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Neonatal fluoxetine exposure affects the action potential properties and dendritic development in cortical subplate neurons of rats☆

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ABSTRACT

Selective serotonin reuptake inhibitor (SSRI)-type antidepressants might be given to depressive pregnant women and the developing fetuses are thus exposed to these drugs. Since serotonin plays important roles in the maturation of the nervous system, early SSRI exposure might influence the fetal brain development. To test this hypothesis, we treated the neonatal rat pups with fluoxetine (Flx) from the day of birth to postnatal day (P) 4, comparable to the third trimester of human gestation, and observed the physiological and morphological features of subplate neurons (SPns), a group of cells important for early cortical development and vulnerable to neonatal neural insults. Using whole-cell patch-clamp recording technique, we examined the passive membrane properties and characteristics of action potential (AP). In SPns of Flx-treated rats, the rheobase for generating an AP was increased and the width of APs was reduced, especially in the falling phase. In the morphological aspect, the dendritic remodeling of SPns including dendritic branching, elongation and pruning were affected by early Flx treatment. Together, our results demonstrate that the teratogenic effect of early SSRI exposure on the structure and function of developing SPns and these changes may lead to undesired brain activity and distorted behaviors later in life.

1. Introduction

Selective serotonin reuptake inhibitor (SSRI)-type antidepressants are often prescribed to depressive pregnant women because of their relative mild adverse side effects (Kallen, 2004; Ververs et al., 2006). Since serotonin plays important roles during brain development (Daubert and Condron, 2010; Gaspar et al., 2003; Vitalis and Parnavelas, 2003), the influence of SSRIs on the developing nervous system is obvious (see Gentile and Galbally, 2011; Oberlander et al., 2009 for recent reviews). For examples, altered pain response and subtle delays in motor development in SSRI-exposed infants have been noted (Casper et al., 2003; Oberlander et al., 2005; Pedersen et al., 2010). In rodent studies, neonatal SSRI treatments could lead to impaired sensory (Lee, 2009), motor (Lee and Lee, 2011), emotional (Ansorge et al., 2004, 2008) as well as sexual behaviores (Maciag et al., 2006) later in life. However, the underlying mechanisms are still poorly understood.

In a previous study, we have demonstrated defects in the function and structure of the somatosensory system in adolescent rats that have neonatally exposed to fluoxetine (Flx), a commonly used SSRI-type antidepressant (Lee, 2009). In the functional aspect, Flx-treated rats exhibit blunted thermal and tactile perceptions, whereas in the structural aspect, the thalamocortical afferents (TCAs) to the somatosensory cortex have altered branches in layer IV and layer IV cortical neurons display impaired dendritic arbors. Due to the structural deformations, sensory information processing in Flx-exposed rats may thus be disturbed. In mature sensory systems, TCAs preferentially make synapses with layer IV cortical neurons. However, during earlier stage, the first cortical target for developing TCAs is the subplate (McConnell et al., 1989). In rodents, TCAs are guided to the subplate by molecule cues (Osheroff and Hatten, 2009) by embryonic day (E) 16 (Erzurumlu and Jhaveri, 1992). A dense meshwork of TCAs is present in the subplate area by postnatal day (P) 2 (Agmon et al., 1993). The growing TCAs make synapses with subplate neurons (SPns) and SPns send axons

Abbreviations: ABC, avidin–biotin complex; ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; AP, action potential; E, embryonic; Flx, fluoxetine; P, postnatal day; PB, phosphate buffer; Rheo, rheobase; R_{in}, input resistance; SPn, subplate neuron; SSRI, selective serotonin reuptake inhibitor; Tau, time constant; TCA, thalamocortical afferents; V_m, resting membrane potential.

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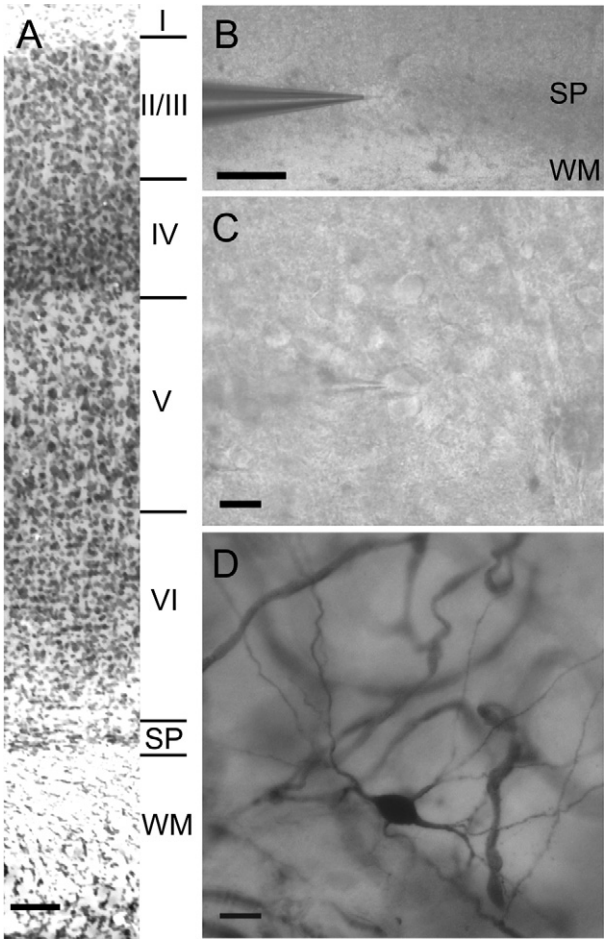

Fig. 1. Identification and labeling of subplate neurons. (A) Nissl staining of somatosensory cortex from a P7 rat. Subplate (SP) is between the cell-sparse region of layer VI and white matter (WM). (B) The subplate in the brain slice can be indentified and approached under a DIC microscope. Note the cell-sparse region of layer VI just above the subplate. (C) A subplate neuron is attached by a glass patch electrode. (D) Neurobiotin is diffused into the patched neuron during recording and revealed by ABC-DAB method. Scale bar = 100 μm in (A); 200 μm in B; 20 μm in (C) and (D).

to the cortical plate, which forms the future cortical layers II–VI (Friauf et al., 1990; McConnell et al., 1989). SP thus serves as a relay station between thalamic and cortical neurons (Allendoerfer and Shatz, 1994; Kanold, 2004; Kanold and Luhmann, 2010). In the visual cortex of cat, for example, disruption of the SP at early stages causes defects in thalamocortical projection (Ghosh et al., 1990), demonstrating the critical role of SPn in establishing thalamocortical connections. In our previous study, Flx was given from the day of birth (P0), when the developing TCAs are in the subplate layer, the venerability of SPns under neonatal Flx treatment is thus concerned. The disrupted TCA pattern in neonatal Flx-exposed rat pups might reflect the defects of SPns following Flx treatment.

In this study, we tested the hypothesis if neonatal Flx treatment alters the development of SPns. We analyzed the physiological properties and morphological features of the developing SPns in the somatosensory cortex of rats. The quantitative data revealed that the AP and dendritic remodeling of SPns are altered by early exposure of Flx. Since the structure and function of SPns is affected by early Flx exposure, the use of SSRIs in pregnant women should be more concerned.

2. Materials and methods

2.1. Animals

All animal handling was in accordance with a protocol approved by Institutional Animal Care and Use Committee of College of Medicine and College of Public Health, National Taiwan University. Newborn (postnatal day 0, P0) Wistar rats of both sexes were used in this study. All rats housed in the Laboratory Animal Center of National Taiwan University College of Medicine under 12-h light/dark cycle with free access to food and water.

2.2. Drug treatment

Rat pups received physiological saline (Con, $n = 22$) or fluoxetine hydrochloride (Flx, $n = 25$, 20 mg/(kg day) in physiological saline) subcutaneously from P0 to P4. The dosage was comparable to the previous neonatal SSRI studies (Ansorge et al., 2004, 2008; Lee, 2009; Maciag et al., 2006; Xu et al., 2004). This period also corresponds to the third trimester in human pregnancy (Clancy et al., 2007). All chemicals were purchased from Sigma (St. Louis, MO, USA), unless specifically mentioned.

2.3. Brain slice preparation

Rat pups were killed by decapitation and the brains were immediately taken, immersed into oxygenized ice-cooled artificial cerebrospinal fluid (ACSF) (in mM: 1.8 $MgSO_4$; 124 NaCl; 3 KCl; 1.3 NaH_2PO_4; $2H_2O$; 1.6 $CaCl_2$; 26 $NaHCO_3$; 20 glucose) and cut into 400 μm slices with a microslicer (DTK-1000, D.S.K., Kyoto, Japan). Slices were then transferred and kept in oxygenized warm (34 °C) ACSF for recovery for at least 1 h before recording.

2.4. Electrophysiological recording

After recovery, brain slices were transferred into the recording chamber under an upright microscope equipped with water-immersion objectives and IR-DIC system (BX51W1, Olympus, Tokyo, Japan). Whole-cell patch micropipettes were pulled from filamented borosilicate glass (Harvard apparatus, Holliston, MA, USA) by a P-87 puller (Sutter instruments, Novato, CA, USA). The patch electrodes (4–6 MΩ) were filled with potassium-based internal solution containing (in mM): 117 K-gluconate, 13 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, 10 HEPES, 2 Na_2-ATP and 0.5 Na-GTP with 0.5% (v/v) neurobiotin (Sigma–Aldrich, CA, USA), pH 7.4, osmolarity 306 mOsm. Experiments were conducted at room temperature (26–27 °C) and the perfusion rate of ACSF was 3 ml/min. Whole-cell recording was performed to record the activity of SPns using Multiclamp 700B amplifier and Digidata 1440 with pClamp 10 software (Axon instruments, Union City, CA, USA). Data was collected with 25 kHz sampling rate with 4 kHz filter and stored in a PC. Input and series resistances were monitored continuously to evaluate the health of the neurons. Clampfit 10.0 software (Axon instruments) was used for off-line data analysis.

2.5. Characterization of intrinsic membrane properties

When the whole-cell patch recording was achieved, the resting membrane potential (V_m), input resistance (R_{in}), and series resistance of SPns were checked. The membrane time constant (tau, τ) was determined by fitting the change of membrane potential in respond to a small hyperpolarizing current to a single exponential function. R_{in} was calculated according to Ohm's law from the voltage change following application of a small hyperpolarizing current pulse to avoid rectification.

For analysis of the action potential (AP) and AP train properties, neurons were given a series of current injection steps (−220 to +250 pA for 2000 ms) and the first AP was used for single AP analyses. Single AP properties, including threshold, amplitude, durations, and the amplitude of afterhyperpolarization (AHP) were measured. The AP threshold was determined as the absolute membrane potential at the onset of an AP. The AP amplitude was measured from the threshold to the peak of the spike. The amplitude of AHP was measured from the baseline to the maximum AHP. The half AP (W_h) duration was measured as the AP width at 50% AP height relative to threshold. The durations of the rising phase (W_1) and falling phase (W_2) of AP were determined from the time at AP threshold to the peak of AP and from the peak of AP to the level of AP threshold, respectively.

2.6. Reconstruction of SPns and morphometric analyses

During recording, neurobiotin was passively diffused into the patched neuron. After recording, the patch electrode was slowly elevated and withdrew from the recorded cell, allowing the cell membrane to reseal. The slice was kept in the same recording chamber for another 20 min for dye diffusion and then fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB) overnight. After fixation, the slices were incubated in PB containing 0.5% H_2O_2 and 0.1% Triton for 10 min and then reacted with avidin–biotin complex (ABC Elite Kit, Vector Labs., Burlingame, CA, USA) for 2 h. Finally, biocytin labeling was visualized by peroxidase-elicited DAB reaction. Only neurons without obvious truncation in their dendritic profile were selected for qualitative and quantitative analyses. Stacks of serial images were taken by a color CCD camera (CX-9000, MicroBrightField, Williston, VT, USA) with the aid