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The postnatal development of intrinsic properties and spike encoding at cortical GABAergic neurons

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ABSTRACT

GABAergic neurons play a critical role in maintaining the homeostasis of brain functions for well-organized behaviors. It is not known about the dynamical change in signal encoding at these neurons during postnatal development. We investigated this issue at GFP-labeled GABAergic neurons by whole-cell recording in cortical slices of mice. Our results show that the ability of spike encoding at GABAergic neurons is improved during postnatal development. This change is associated with the reduction of refractory periods and threshold potentials of sequential spikes, as well as the improvement of linear correlations between intrinsic properties and spike capacity. Therefore, the postnatal maturation of the spike encoding capacity at GABAergic neurons will stabilize the excitatory state of cerebral cortex.

Sequential action potentials at the neurons are one type of essential neural codes, in which different spike patterns, likely on–off patterns in the silicon-based switch of the computers [1–4,8], encode the messages to control well-organized behaviors and cognition, such as learning and memory, consciousness and thinking. Cortical neurons are generated from progenitor cells in the ventricular zone [5], and then migrate to the different layers of cerebral cortex along with a dendrite of radial glia cells [6]. Less is known about the developmental profile in the functions of these neurons after their differentiation.

GABAergic inhibitory neurons, in spite of a small population in the brain, play an important role in maintaining a functional homeostasis of the brain through encoding spike patterns. It has been proposed that their spike encoding is influenced by synapse dynamics [7–9], development [10] and membrane intrinsic property [11–15]. However, it is not known about quantitative correlations among the spike encoding, membrane intrinsic properties and development. We investigated dynamical changes in the spike encoding and intrinsic property of cortical GABAergic neurons, which are genetically labeled with GFP, by whole-cell patch recording in cortical slices during postnatal development.

Methods and materials

Brain slices and neurons. Cortical slices (400 μm) were made from FVB-Tg(Gad GFP)4570Swn/J mice (Jackson Lab, Bar Harbor, ME 04609, USA) of eyes-opening (postnatal day, PND 15–22) or eyes-unopening (PND 6–12). Mice were anesthetized by inhaling isoflurane and decapitated with a guillotine. Cortical slices were cut with a Vibratome in oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) in the concentrations (mM) of 124 NaCl, 3 KCl, 1.2 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 4 $MgSO_4$, 10 dextrose, and 5 HEPES, pH 7.35 at 4 °C. The slices were held in (95% O_2 and 5% CO_2) ACSF (124 NaCl, 3 KCl, 1.2 NaH_2PO_4, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 10 dextrose, and 5 HEPES, pH 7.35) at 25 °C for 2 h. A slice was then transferred to a submersion chamber (Warner RC-26G) that was perfused with ACSF oxygenated at 31 °C for whole-cell recording [8,10,16–17]. Chemical reagents were from Sigma. The entire procedures were approved by IACUC in Anhui, China.

The neurons for whole-cell recording in layer II–III of sensory cortex were selected based on GFP-labeled neurons under fluorescent microscope (Nikon, FN-E600), in which excitation wavelength was 488 nm. These neurons demonstrated the typical properties of interneurons, such as fast-spiking and less adaptation in spike amplitude and frequency [10,18–19].

Whole-cell recording. Electrical signals were recorded by using an Axoclamp-2B amplifier under current-clamp, and were inputted into pClamp 9 (Axon Instrument Inc., Foster CA, USA) for data acquisition and analysis. Output bandwidth in the amplifier was 3 kHz. The spike patterns were evoked by depolarization current

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pulses, in which the amplitude and duration were based on the aim of experiments. Pipettes for whole-cell recordings were filled with the standard solution that contained (mM) 150 K-gluconate, 5 NaCl, 5 HEPES, 0.4 EGTA, 4 Mg-ATP, 0.5 Tris–GTP, and 5 phosphocreatine (pH 7.35 adjusted by 2 M KOH). Fresh pipette solution was filtered with centrifuge filters (0.1 μm) before the use, and osmolarity was 295–305 mOsmol. Pipette resistance was 5–6 MΩ.

The intrinsic properties of cortical GABAergic neurons in our investigation include the threshold potentials (Vts) of firing spikes and absolute refractory periods (ARP) following each spike. Vts are a start point of the rising phase of spikes [15,20]. The ARP of sequential spikes are measured by injecting multiple depolarization current pulses (3 ms) into GABAergic neurons following each of spikes (please see Fig. 2). By changing inter-pulse intervals, we define ARP as the duration from a complete spike to a subsequent spike at 50% probability [15]. Spike programming (capacity and timing precision) is represented as inter-spike interval (ISI) and the standard deviation of spike timing (SDST), respectively.

Data were analyzed if the recorded neurons had the resting membrane potentials negatively more than −60 mV. The criteria for the acceptation of each experiment also included less than 5% changes in resting membrane potential, spike magnitude, and input resistance throughout each of experiments. Input resistance was monitored by measuring cell responses to the hyperpolarization pulses at the same values as the depolarization that evoked spikes. Vts, ARP, ISI, and SDST are presented as mean ± SE. The comparisons before and after eyes-opening are done by *t*-test.

Results

Developmental change in spike capacity and timing precision at GABAergic cells

Whole-cell recordings were conducted at GABAergic neurons of cortical slices from mice in the two ages, postnatal day (PND) 6–12 and 15–22. Inter-spike intervals (ISI, an index of spike capacity) and the standard deviation of spike timing (SDST, an index of spike timing precision) were measured by evoking sequential spikes with depolarization currents [15,18–19].

Fig. 1A–B demonstrates sequential spikes at GABAergic neurons from the mice of PND 18 (A) and 8 (B), in which the matured neurons appear to fire more spikes in a given time. The ISI values of sequential spikes are 11.7 ± 0.39, 12.9±0.46, 14.4 ± 0.53, and 15.7 ± 0.56 ms in PND 6–12 (filled symbols in Fig. 1C, $n = 29$); and ISI values are 9.5 ± 0.25, 10.1 ± 0.31, 11.6 ± 0.37, and 13.1 ± 0.43 ms in PND 15–22 mice (open symbols, $n = 24$). ISI values for corresponding spikes in these two ages are statistically different ($p < 0.01$). Therefore, the spike capacity of cortical GABAergic neurons is improved during postnatal development.

SDST values of sequential spikes at cortical GABAergic neurons are 0.33 ± 0.03, 0.43 ± 0.04, 0.56 ± 0.06, 0.74 ± 0.09 and 0.91 ± 0.1 ms in PND 6–12 mice (filled symbols in Fig. 1D, $n = 29$); and SDST values are 0.21 ± 0.01, 0.32 ± 0.017, 0.44 ± 0.02, 0.61 ± 0.03, and 0.81 ± 0.04 ms in PND 15–22 (open symbols in Fig. 1D, $n = 24$). SDST values for corresponding spikes in such two ages are not statistically different ($p > 0.176$).

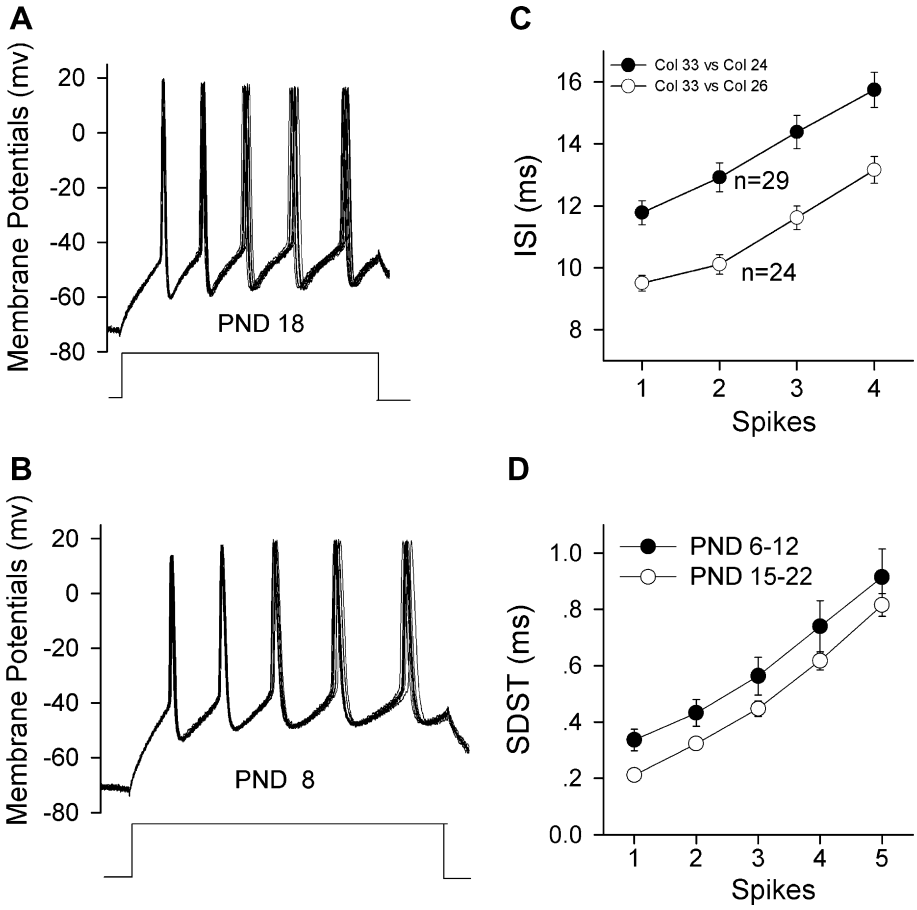

Fig. 1. The postnatal maturation of spike capacity and timing precision at cortical GABAergic neurons of brain slices in FVB-Tg(Gad GFP)4570Swn/J mice. (A) The superimposed waveforms of sequential spikes are evoked by depolarization current pulses (100 ms) at PND 18. (B) The superimposed waveforms of sequential spikes are evoked by depolarization currents (140 ms) at PND 8. (C) The inter-spike intervals (ISI) of sequential spikes during PND 15–22 (open symbols, $n = 24$) and PND 6–12 (filled symbols, $n = 29$). (D) The standard deviation of spike timing (SDST) for spikes one to five during PND 15–22 (open symbols) and PND 6–12 (filled symbols).

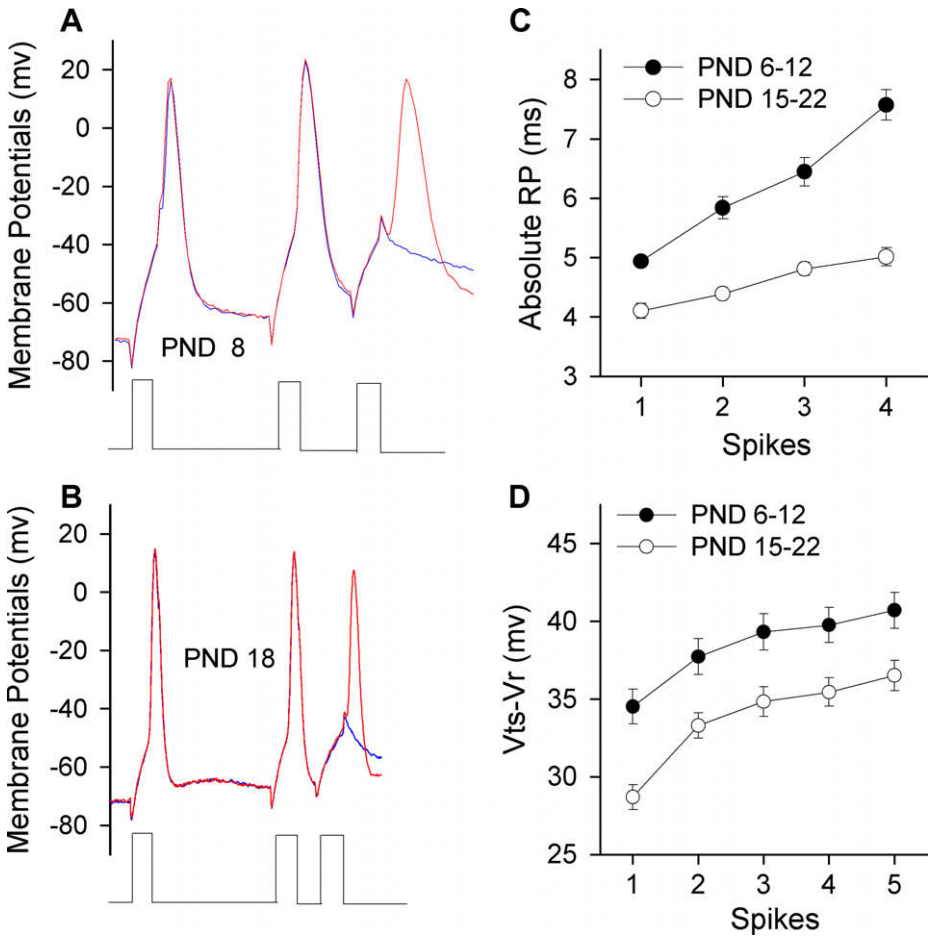

Fig. 2. Postnatal changes in the absolute refractory periods and threshold potentials of sequential spikes at cortical GABAergic neurons of brain slices in FVB-Tg(Gad GFP)4570Swn/J mice. (A) The superimposed waveforms show ARP measurement by changing inter-pulse interval of depolarization currents (3 ms) at PND 8. (B) The superimposed waveforms show ARP measurement by changing inter-pulse interval of depolarization currents (3 ms) at PND 18. (C) The comparisons of ARP of sequential spikes during PND 15–22 (open symbols, $n = 30$) versus PND 6–12 (filled symbols, $n = 36$). (D) The comparisons of threshold potentials (Vts–Vr) of sequential spikes during PND 15–22 (open symbols, $n = 24$) versus PND 6–12 (filled symbols, $n = 29$).

The results above indicate that excitatory inputs on cortical GABAergic neurons are able to drive them to fire more action potentials after mice open their eyes (usually PND 13–14). That is, mouse eyes-opening during postnatal development is critical for cortical GABAergic neuron to be matured for firing sequential spikes.

Developmental changes in refractory periods and threshold potentials at cortical GABAergic neurons

As sequential spikes are navigated by refractory periods and threshold potentials [15,18–19], we hypothesize that the postnatal improvement of GABAergic neuron spike capacity is due to the changes in ARP and Vts. Postnatal changes in ARP were measured by injecting depolarization pulses (3 ms) into cortical GABAergic neurons following each of spikes. The ARPs of sequential spikes appear longer in PND 15–22 mice (Fig. 2A) than PND 6–12 mice (Fig. 2B). ARP values of spikes one to four are 4.98 ± 0.07, 5.78 ± 0.07, 6.46 ± 0.06, and 7.63 ± 0.06 ms in PND 6–12 (filled symbols in Fig. 2C, $n = 36$); and the values are 4.12 ± 0.12, 4.37 ± 0.07, 4.8 ± 0.05, and 4.99 ± 0.05 ms in PND 15–22 (open symbols in Fig. 2C, $n = 30$). ARP values for corresponding spikes at GABAergic neurons in these two ages of mice are statistically different ($p < 0.01$). Spikes' refractory periods become short during postnatal development.

We also examined whether plastic change in threshold potentials occurred during postnatal development. Threshold potentials are presented as the gap between resting membrane potential (Vr) and threshold potential (Vts) [15,18–19]. The values of Vts–Vr for spikes one to five are 34.5 ± 1.11, 37.72 ± 1.16, 39.31 ± 1.17, 39.75 ± 1.13, and 40.71 ± 1.15 mV in PND 6–12 mice (filled symbols in Fig. 2D, $n = 29$); and the values are 28.7 ± 0.8, 33.3 ± 0.82, 34.8 ± 0.95, 35.44 ± 0.91, and 36.51 ± 0.98 mV in PND 15–22 (open symbols in Fig. 2D, $n = 24$). Vts–Vr values for corresponding spikes at cortical GABAergic neurons in these two ages are statistically different ($p < 0.05$). Threshold potentials become low during postnatal development.

Developmental change in the correlation between intrinsic properties and spike encoding

To clarify the effects of threshold potentials and refractory periods on sequential spike encoding during postnatal development, we analyzed the relationships between membrane intrinsic properties (ARP and Vts–Vr) and spike encoding indices (ISI and SDST) at the cortical GABAergic neurons of PND 6–12 and 15–22. The correlations between ARP and SDST are linear in PND 6–12 ($r^2 = 0.96$, slope = 0.18, filled symbols in Fig. 3A) and PND 15–22 ($r^2 = 0.98$, slope = 0.51, open symbols, $p < 0.05$). The linear correlations are present between ARP and ISI in PND 6–12 ($r^2 = 0.95$, slope = 1.55,

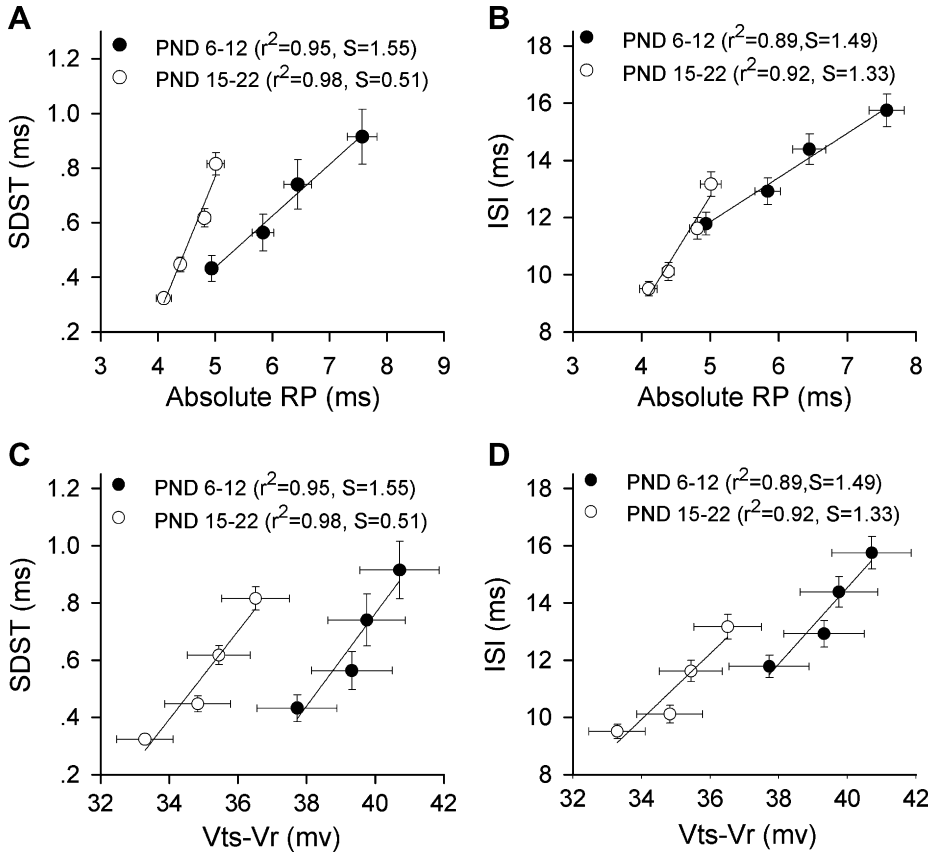

Fig. 3. Dynamic changes in the relationship between intrinsic properties (ARP and Vts–Vr) and spike parameters (ISI and SDST) at cortical GABAergic neurons of brain slices in FVB-Tg(Gad GFP)4570Swn/J mice. (A)The correlations between ARP and SDST are linear during PND 6–12 (r^2 = 0.96, slope = 0.18, filled circles) and PND 15–22 (r^2 = 0.98, slope = 0.51, open circles, $p < 0.05$). The linear slope of ARPs versus SDST changes to bigger. (B) The relationship between ARP and ISI are linear during PND 6–12 (r^2 = 0.95, slope = 1.55, filled circles) and PND 15–22 (r^2 = 0.96, slope = 3.89, open circles, $p < 0.05$). The linear slope of ARPs versus ISI changes to bigger. (C) The linear correlation between Vts–Vr and SDST during PND 6–12 (r^2 = 0.94, slope = 0.15, filled circles) and PND 15–22 (r^2 = 0.91, slope = 0.16, open circles). (D) Linear correlations between Vts–Vr and ISI during PND 6–12 (r^2 = 0.89, slope = 1.49, filled circles) and PND 15–22 (r^2 = 0.92, slope = 1.33, open circles, $p < 0.05$).

filled symbols in Fig. 3B) and PND 15–22 (r^2 = 0.96, slope = 3.89, open circles, $p < 0.05$). The correlations between Vts–Vr and SDST are linear in PND 6–12 (r^2 = 0.94, slope = 0.15, filled symbols in Fig. 3C) and PND 15–22 (r^2 = 0.91, slope = 0.16, open symbols). The correlations between Vts–Vr and ISI are linear in PND 6–12 (r^2 = 0.89, slope = 1.49, filled circles in Fig. 3D) and PND 15–22 (r^2 = 0.92, slope = 1.33, open symbols).

The results above support a notion that refractory periods and threshold potentials control spike timing precision and capacity [15]. In addition, as the slopes of linear correlations between ARP and spiking encoding increase postnatally (Fig. 3A–B), the influences of ARPs on the spike capacity and timing precision at cortical GABAergic neurons become more efficient during postnatal maturation.

Discussion

With measuring threshold potentials and absolute refractory periods of sequential spikes at cortical GABAergic neurons during postnatal maturation, we found that their threshold potentials to initiate spikes become lower (Fig. 2D) and refractory periods to evoke subsequent spikes are shorter (Fig. 2C). Lower threshold potentials in these neurons of eyes-opening mice allow them being more sensitive to excitatory synaptic inputs and firing spikes in high capacity. Shorter refractory periods make subsequent spikes shift toward initial ones, increasing the number of spikes in a given duration. The increase of spike capacity during the postnatal development (Fig. 1) grants these predictions. Thus, postnatal eyes-opening in mice may be a critical period for cortical GABAergic neurons to be matured in firing sequential spikes. It is noteworthy that the standard deviation of spike timing does not change postnatally, indicating that spike timing precision at GABAergic neurons has been matured before eyes-opening.

The capacity and timing precision of sequential spikes is linearly correlated with threshold potentials and refractory periods. This datum grants an indication that spike capacity and timing precision at cortical GABAergic neurons are under the control of membrane intrinsic properties [15]. Although the linear correlation between intrinsic properties and spike encoding is not improved dramatically during postnatal period, the slope of linear correlations between ARP and spike encoding (capacity and timing precision) become bigger (Fig. 3A–B), indicating that ARP is a major factor to control spike capacity and timing precision during postnatal maturation. On the other hand, threshold potentials seem to be more critical to navigate spike encoding during the early development of cortical GABAergic neurons.

The activities of potassium channels are thought to influence neuronal excitability and spike timing [11–12]. The dynamics of voltage-gated sodium channels underlies threshold potentials and refractory periods [18–19,21–22], which navigate spike encoding [15]. With such postnatal changes in spike firing and intrinsic property, we propose that the kinetics of voltage-gated ion channels undergoes plastic change during postnatal maturation, which is under study.

Our studies provide the developmental profiles of intrinsic properties at cortical GABAergic neurons for understanding the maturation of the cerebrum in encoding sequential spikes. With the postnatal development, GABAergic inhibitory neurons are more sensitive to the driving force from excitatory inputs to fire sequential spikes, i.e., their programming of sequential spikes is improved. This improvement of writing digital signals at GABAergic neurons of neural network strengthens an establishment of homeostasis in the central nervous system [23] to guide well-organized behaviors.

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