Maturation of layer 5 pyramidal neurons in the rat prefrontal cortex: intrinsic properties and synaptic function

Zhong-wei Zhang

Centre de recherche Université Laval Robert-Giffard, Département de psychiatrie, Faculté de médecine, Université Laval, Québec City, Canada

**Running Head:** Development of neurons in the prefrontal cortex

**Correspondence:**
Zhong-wei Zhang, Ph.D.
Centre de recherche U-Laval Robert-Giffard
2601, de la canadière, F-6500
Québec, QC, G1J 2G3, Canada
Tel : 418-663-5000, extension 6899
Fax : 418-663-8756
Email : zhongwei.zhang@crulrg.ulaval.ca

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ABSTRACT

Layer 5 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 5 pyramidal neurons had low resting potentials, high input resistance and long membrane time constant. During the next 2 weeks, 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 weeks, 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 2/3 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 5 pyramidal neurons in the PFC undergo a period of rapid development during the first three weeks after birth. These findings suggest that the intrinsic properties of neurons and the properties of synaptic inputs develop concomitantly during early life.

Key Words: neocortex, slice, patch-clamp, layer 5 pyramidal neuron, development
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 (Uylings and van Eden 1990; Ongur and Price 2000). In rats, the prelimbic, infralimbic and dorsal anterior cingulate areas are the major subdivisions of the PFC (Krettek and Price 1977; Groenewegen 1988).

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 (Goldman-Rakic 1990; Fuster 1991). 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, in particular 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 abnormalities that occur during early postnatal development (Jones 1997; Raedler et al. 1998; Lewis and Levitt 2002).

Electrical activities play important roles in developmental processes including neuronal differentiation, cell migration, formation and refinement of synaptic connections (Spitzer 1991; Rakic and Komuro 1995; Katz and Shatz 1996). 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 (Kriegstein et al. 1987; McCormick and Prince 1987; Burgard and Hablitz 1993; Ramoa and McCormick 1994a; Picken Bahrey and Moody 2003).

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 the present 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 (Jones et al. 1977; Berendse et al. 1992; Levesque and Parent 1998).
MATERIALS AND METHODS

Slice Preparations

Brain slices were prepared from Sprague-Dawley rats of either sex aged P1 to P36 (with the day of birth as P0) as described previously (Zhang 2003). All procedures were performed according to the guidelines of the Canadian Council on Animal Care, and were approved by the Animal Care Committee at Laval University. Briefly, rats were deeply anaesthetized 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, 10 glucose, saturated with 95% O₂ and 5% CO₂ (Aghajanian and Rasmussen 1989; Moyer and Brown 1998). Coronal slices including the prelimbic area were cut at 300 to 350 µm on a vibrating tissue slicer (VT 1000s, Leica, Germany), and kept in artificial cerebral spinal fluid (ACSF) containing (in mM): 124 NaCl, 3.0 KCl, 2.0 CaCl₂, 1.3 MgSO₄, 1.0 NaH₂PO₄, 26 NaHCO₃, 20 glucose, saturated with 95% O₂ and 5% CO₂ at room temperature. Slices were allowed to recover for at least 1 hr before any recording.

A slice was transferred to a submerge-type chamber where it was continuously exposed to ACSF at 30-32 °C, saturated with 95% O₂ and 5% CO₂ and flowing at rate of 1.8 ± 0.2 ml/min. The slice was viewed first with a 4x 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 then viewed under near infrared illumination with a 40x water-immersion objective (Fluor, 40X/0.80W, Nikon, Japan) and a CCD camera (CCD-300-RC, MTI, Michigan...
Layer I can be easily identified due to a very low density of cells. The border between layer 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-glucconate, 15 KCl, 0.5 EGTA, 4 ATP-Mg, 0.3 GTP-Na₂, 20 HEPES (pH 7.4 with KOH, 280-290 mOsm). Electrodes had resistances between 3 and 7 MΩ. The seal resistance was greater than 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 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, PA).

**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 (Zhang and Deschenes 1997; Reyes and Sakmann 1999). Briefly, slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PBS) for at least 12 hrs. After 2 wash in PBS, slices were treated with 0.3% H$_2$O$_2$ 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 hr. Slices were then incubated with the avidin biotin peroxidase complex (ABC Elite Standard, Vector Laboratories, Burlingame, CA) for 4 hrs at room temperatures. Slices were reacted with 3,3-diaminobenzidine (1 mg/ml) for 5 to 10 min. Slices were then mounted onto slides, dehydrated, embedded in Permount, and coverslipped. Slices were viewed with a 40x objective, and cells were reconstructed and analyzed with the Neurolucida system (MicroBrightfield, Colchester, VT). The total lengths of the apical and basal dendrites, and the major and minor diameters ($a$ & $b$) of the cell body were estimated with NeuroExplorer. The surface area (SA) of cell body was calculated as: $SA = \pi a b$. 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, Nothampton, MA). Throughout, means are given ± SEM. Means were compared by two-tailed
Students’ t Test

RESULTS

Data were collected from 294 layer 5 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. Fig. 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.

Resting membrane potential. The resting membrane potential (V_r) was measured
immediately after achieving whole-cell configuration. At P1 and P3, about 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 $V_r$ stayed 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, 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. 2A1 and 2A2. $V_r$ became more negative during the first two weeks after birth. At P3, $V_r$ was $-65.6 \pm 1.6$ mV ($n = 9$). At P16, $V_r$ was $-76.7 \pm 0.7$ mV ($n = 10$), about 11 mV more negative than at P3 ($p < 0.001$). There was little change in $V_r$ after P16; at P36, $V_r$ was $-76.5 \pm 0.9$ mV ($n = 8$), not different from that at P16 ($p > 0.8$).

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

**Membrane input resistance ($R_N$).** There was a large reduction in $R_N$ during early life (Fig. 2B1 & 2B2). Although the largest reduction occurred during the first 2 weeks after birth, $R_N$ decreased significantly during the third week. After P21, there was little change in $R_N$. $R_N$ was $1139 \pm 100$ MΩ at P3 ($n = 9$), $384 \pm 36$ MΩ at P7 ($n = 9$), $168 \pm 20$ MΩ at P13 ($n = 12$), $70 \pm 4$ MΩ at P21 ($n = 11$, $p < 0.001$ vs. P13), and $70 \pm 7$ MΩ at P30 ($n = 12$, $p > 0.8$ vs. P21). Data can be fitted to a single exponential function: $y = A1*\exp(-x/t1) + y0$, where $y0$ is the $R_N$ in adult, $A1$ the postulated $R_N$ at birth minus $y0$, and $t1$ the decay time constant. The results give $63 \pm 2$ MΩ, $1733 \pm 145$ MΩ, and $4.3 \pm 0.2$ day for $y0$, $A1$ and $t1$, respectively (Fig. 2B2).
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. 2C₁ & 2C₂). $T_m$ 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 reconstructed. Fig. 3 illustrates examples of cells at various ages. The surface area (SA) of the cell body was estimated as $SA = \pi ab$, with $a$, $b$ being the major and minor diameter of the cell body respectively. SA doubled between P3 and P21, with most change occurred before the end of the second week (Fig. 4A). The mean SA was 626 ± 30 $\mu$m$^2$ at P3 ($n = 10$), 1163 ± 60 $\mu$m$^2$ at P13 ($n = 8$; $p < 0.0001$, vs. P3), and 1248 ± 79 $\mu$m$^2$ at P21 ($n = 8$; $p > 0.3$, vs. P13). There was a rapid growth of both apical and basal dendrites during the first 3 weeks. The total length of apical dendrite increased 5 fold between P3 and P21 (Fig. 4B), with the mean values of 491 ± 76 $\mu$m at P3 ($n = 10$) and 3229 ± 437 $\mu$m at P21 ($n = 8$; $p < 0.0001$ vs. P3). The total length of basal dendrite showed an even larger increase: from 185 ± 12 $\mu$m at P3 ($n = 10$) to 2697 ± 241 $\mu$m at P21 ($n = 8$; $p < 0.0001$ vs. P3), a 13-fold increase (Fig. 4C).

Postnatal 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. Fig. 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** ($V_{thres}$) of action potential. There was a substantial negative shift in $V_{thres}$ during the first 3 weeks after birth (Fig. 5B1 & 5B2). $V_{thres}$ was $-42.1 \pm 0.5$ mV at P3 ($n = 9$), $-47.4 \pm 0.8$ mV at P7 ($n = 8$), and $-54.8 \pm 0.5$ mV at P21 ($n = 11$). Data can be fitted to an exponential function: $y = A1*\exp(-x/t1) + y0$, where $y0$ is the $V_{thres}$ in adult, $A1$ the postulated $V_{thres}$ at birth minus $y0$, and $t1$ the decay time constant. The results give $-56.1 \pm 0.3$ mV, $23.5 \pm 1.2$ mV, and $6.2 \pm 0.5$ day for $y0$, $A1$, and $t1$, respectively.

I also measured the difference between $V_{thres}$ and $V_r$ ($V_{thres} - V_r$), and the results are illustrated in Fig. 5C1 & 5C2. There was considerable variation among neurons of the same age, but little difference between the mean values estimated at various ages (P3 through P36). For example, ($V_{thres} - V_r$) was $23.1 \pm 1.8$ mV at P3 ($n = 7$); $22.0 \pm 0.6$ mV at P7 ($n = 9$); $20.0 \pm 0.7$ mV at P12 ($n = 9$); $21.9 \pm 0.5$ mV at P21 ($n = 11$); $20.5 \pm 1.2$ mV at P36 ($n = 8$). A linear fit of the mean values gives a slope of $-0.006$ mV/day (Fig. 5C2), suggesting the difference between $V_{thres}$ and $V_r$, thus the amount of depolarization required to reach spike threshold, is stable between P3 and P36.
Peak amplitude of action potential. There was a substantial increase in the peak amplitude of action potential during the first 2 weeks after birth (Fig. 6A₁ & 6A₂). The peak amplitude was 51.7 ± 1.9 mV at P3 (n = 10), 67.2 ± 1.3 mV at P10 (n = 12), and 77.8 ± 1.1 mV at P13 (n = 12). There was little change after P13: the peak amplitude was 79.5 ± 2.2 mV at P21 (n = 11), and 80.9 ± 1.0 mV at P36 (n = 8).

Rise time of action potential. Action potential rise time was measured as the time required from 10 to 90% of the total height. The rise time reduced by about 40% between P3 (0.73 ± 0.04 ms, n = 11) and P13 (0.42 ± 0.03 ms, n = 12, p < 0.005 vs. P3), but changed little after P13 (Fig. 6B₁ & 6B₂).

Duration of action potential. The duration of action potential was estimated as the width at half maximum height of an action potential (t₁/₂). There was a 75% reduction in t₁/₂ during the first 3 weeks after birth (Fig. 6C₁ & 6C₂). T₁/₂ 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 > 0.5 vs. P21). Data can be fitted to a single exponential function: y = A₁*exp(-x/t₁) + y₀, where y₀ is the estimated t₁/₂ in adult, A₁ the estimated t₁/₂ at birth minus y₀, and t₁ the decay time constant. The results give 0.86 ± 0.01 ms, 5.83 ± 0.42 ms, and 5.1 ± 0.2 day for y₀, A₁, and t₁, 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. 7A through 7C). 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 $580 \pm 48$ Hz/nA (n = 5). The slope flattened progressive over the next 10 days (Fig. 7E), with means of $164 \pm 16$ Hz/nA at P7 (n = 9, p < 0.0001 vs. P3) and $88 \pm 9$ 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 $70 \pm 3$ Hz/nA at P16 (n = 6, p > 0.09 vs. P13); $76 \pm 4$ Hz/nA at P21 (n = 9, p > 0.2 vs. P13); $84 \pm 6$ Hz/nA at P28 (n = 6, p > 0.5 vs. P13). Data can be fitted to an exponential function with a decay constant of $2.3 \pm 0.2$ day (Fig. 7E).

Besides the changes of the input-output curve, there was a 100% increase in the maximum frequency during the first 3 weeks after birth (Fig. 7F). The maximum frequency was at $30 \pm 4$ Hz at P3 (n = 6), $45 \pm 4$ Hz at P13 (n = 5, p < 0.02 vs. P3), and $57 \pm 2$ Hz at P21 (n = 10, p < 0.02 vs. P13). There was no change in the maximum frequency between P21 and P28 ($59 \pm 3$ 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. Fig. 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 2/3 pyramidal neurons provide a major source of excitatory inputs to layer 5 pyramidal 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 2/3 to layer 5 connections. We examined synaptic responses in neurons voltage-clamp at -72 mV, near the resting membrane potential (Fig. 9A). At P3, stimulation of layer 2/3 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 2/3 was stimulated (n = 13). In 5 cells, bath application of 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 µM) and DL-2-amino-5-phosphonopentanoic acid (DL-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 two weeks, 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; Reyes and Sakmann 1999; Kumar and Huguenard 2001). We examined paired-pulse responses evoked at 50 ms interval in neurons voltage-clamp at -72 mV (Fig. 10A). The stimulation intensity was set to produce less than 1/3 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 weeks (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). At P30, the paired-pulse ratio was 1.51 ± 0.07 (n = 10), similar to that of P21 (p > 0.5, P21 vs. P30).

DISCUSSION

In this study, I showed that layer 5 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 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 5 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 weeks after birth. This is comparable with the results obtained from hippocampal pyramidal neurons (Spigelman et al. 1992), from layer 2/3 pyramidal neurons and layer 1 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, 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 ($R_N$) decreased by about 15 fold during the first 3 weeks, with the largest reduction happened during the first 10 days. This is comparable with those observed in layer 1 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 $R_N$ 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 ($t_m$) followed the same time course as $R_N$, 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 dendrite increased by 5 fold. These findings are in line with the results obtained by Zhu (2000) in layer 5 pyramidal neurons in the rat somatosensory cortex. In addition, I showed that the length of basal dendrite increased by 13 fold during the same period. Taking into account the decrease in the specific membrane resistance during development (see discussion 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 (Ramoa and McCormick 1994a; Zhou and Hablitz 1996; Belleau and Warren 2000), our results showed that the amplitude of AP increased by 30 mV during the first 3 weeks, 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 APs, 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 (see discussion below).

An interesting finding of this study is that although the spike threshold \( V_{\text{thres}} \) shifted -14 mV during the first 3 weeks, 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, 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 \( \text{Na}^+ \) conductance, and the latter largely by \( \text{K}^+ \) conductance), cellular mechanisms are required to coordinate the development of various membrane conductance (Spitzer 1991; Moody 1998; 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 to 50% of pyramidal neurons were found to be IB type (Chagnac-Amitai et al. 1990; Agmon and Connors 1992; Franceschetti et al. 1998; Larkum and Zhu 2002). This discrepancy is probably not due to the differences in methods, because under similar conditions, Larkum et al. (2002) found that 50% of layer 5 pyramidal neurons in the rat somatosensory 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 P18-36, 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 5 pyramidal neurons in rats

The developmental change in $V_r$ reported here is similar to those observed in the visual and sensorimotor cortices (Kasper et al. 1994; Franceschetti et al. 1998; Zhu 2000). On the other hand, $R_N$ and $t_m$ of immature neurons reported here were much higher than those obtained with sharp electrode intracellular recordings in the sensorimotor cortex (McCormick and Prince 1987; Franceschetti et al. 1998) 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 AP properties of layer 5 pyramidal neurons have been examined in detail with sharp electrodes in the rat visual cortex (Kasper et al. 1994). The changes in AP amplitude, duration, rise time were comparable to those reported here, suggesting a similar time course of maturation for these two areas. However, unlike
Development of synaptic function

Our results showed that the layer 2/3 to layer 5 projection became functional around P6. This is at least 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 2/3 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 2/3 to layer 5 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 9-fold 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 2/3 to layer 5 synapses, the change in the short-term plasticity is complete by P21.

*Developmental regulation of neuronal activity*

The developmental changes in $R_N$ and $t_m$ 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; Ramoa and McCormick 1994b; Crair and Malenka 1995; Kim et al. 1995). 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 $t_m$, 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 $V_r$ and $R_N$ by over-expressing an inward rectifier $K^+$ channel lead 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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REFERENCES


FIGURE LEGENDS

Figure 1. Changes in membrane potential in response to current injections in neurons at P3, P12, and P30. The amplitudes of current steps (lower traces) were -20, -10, and +20 pA at P3; -100, -60, -20, and +140 pA at P12; -300, -100, +100, and +500 pA at P30. There is a progressive decrease in cell input resistance and in membrane time constant. Data were filtered at 1 kHz, and digitized at 4 kHz.

Figure 2. Developmental changes in resting membrane potential (A₁ & A₂), cell input resistance (B₁ & B₂), and membrane time constant (C₁ & C₂). Each point in A₁, B₁, and C₁ represents the result from a single neuron, whereas A₂, B₂, C₂ illustrate the mean ± sem from cells recorded at a given age. Neurons become progressively hyperpolarized during the first 2 weeks, and during the same period, there is a substantial reduction in cell input resistance and time constant. The decay of input resistance can be fit to a single exponential function with a time constant of 4.3 day (B₂, R² = 0.95).

Figure 3. Morphology of layer 5 pyramidal neurons in the PFC at various ages. Neurons were labeled with Neurobiotin, and reconstructed using the Neurolucida system. All neurons shown here were regular spiking cells.

Figure 4. Developmental changes in the morphology of layer 5 pyramidal neurons. A through C illustrate the developmental changes in the surface area of cell body, and the
total length of apical and basal dendrites, respectively. Each data point is the average of the results from 7 to 10 cells. 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.

Figure 5. Developmental changes in action potential. A: Examples of action potential recorded at P3, P9, P13, and P21. The thin line indicates 0 mV level, and the spike threshold is shown by the dashed lines. Filtered at 2 kHz; digitized at 8 kHz. Please note the increase in spike amplitude and the reduction in spike duration during development. B1: scatter plot of spike threshold at various ages. B2: the mean ± sem from cells recorded at a given age. Data can be fit to a single exponential function with a time constant of 6.2 day (R² = 0.98). C1: the difference between spike threshold (V_thres) and resting potential (V_r) measured at various ages. C2 illustrates the mean ± sem. A linear fit gives a slope of -0.006 mV/day, suggesting that the difference between V_thres and V_r remains stable during the first 5 weeks after birth.

Figure 6. Developmental changes in spike amplitude, rise time, and duration at half-maximum height. A1, B1, and C1 illustrate results from neurons at various ages, whereas A2, B2, C2 show the mean ± sem from neurons at a given age. Spike amplitude increased by 30 mV during the first 2 weeks, whereas the rise time and duration at half-maximum height decreased substantially during the same period. The decay of ½ height duration can be fitted to a single exponential function with a time constant of 5.1 day (C2, R² = 0.97).
Figure 7. Developmental changes in spike trains. A through C: responses in membrane potential to 600 ms current steps in neurons at P3 (A), P13 (B), and P21 (C). The amplitudes of current steps are indicated at the beginning of each trace. The second trace in each panel illustrates near maximum frequency response. D: input-output curves obtained from neurons aged P3 (filled square), P7 (empty triangle), and P13 (filled circle). E: the initial slopes of the input-output curves at various ages. Data can be fitted to an exponential function with a decay time constant of 2.3 day ($R^2 = 0.95$). F: the maximum frequency of spike train at various ages. There was a 100% increase between P3 and P12. In E & F, the number of neurons tested at each age is given in round brackets.

Figure 8. Firing pattern and morphology of an intrinsic bursting neuron. A: a burst of AP 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.

Figure 9. Developmental changes in synaptic responses. A: examples of evoked synaptic currents in neurons aged P3, P5, P7, P10, and P16. Stimuli (40 µs, 4 – 15 µA) were delivered to layer 2/3. The stimulus intensity is indicated beside the stimulation artifact. No or little response was observed at P3 and P5. Holding potential at -72 mV. Each trace is the average of 5 consecutive trials. B: input-output curves of evoked monosynaptic responses obtained from 4 neurons at P5 (open square), P7 (filled square), P13 (open circle), and P21 (filled circle), respectively. Holding potential at -72 mV. $R_s$
was compensated at 80%. C: the maximum monosynaptic responses at various ages. Each data point is the average of the results from 5 to 7 cells. The response increased by 9 fold between P7 and P17. The maximum responses at P17, P21 and P30 were not significantly different (p > 0.1).

Figure 10. Developmental changes in short-term synaptic plasticity. A: paired-pulse responses recorded in neurons aged P9, P13, and P21. The inter-stimulus interval was 50 ms. Each trace is the average of 10 consecutive trials. B: The mean paired-pulse ratio at various ages. The response shifted from depression at P7 to facilitation at P21. The dashed line indicates the level where the second response equals the first one. The number of neurons tested at each age is given in round brackets.
Fig. 1, Zhang

P3

P12

P30

Currents

0.5 nA  25 mV
200 ms
Fig. 4, Zhang

A

Cell body

Surface area (μm²)

Age (days)

B

Apical dendrite

Total length (μm)

Age (days)

C

Basal dendrite

Total length (μm)

Age (days)
Fig. 5, Zhang

A

B1

B2

C1

C2
Fig. 6, Zhang

A1

AP amplitude (mV)

Age (days)

50 55 60 65 70 75 80 85

40 50 60 70 80

0 5 10 15 20 25 30 35 40

A2

AP amplitude (mV)

Age (days)

50 55 60 65 70 75 80 85

45 50 55 60

0 5 10 15 20 25 30 35 40

B1

AP rise time (ms)

Age (days)

0.2 0.4 0.6 0.8 1.0

0 5 10 15 20 25 30 35 40

B2

AP rise time (ms)

Age (days)

0.2 0.3 0.4 0.5 0.6 0.7 0.8

0 5 10 15 20 25 30 35 40

C1

AP duration (ms)

Age (days)

0 1 2 3 4 5

0 5 10 15 20 25 30 35 40

C2

AP duration (ms)

Age (days)

0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5

0 5 10 15 20 25 30 35 40
Fig. 7, Zhang

A. P3
+20 pA
+60 pA

B. P13
+30 pA
+0.99 nA

C. P21
+0.12 nA
+1.48 nA

D. AP frequency (Hz)

E. Initial slope (Hz/nA)

F. Maximum frequency (Hz)

200 ms
Fig. 8, Zhang

A

-76 mV

200 ms

20 mV

B

100 µm

I

II/III

V
Fig. 9, Zhang

A

P3 15 μA
P5 10 μA
P7 8 μA
P10 6 μA
P16 4 μA

40 pA
20 ms

B

EPSCs (nA)

Stimulus intensity (μA)
P21
P13
P7
P5

C

Maximum EPSCs (nA)

Age (days)
4 8 12 16 20 24 28 32
Fig. 10, Zhang

A

P9

P13

P21

10 pA

30 pA

50 pA

30 ms

B

Paired-pulse ratio

Age (days)

8 12 16 20 24 28 32

(11) (10)

(8)

(9) (7)