicer (VT 1000s, Leica) and kept in artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3.0 KCl, 2.0 CaCl₂, 1.3 MgSO₄, 1.0 NaH₂PO₄, 26 NaHCO₃, and 2.0 glucose, saturated with 95% O₂-5% CO₂ at room temperature. Slices were allowed to recover for ≥1 h before any recording.

A slice was transferred to a submerger-type chamber where it was continuously exposed to ACSF at 30–32°C saturated with 95% O₂-5% CO₂ and flowed at a rate of 1.8 ± 0.2 ml/min. The slice was viewed first with a 4× objective, and the prelimbic area of the PFC was localized as the area between the forceps minor corpus callosum and the midline (Paxinos and Watson 1998). Layers I, II–III, V, and VI of the prelimbic area were viewed under near infrared illumination with a 40× water-immersion objective (Fluor, 40X/0.80W, Nikon) and a CCD camera (CCD-300-RC, MTI, Michigan City, IN). Layer I can be easily identified due to a very low density of cells. The border between layers II/III and layer V is not as clear-cut, but as a general rule, the thickness of layer II/III is about the same as that of layer I in the prelimbic area throughout postnatal development (Van Eden and Uylings 1985). Layer V pyramidal neurons were identified by their large size and apical dendrite.

**Patch-clamp recording**

All experiments were conducted at 30–32°C. Electrodes were pulled from thick wall borosilicate glass (1.5/0.84 mm, WPI, Sarasota, FL) on a horizontal puller (P-97, Sutter Instruments, Novato, CA). The pipette solution contained (in mM) 120 K-glutamate, 15 KCl, 0.5 EGTA, 4 ATP-Mg, 0.3 GTP-Na₂, and 20 HEPES (pH 7.4 with KOH, 280–290 mOsm). Electrodes had resistances between 3 and 7 MΩ. The seal resistance was >5 GΩ. Whole cell recordings were made from the soma with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Current-clamp recording was performed in the Fast Current Clamp mode. The series resistance (Rₛ), usually between 10 and 40 MΩ, was monitored throughout the recording and was compensated 100%. For voltage-clamp recording, Rₛ was usually between 10 to 25 MΩ and was not compensated unless specified otherwise. Rₛ was monitored throughout the recording, and data were discarded if there was over 20% change in Rₛ. Experiments were conducted using the Clampex program (pClamp 6, Axon Instruments), and data were filtered at 1 or 2 kHz and digitized at 4 or 8 kHz.

For stimulation, a pipette with a large tip (~3 μm) was filled with ACSF and placed in upper Layer II/III, 100–200 μm away from the apical dendrite of the recorded layer V neuron. Brief current pulses (40 μs, 1–36 μA) were delivered with a stimulation-isolation unit (PG4000A, SIU90, Cygnus Technology).

**Biocytin histochemistry and cell reconstruction**

Cells were labeled with 0.25% neurobiotin (Vector Laboratories, Burlingame, CA) included in the patch pipette. Slices were processed as described previously (Reyes and Sakmann 1999; Zhang and Beschens 1997). Briefly, slices were fixed in 4% paraformaldehyde in 0.1 M PBS for ≥12 h. After 2 wash in PBS, slices were treated with 0.3% H₂O₂ in 10% MeOH and 90% PBS for 30 min. After 5 wash in PBS, slices were treated with 2% Triton X-100 and 0.5% normal rabbit serum in PBS for 1 h. Slices were incubated with the avidin-biotin peroxidase complex (ABC Elite Standard, Vector Laboratories, Burlingame, CA) for 4 h at room temperature. Slices were reacted with 3,3-diaminobenzidine (1 mg/ml) for 5–10 min. Slices were then mounted onto slides, dehydrated, embedded in Pertum, and coverslipped. Slices were viewed with a 40× objective, and cells were reconstructed and analyzed with the Neuronlucida system (Micro- Brightfield, Colchester, VT). The total lengths of the apical and basal dendrites, and the major and minor diameters (a and b) of the cell body were estimated with NeuroExplorer. The surface area (SA) of cell body was calculated as: SA = πab. No shrinkage correction was used.

**Data analysis**

AxoGraph 4 and Clampfit 6 (Axon Instruments) were used for analysis. Input resistance and membrane time constant were estimated in current-clamp mode by applying small hyperpolarizing current steps. Spike threshold, rise time, peak amplitude, and half time were measured for the first action potential evoked by a short depolarizing current pulse. The liquid junction potential was estimated to be 12 mV, and corrections were made accordingly to all membrane potentials.

Statistics and data plotting were done with Origin 7 (OriginLab, Northampton, MA). Throughout, means are given as mean ± SE. Means were compared by two-tailed Student’s t-test.

**Results**

Data were collected from 294 layer V pyramidal neurons from 78 rats aged between P1 and P36 (with the day of birth as P0). Only neurons with resting potentials more negative than −45 mV were included. Figure 1 illustrates typical voltage responses to intracellular current injections in neurons at P3, P12, and P30.

**Postnatal changes in resting membrane properties**

We examined the resting membrane potential, input resistance, and time constant of neurons aged from P1 through P36. The resting membrane potential (Vᵣ) was measured immediately after achieving whole cell configuration. At P1 and P3, about one-half of the neurons recorded showed persistent spontaneous firing after break in. Most of these spontaneously active cells lost their activities within minutes and became depolarized, with Vᵣ staying around −20 mV. After P5, fewer than 15% of neurons were spontaneously active. It is possible that at least some of the spontaneous activities seen at P1 and P3 are experimental artifacts, because brains at these ages are very fragile, and therefore, more venerable to damages associated with slice sectioning. Accordingly, only cells with little or no spontaneous firing (<0.2 Hz) were included. The results are summarized in Fig. 2. At P1, Vᵣ increased more negative during the first 2 wk after birth. At P3, Vᵣ was −65.6 ± 1.6 mV (n = 9). At P16, Vᵣ was −76.7 ± 0.7 mV (n = 10), about 11 mV more negative than at P3 (P < 0.001). There was little change in Vᵣ after P16; at P36, Vᵣ was −76.5 ± 0.9 mV (n = 8), which was not different from that at P16 (P > 0.8).

Membrane input resistance and time constant were estimated with small current pulses of 600 ms duration near the resting membrane potential.

**Membrane input resistance (Rᵣ).** There was a large reduction in Rᵣ during early life (Fig. 2, B₁ and B₂). Although the largest reduction occurred during the first 2 wk after birth, Rᵣ decreased significantly during the third week. After P21, there was little change in Rᵣ. Rᵣ was 1,139 ± 100 MΩ at P3 (n = 9), 384 ± 36 MΩ at P7 (n = 9), 168 ± 20 MΩ at P13 (n = 12), 70 ± 4 MΩ at P36.
Postnatal changes in cell morphology

To correlate changes in membrane properties with those in morphology, 66 neurons aged between P3 and P30 were reconstructed. Figure 3 illustrates examples of cells at various ages. The SA of the cell body was estimated as \( \text{SA} = \pi ab \), with \( a \) and \( b \) being the major and minor diameter of the cell body, respectively. SA doubled between P3 and P21, with most change occurring before the end of the second week (Fig. 4A). The mean SA was 626 ± 30 \( \mu \text{m}^2 \) at P3 (\( n = 10 \)), 1,163 ± 60 \( \mu \text{m}^2 \) at P13 (\( n = 8 \); \( P < 0.0001 \) vs. P3), and 1,248 ± 79 \( \mu \text{m}^2 \) at P21 (\( n = 8 \); \( P > 0.3 \) vs. P13). There was rapid growth of both apical and basal dendrites during the first 3 wk. The total length of apical dendrites increased fivefold between P3 and P21 (Fig. 4B), with mean values of 491 ± 76 \( \mu \text{m} \) at P3 (\( n = 10 \)) and 3,229 ± 437 \( \mu \text{m} \) at P21 (\( n = 8 \); \( P < 0.0001 \) vs. P3). The total length of basal dendrite showed an even larger increase; from 185 ± 12 \( \mu \text{m} \) at P3 (\( n = 10 \)) to 2,697 ± 241 \( \mu \text{m} \) at P21 (\( n = 8 \); \( P < 0.0001 \) vs. P3), there was a 13-fold increase (Fig. 4C).

Threshold changes in action potential properties

Threshold, rise time, peak amplitude, and duration at half-maximum height were measured for the first action potential evoked by depolarizing current pulses of 50 ms in duration. Figure 5A illustrates typical action potentials at P3, P9, P13, and P21. Action potential threshold was difficult to determine at P1 because the transition to action potential was not as clear cut as at older ages. Therefore neurons at P1 were not included here.

Threshold (\( V_{\text{thres}} \)) of action potential. There was a substantial negative shift in \( V_{\text{thres}} \) during the first 3 wk after birth (Fig. 5, \( B_1 \) and \( B_2 \)). \( V_{\text{thres}} \) was −42.1 ± 0.5 mV at P3 (\( n = 9 \)), −47.4 ± 0.8 mV at P7 (\( n = 8 \)), and −54.8 ± 0.5 mV at P21 (\( n = 11 \)). Data can be fitted to an exponential function: \( y = A_1 \times \exp(-x/r_1) + y_0 \), where \( y_0 \) is the \( V_{\text{thres}} \) in adult, \( A_1 \) is the postulated \( R_N \) at birth minus \( y_0 \), and \( r_1 \) the decay time constant. The results give 63 ± 2 M\( \Omega \), 1733 ± 145 M\( \Omega \), and 4.3 ± 0.2 d for \( y_0 \), \( A_1 \), and \( r_1 \), respectively (Fig. 2B).

Membrane time constant (\( t_m \)). The \( t_m \) was estimated by fitting the change in membrane potential induced by a small hyperpolarizing current step to a single exponential function. The changes in \( t_m \) follow a time course similar to that of \( R_N \), but with much smaller magnitude (Fig. 2, \( C_1 \) and \( C_2 \)). \( t_m \) was at 69 ± 8 ms at P3 (\( n = 9 \)), 47 ± 3 ms at P7 (\( n = 9 \)), 28 ± 2 ms at P13 (\( n = 12 \)), 13 ± 1 ms at P21 (\( n = 11 \)), and 12 ± 1 ms at P30 (\( n = 12 \); \( P > 0.6 \) vs. P21).

Postnatal changes in cell morphology

To correlate changes in membrane properties with those in morphology, 66 neurons aged between P3 and P30 were reconstituted. Figure 3 illustrates examples of cells at various ages. The SA of the cell body was estimated as \( \text{SA} = \pi ab \), with \( a \) and \( b \) being the major and minor diameter of the cell body, respectively. SA doubled between P3 and P21, with most change occurring before the end of the second week (Fig. 4A). The mean SA was 626 ± 30 \( \mu \text{m}^2 \) at P3 (\( n = 10 \)), 1,163 ± 60 \( \mu \text{m}^2 \) at P13 (\( n = 8 \); \( P < 0.0001 \) vs. P3), and 1,248 ± 79 \( \mu \text{m}^2 \) at P21 (\( n = 8 \); \( P > 0.3 \) vs. P13). There was rapid growth of both apical and basal dendrites during the first 3 wk. The total length of apical dendrites increased fivefold between P3 and P21 (Fig. 4B), with mean values of 491 ± 76 \( \mu \text{m} \) at P3 (\( n = 10 \)) and 3,229 ± 437 \( \mu \text{m} \) at P21 (\( n = 8 \); \( P < 0.0001 \) vs. P3). The total length of basal dendrite showed an even larger increase; from 185 ± 12 \( \mu \text{m} \) at P3 (\( n = 10 \)) to 2,697 ± 241 \( \mu \text{m} \) at P21 (\( n = 8 \); \( P < 0.0001 \) vs. P3), there was a 13-fold increase (Fig. 4C).

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DURATION OF ACTION POTENTIAL. The duration of action potential was estimated as the width at half-maximum height of an action potential ($t_{1/2}$). There was a 75% reduction in $t_{1/2}$ during the first 3 wk after birth (Fig. 6, C1 and C2). $t_{1/2}$ was 3.96 ± 0.24 ms at P3 ($n = 11$), 2.33 ± 0.15 ms at P7 ($n = 9$), 1.28 ± 0.04 ms at P13 ($n = 12$), 0.91 ± 0.03 ms at P21 ($n = 11$), and 0.93 ± 0.05 ms at P36 ($n = 8, P < 0.05$ vs. P21). Data can be fitted to a single exponential function: $y_0, A_1, \text{and} t_1$ are the estimated $t_{1/2}$ in adults, the estimated $t_{1/2}$ at birth minus $y_0$, and the decay time constant, respectively. The results give 0.86 ± 0.01 ms, 5.83 ± 0.42 ms, and 5.1 ± 0.2 d for $y_0, A_1, \text{and} t_1$, respectively.

TRAINS OF ACTION POTENTIAL. I examined spike trains evoked by depolarizing current pulses of 600 ms long, applied at 0.1 Hz. At all ages tested, neurons were capable to respond with a train of spikes (Fig. 7, A–C). About 98% of neurons were regular spiking cells that showed moderate adaptation in spike frequency. Spike frequency was estimated by counting the number of spikes in the last 500 ms of the current step. The minimum current required for spike train induction increased significantly during development: at P3, a 20-pA step induced spike train in all cells tested ($n = 6$); at P21, 100 pA or more was needed ($n = 9$). The input-output relationship was examined by plotting spike frequency versus the amplitude of injected current (Fig. 7D). The input-output curve was very steep at P3; a linear fit of the initial portion of the curve gives a slope of 580 ± 48 Hz/nA ($n = 5$). The slope flattened progressive over the next 10 days (Fig. 7E), with means of 164 ± 16 Hz/nA at P7 ($n = 9, P < 0.0001$ vs. P3) and 88 ± 9 Hz/nA at P13 ($n = 6, P < 0.005$ vs. P7). There was little change in the slope of the input-output curves after P13. The slopes were 70 ± 3 Hz/nA at P16 ($n = 6, P > 0.09$ vs. P13); 76 ± 4 Hz/nA at P21 ($n = 9, P > 0.2$ vs. P13); and 84 ± 6 Hz/nA at P28 ($n = 6, P > 0.5$ vs. P13). Data can be fitted to
an exponential function with a decay constant of 2.3 ± 0.2 d (Fig. 7E).

Besides the changes of the input-output curve, there was a 100% increase in the maximum frequency during the first 3 wk after birth (Fig. 7F). The maximum frequency was at 30 ± 4 Hz at P3 (n = 6), 45 ± 4 Hz at P13 (n = 5, P < 0.02 vs. P3), and 57 ± 2 Hz at P21 (n = 10, P < 0.02 vs. P13). There was no change in the maximum frequency between P21 and P28 (59 ± 3 Hz, P > 0.5 vs. P21).

INTRINSICALLY BURSTING NEURONS. Only five neurons fired bursts of AP in response to current steps, and all of them were from rats aged P18 or older. Figure 8 illustrates an example of such neurons. The neuron fired a burst of AP at the beginning of a spike train (Fig. 8A). Reconstruction revealed a thick-tufted pyramidal neuron (Fig. 8B).

**Postnatal changes in synaptic responses**

Layer II/III pyramidial neurons provide a major source of excitatory inputs to layer V pyramidial neurons in the neocortex (Thomson and Bannister 2003). In the PFC, this projection is believed to play a key role in goal-directed behaviors (Fuster 1991; Goldman-Rakic 1995). Little is known however, about the development of layer II/III to layer V connections. We examined synaptic responses in neurons voltage-clamp at −72 mV, near the resting membrane potential (Fig. 9A). At P3, stimulation of layer II/III produced no response (n = 8), even at high stimulus intensities (15–20 μA, 40 μs). At P5, 3 of 12 cells tested showed monosynaptic responses, and the responses were small in amplitude, in the range of −10 to −25 pA. At P7, all neurons showed monosynaptic responses when layer II/III was stimulated (n = 13). In five cells, bath application of 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μM) and d-2-amino-5-phosphonopentanoic acid (d-AP5; 100 μM) blocked the monosynaptic response, suggesting that the response results from glutamatergic transmission. The input-output relationship was examined by plotting the peak amplitude of the monosynaptic response versus the intensity of the stimulation (Fig. 9B). The threshold decreased slightly from about 6 μA at P7 to about 3 μA at P21. The maximum response was reached at around 28 μA for all ages examined. The maximum response at P7 was small, with the mean peak amplitude of 137 ± 22 pA (n = 6). Over the next 2 wk, there was a substantial increase in the response (Fig. 9C). At P21, the maximum response was 1.35 ± 0.16 nA (n = 7; P < 0.0001 vs. P7).

**SHORT-TERM PLASTICITY OF SYNAPSES.** Postnatal changes in short-term synaptic plasticity have been observed in a number of structures including the striatum and other areas of the neocortex (Choi and Lovinger 1997; Kumar and Huguenard 2001; Reyes and Sakmann 1999). We examined paired-pulse responses evoked at 50-ms intervals in neurons voltage clamped at −72 mV (Fig. 10A). The stimulation intensity was set to produce less than one-third of the maximum response. At P7, the responses showed a small depression, with a paired-pulse ratio (p2/p1) of 0.84 ± 0.06 (n = 9). This ratio increased gradually over the next 2 wk (Fig. 10B), so that by P21, there was a considerable facilitation in the paired pulse response with a ratio of 1.57 ± 0.08 (n = 11, P < 0.001 vs. P7).
the paired-pulse ratio was $1.51 \pm 0.07 (n = 10)$, similar to that of P21 ($P > 0.5$, P21 vs. P30).

DISCUSSION

In this study, I showed that layer V pyramidal neurons in the medial PFC undergo remarkable changes in functional properties and morphology during early postnatal life. Although the largest changes were observed during the first 10 days after birth, adult-like properties were not reached until the end of the third week (P21). The results are consistent with previous studies in other parts of the brain and suggest that the maturation of layer V pyramidal neurons in the PFC follows a time course similar to that described for other neurons in the brain.

Development of passive membrane properties

The resting membrane potential ($V_r$) shifted by about $-14$ mV during the first 2 wk after birth. This is comparable with the results obtained from hippocampal pyramidal neurons (Spigelman et al. 1992), from layer II/III pyramidal neurons and layer I interneurons in the neocortex (Burgard and Hablitz 1993; Zhou and Hablitz 1996), and from neurons in the thalamus (Ramoa and McCormick 1994a) and striatum (Belleau and Warren 2000). It is not clear why younger neurons showed lower $V_r$. One possibility is that neurons in newborn rats are more venerable to damages induced by slice cutting and therefore may have lower $V_r$ in vitro. A second possibility is that the lower $V_r$ can be attributed to the internal dialysis introduced by whole cell patch-clamp recordings, and that the difference between young and mature neurons is due to the much reduced extent of dialysis in adult neurons. A third possibility is that the higher ratio of cell input resistance to the seal resistance in neonatal neurons causes significant error in $V_r$ measurement. The last and more interesting possibility is that the shift of $V_r$ is physiological and reflects a developmental change in the ionic basis of $V_r$ in neurons during early life. Future studies are needed to examine these possibilities.

The input resistance ($R_I$) decreased by about 15-fold during the first 3 wk, with the largest reduction happened during the first 10 days. This is comparable with those observed in layer I interneurons in the rat neocortex (Zhou and Hablitz 1996) and in deep layer pyramidal neurons in the mouse neocortex (Picken Bahrey and Moody 2003). Interestingly, neurons in the mouse neocortex showed little change in $R_I$ from E16 through P2 (Picken Bahrey and Moody 2003). Thus the birth marks the beginning of a period of rapid development for neurons in the brain.

Consistent with other studies, the decrease of membrane time constant ($\tau_m$) followed the same time course as $R_I$, but with much less reduction (from 70 ms at P3 to 13 ms at P21). Assuming that the specific capacitance of the membrane remains constant during development, the specific membrane resistance at P21 was estimated to be one-fifth of that at P3. Both the growth of neurons and an increase in membrane conductance therefore contribute to the decline of $R_N$ during development.

Correlations between changes in morphology and input resistance

My results showed that, between P3 and P21, the surface area of the cell body doubled, while the lengths of apical and basal dendrites increased by 5- and 13-fold, respectively.
above), the 100% increase in the surface area of the cell body between P3 and P21 would account for about 60% of the decrease in $R_N$ during the same period. The remaining 40% may be attributed to the changes of apical and basal dendrites.

**Development of AP properties**

Consistent with other studies (Belleau and Warren 2000; Ramoa and McCormick 1994a; Zhou and Hablitz 1996), our results showed that the amplitude of action potentials increased by 30 mV during the first 3 wk, while the rise time and duration decreased by 50 and 75%, respectively. These changes clearly reflect the up-regulation of voltage-dependent sodium and potassium conductance during development (Huguenard et al. 1988; Picken Bahrey and Moody 2003). Associated with the changes in single action potentials, the properties of spike trains also changed remarkably: older neurons required higher currents for train generation, with less steep input-output curves and higher maximum frequency. Thus neurons at different ages are tuned to respond to the developmental changes in the properties of synaptic inputs.

An interesting finding of this study is that, although the spike threshold ($V_{\text{thres}}$) shifted $-14$ mV during the first 3 wk, the difference between $V_{\text{thres}}$ and $V_r$ remained stable at 21 mV throughout the postnatal development. A similar result has been reported in the visual thalamus of the ferret (Ramoa and McCormick 1994a). Together, these results suggest that the amount of depolarization required for spike generation, and thus the responsiveness of a neuron, is a constant during development. Since $V_{\text{thres}}$ and $V_r$ are determined by distinct mechanisms (the former by voltage-gated $Na^+$ conductance and the latter largely by $K^+$ conductance), cellular mechanisms are required to coordinate the development of various membrane conductance (Moody 1998; Spitzer 1991; Spitzer and Ribera 1998).

Less than 2% of neurons examined here were intrinsically bursting (IB) neurons. This is much less than what has been found in other studies conducted in adult or young adult neocortex, where 30–50% of pyramidal neurons were found to be IB type (Agmon and Connors 1992; Chagnac-Amitai et al. 1990; Franceschetti et al. 1998; Larkum and Zhu 2002). This discrepancy is probably not due to the differences in methods, because under similar conditions, Larkum and Zhu (2002) found that 50% of layer V pyramidal neurons in the rat so-

![FIG. 5. Developmental changes in action potential. A: examples of action potential recorded at P3, P9, P13, and P21. Thin line indicates 0 mV level, and spike threshold is shown by dashed lines; filtered at 2 kHz and digitized at 8 kHz. Please note increase in spike amplitude and reduction in spike duration during development. B: scatter plot of spike threshold at various ages. B: mean ± SE from cells recorded at a given age. Data can be fit to a single exponential function with a time constant of 6.2 d ($R^2 = 0.98$). C: difference between spike threshold ($V_{\text{thres}}$) and resting potential ($V_r$), measured at various ages. C: mean ± SE. A linear fit gives a slope of $-0.006$ mV/d, suggesting that the difference between $V_{\text{thres}}$ and $V_r$ remains stable during the 1st 5 wk after birth.](image-url)
matosensory cortex were IB cells. In the rat sensorimotor cortex, IB neurons start to appear after P14, and the proportion of IB neurons increases steadily until adulthood (Franceschetti et al. 1998). In this study, all IB neurons were found after P18, and they represented about 10% of all neurons aged P18–P36, but the proportion of IB did not increase between P18 and P36. A recent study has shown that about 30% of pyramidal neurons in the adult rat PFC are IB cells (Degenetais et al. 2002). It is possible that in the PFC, the maturation of IB neurons requires a longer period.

Comparison with previous developmental studies of layer V pyramidal neurons in rats

The developmental change in \( V_r \) reported here is similar to those observed in the visual and sensorimotor cortices (Franceschetti et al. 1998; Kasper et al. 1994; Zhu 2000). On the other hand, \( R_S \) and \( V_m \) of immature neurons reported here were much higher than those obtained with sharp electrode intracellular recordings in the sensorimotor cortex (Franceschetti et al. 1998; McCormick and Prince 1987) and in the visual cortex (Kasper et al. 1994), but are comparable with those obtained with patch-clamp recordings in the somatosensory cortex (Kim et al. 1995; Zhu 2000). Our results may represent a better estimation because of the much reduced leak current with patch-clamp recordings (Spruston and Johnston 1992). The quality of seals may be particularly important for neonatal neurons.

Developmental changes in action potential properties of layer V pyramidal neurons have been examined in detail with sharp electrodes in the rat visual cortex (Kasper et al. 1994). The changes in action potential amplitude, duration, and rise time were comparable to those reported here, suggesting a similar time course of maturation for these two areas. However, unlike what’s been shown in the visual thalamus (Ramoa and McCormick 1994a) and my results in the PFC, the difference between \( V_{thres} \) and \( V_r \) appeared to decrease in the visual cortex during development. It is not clear whether this discrep-

FIG. 6. Developmental changes in spike amplitude, rise time, and duration at half-maximum height. A1–C1: results from neurons at various ages. A2–C2: mean ± SE from neurons at a given age. Spike amplitude increased by 30 mV during the 1st 2 wk, whereas the rise time and duration at half-maximum height decreased substantially during the same period. Decay of one-half height duration can be fitted to a single exponential function with a time constant of 5.1 d (\( \tau = 0.97 \)).
FIG. 7. Developmental changes in spike trains. A–C: responses in membrane potential to 600-ms current steps in neurons at P3 (A), P13 (B), and P21 (C). Amplitudes of current steps are indicated at beginning of each trace. The 2nd trace in each panel shows near maximum frequency response. D: input-output curves obtained from neurons aged P3 (○), P7 (△), and P13 (●). E: initial slopes of input-output curves at various ages. Data can be fitted to an exponential function with a decay time constant of 2.3 d ($R^2 = 0.95$). F: maximum frequency of spike train at various ages. There was a 100% increase between P3 and P12. In E and F, number of neurons tested at each age is given in parentheses.

FIG. 8. Firing pattern and morphology of an intrinsic bursting neuron. A: burst of action potential leads the spike train evoked by a current step of +400 pA in a P18 neuron. B: morphological reconstruction of the neuron shown in A.
Development of synaptic function

Our results showed that the layer II/III to layer V projection became functional around P6. This is ≥3 days later than the thalamocortical and other intracortical synapses (Burgard and Hablitz 1993; Crair and Malenka 1995; Kim et al. 1995). A previous study has shown that layer II/III of the rat medial PFC is still poorly differentiated at P6, being composed of neurons with a large nucleus and little cytoplasm (Van Eden and Uylings 1985). It is therefore possible that the layer II/III to layer V projection is established relatively late during development. The input-output relationship of the monosynaptic response did not change significantly between P7 and P21, although the maximum response increased by about ninefold during the same period. This developmental change may be attributed to an increase in the number of synapses and an up-regulation of function at individual synapses.

The paired-pulse responses changed significantly during development: a short-term depression was observed at P9 or younger, whereas a clear facilitation was seen at P21. A similar developmental switch in short-term plasticity has been observed at other synapses in the striatum, cerebellum, and neocortex (Choi and Lovinger 1997; Pouzat and Hestrin 1997; Reyes and Sakmann 1999). However, it is not clear whether different synapses follow the same time course. Our results suggest that, at layer II/III to layer V synapses, the change in the short-term plasticity is complete by P21.

Developmental regulation of neuronal activity

The developmental changes in $R_N$ and $t_{\text{rise}}$ imply that younger neurons respond well to small synaptic currents with slow rise and decay time constant, whereas large synaptic currents with...
fast kinetics are effective in older neurons. Interestingly, such a shift of excitatory synaptic input has been observed in many parts of the brain: synaptic responses at early ages have a large NMDA component with slow kinetics, whereas a fast AMPA component becomes dominant at later stages (Burgard and Hablitz 1993; Crair and Malenka 1995; Kim et al. 1995; Ramoa and McCormick 1994b). The short-term synaptic depression and the lower maximum spike train frequency at young ages suggest that low frequency synaptic inputs dominate in immature neocortex. On the other hand, immature neurons, because of their long $r_m$, should have greater capacity for temporal integration. Therefore the postnatal development of the intrinsic properties of neurons appears to be interrelated with the maturation of synaptic inputs. A recent study in culture has shown that lowering $V_t$ and $R_N$ by overexpressing an inward rectifier $K^+$ channel led to a reduction in excitatory inputs (Burrone et al. 2002). It would be interesting to see whether modifications of synaptic inputs affect the development of the intrinsic properties of neurons.

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