

Layer II/III of the Prefrontal Cortex: Inhibition by the Serotonin 5-HT_{1A} Receptor in Development and Stress

Nathalie M. Goodfellow,¹ Madhurima Benekareddy,³ Vidita A. Vaidya,³ and Evelyn K. Lambe^{1,2}

¹Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada, ²Department of Obstetrics and Gynaecology, University of Toronto, Toronto, Ontario M5G 1L4, Canada, and ³Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai 400 005, India

The modulation of the prefrontal cortex by the neurotransmitter serotonin (5-HT) is thought to play a key role in determining adult anxiety levels. Layer II/III of the prefrontal cortex, which mediates communication across cortical regions, displays a high level of 5-HT_{1A} receptor binding in normal individuals and a significantly lower level in patients with mood and anxiety disorders. Here, we examine how serotonin modulates pyramidal neurons in layer II/III of the rat prefrontal cortex throughout postnatal development and in adulthood. Using whole cell recordings in brain slices of the rat medial prefrontal cortex, we observed that serotonin directly inhibits layer II/III pyramidal neurons through 5-HT_{1A} receptors across postnatal development (postnatal days 6–96). In adulthood, a sex difference in these currents emerges, consistent with human imaging studies of 5-HT_{1A} receptor binding. We examined the effects of early life stress on the 5-HT_{1A} receptor currents in layer II/III. Surprisingly, animals subjected to early life stress displayed significantly larger 5-HT_{1A}-mediated outward currents throughout the third and fourth postnatal weeks after elevated 5-HT_{1A} expression during the second postnatal week. Subsequent exposure to social isolation in adulthood resulted in the almost-complete elimination of 5-HT_{1A} currents in layer II/III neurons suggesting an interaction between early life events and adult experiences. These data represent the first examination of functional 5-HT_{1A} receptors in layer II/III of the prefrontal cortex during normal development as well as after stress.

Introduction

A high level of serotonin 5-HT_{1A} receptors in the adult prefrontal cortex appears to be protective against certain psychiatric disorders. Adult human imaging studies show that the binding of prefrontal 5-HT_{1A} receptors is inversely correlated with anxiety levels in healthy individuals (Tausher et al., 2001) and significantly lowered in patients with mood and anxiety disorders (Drevets et al., 1999). In development, anxiety levels appear to become stable in early infancy (Kagan and Snidman, 1999) and exposure to stress during the perinatal period leads to a predisposition to adult psychopathology (Heim and Nemeroff, 2001; Gross and Hen, 2004). Together, these findings suggest the potential for early programming of adult anxiety levels. For the rodent brain, the early postnatal period is thought to be developmentally equivalent to the last trimester *in utero* and the perinatal period of human brain development (Romijn et al., 1991; Watson et al., 2006), allowing for the use of postnatal rodent models in the investigation of the early programming of anxiety. Paralleling the human data, studies in rodents have shown that exposure to early life stress (Anisman et al., 1998) or alterations to

the expression of the 5-HT_{1A} receptor in the forebrain (Gross et al., 2002) during the early postnatal period is sufficient to produce an anxiety-like phenotype in adulthood.

Despite the strong association between prefrontal 5-HT_{1A} receptors and adult psychopathology, it has yet to be established whether the 5-HT_{1A} receptor modulates the prefrontal cortex during the early postnatal developmental period. Data from human studies suggests that 5-HT_{1A} receptors are expressed (del Olmo et al., 1998) and can bind substrate (Bar-Peled et al., 1991) in the human fetal prefrontal cortex. Yet, a previous study in rodents indicates that the 5-HT_{1A} receptor does not regulate the activity of the prefrontal cortex until relatively late in postnatal development (Béique et al., 2004). However, this work only examined neurons in layer V, the main output layer of the prefrontal cortex. It has yet to be determined how serotonin modulates layer II/III, the main intracortical processing layer of the prefrontal cortex, during the early postnatal developmental period. Layer II/III of the prefrontal cortex is the last cortical layer to mature (Clancy et al., 2001) and, in the rodent, this layer shows substantial postnatal maturation in its connectivity within the cortex and with the amygdala (Bouwmeester et al., 2002; Zhang, 2004). This superficial layer of the prefrontal cortex also appears to be innervated early and preferentially by serotonin fibers (Lidov et al., 1980; Lidov and Molliver, 1982).

Here, we demonstrate that the 5-HT_{1A} receptor plays an early and seemingly exclusive role in the serotonergic modulation of layer II/III pyramidal neurons of the prefrontal cortex. In addition, these 5-HT_{1A} receptors are sensitive to stress early in life and social isolation in adulthood. Characterizing the properties of the 5-HT_{1A} receptor in layer II/III is critical to our understanding of

Received April 24, 2009; revised July 8, 2009; accepted July 9, 2009.

This research was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant (E.K.L.), the Canadian Foundation for Innovation (E.K.L.), and the Canada Research Chairs program (E.K.L.). N.M.G. was supported by a Margaret Santalo fellowship. V.A.V. was supported by a Wellcome Trust Senior Overseas Fellowship (0408200314133). M.B. received support from the Sarojini Damodaran Fellowship. We thank Dr. Paul Fletcher, Dr. Richard Horner, Sameera Kassam, and Dr. Craig Bailey for their valuable comments in preparation of this manuscript.

Correspondence should be addressed to Dr. Evelyn K. Lambe, Department of Physiology, University of Toronto, 1 King's College Circle, Toronto, ON M5S 1A8, Canada. E-mail: evelyn.lambe@utoronto.ca.

DOI:10.1523/JNEUROSCI.1960-09.2009

Copyright © 2009 Society for Neuroscience 0270-6474/09/2910094-10\$15.00/0

the role of this receptor in the maturation of the prefrontal cortex and its involvement in establishing adult psychiatric disorders.

Materials and Methods

Brain slice preparation

The experiments were performed at the University of Toronto and approved by the University of Toronto Animal Care and Use Committee. These protocols conformed to the international guidelines on the ethical use of animals. In brief, coronal slices (400 μ m thick) of the medial prefrontal cortex were prepared from Charles River Sprague Dawley rats ranging from postnatal day 6 (P6) to P96. At all ages, the brain was cooled as rapidly as possible with 4°C oxygenated sucrose artificial CSF (ACSF) (254 mM sucrose was substituted for NaCl). Prefrontal slices were cut from anterior to posterior using the appearance of white matter and the corpus callosum as anterior and posterior guides (4.20–2.52 mm from bregma) to target recording to the cingulate (Cgl) and prelimbic regions (PrL) (see Fig. 1A). The slices were cut on a Dosaka Linear Slicer (Sci-Media) and were transferred to 32°C oxygenated ACSF (128 mM NaCl, 10 mM D-glucose, 24 mM NaHCO₃, 2 mM CaCl₂, 2 mM MgSO₄, 3 mM KCl, 1.25 mM NaH₂PO₄; pH 7.4) in a prechamber (Automate) and allowed to recover for at least 2 h before the beginning of an experiment. For whole-cell recordings, slices were placed in a modified superfusion chamber (Warner Instruments) and mounted on the stage of an Olympus BX50WI microscope. Regular ACSF at 31–33°C was bubbled with 95% oxygen and 5% carbon dioxide and flowed over the slice at a rate of 3–4 ml/min. The temperatures used during brain removal and recordings were found to be critical for measuring serotonin currents.

Electrophysiology

Whole-cell recording electrodes (2–3 M Ω) contained 120 mM potassium gluconate, 5 mM KCl, 2 mM MgCl, 4 mM K₂-ATP, 0.4 mM Na₂-GTP, 10 mM Na₂-phosphocreatine, and 10 mM HEPES buffer (adjusted to pH 7.33 with KOH). Prefrontal cortex layer II/III pyramidal neurons in PrL and Cgl were recorded under visual control using infrared differential interference contrast microscopy. These neurons were identified based on their pyramidal shape and presence of a prominent apical dendrite. In a subset of experiments, layer V pyramidal neurons in the PrL and Cgl were also patched in the same prefrontal cortical slices to compare the serotonergic responses between pyramidal neurons in layer II/III and pyramidal neurons in layer V.

In current clamp, neurons were recorded at their resting potential. In voltage clamp, neurons were held at -75 mV, near the equilibrium potential for chloride under these conditions, and currents were recorded using continuous single electrode voltage-clamp mode with a Multi-clamp 700b (Molecular Devices), acquired and lowpass filtered at 3 kHz using pClamp10.2/Digidata1440 software (Molecular Devices).

Pharmacology

Serotonergic currents were probed by recording for a 1-min baseline period, then adding 10 μ M serotonin to the bath perfusion for a period of 30 s, followed by a 5 min washout period. Other drugs were also added to the bath in specific experiments: 2 μ M tetrodotoxin (TTX), 20 μ M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 30 nM [*O*-methyl-3*H*]-*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635), 10 μ M bicuculline (BCC), 50 μ M D-($-$)-2-amino-5-phosphonopentanoic acid (AP-5), 1 mM barium chloride (BaCl₂), and 10 μ M (*R*)-(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT). All compounds were obtained from Sigma or Tocris Bioscience and stored in stock solutions at -20° C before being diluted and applied to the slice in oxygenated ACSF.

Analysis of electrophysiological data

The peak amplitude of the serotonergic current was measured using Clampfit software (Molecular Devices) by subtracting the mean current at the peak of the serotonergic response from the mean current at baseline. Statistical comparisons of within-cell responses to different pharmacological agents were determined using Student's two-tailed paired *t* tests, at a significance level of 0.05. Statistical comparison between re-

sponses from different experimental groups (male vs female; control vs early life stress) was determined using unpaired *t* tests, at a significance level of 0.05. All developmental changes in the serotonin currents were assessed with parametric one-way or two-way ANOVA and tested *post hoc* with Newman–Keuls or Bonferroni test, at a significance level of 0.05.

Behavioral paradigm

Early life stress. Charles River Sprague Dawley dams delivered pups within the University of Toronto animal housing facility. The animals were kept under constant temperature (25 \pm 2°C) and a 12 h light/dark cycle. On P1, litters were counted and, when required, cross-fostered to equalize for the total number of pups and the number of males and females in each litter. Litters were then randomly assigned to one of the following experimental groups: early life stress or control, as previously described (Nair et al., 2007). Pups in the early life stress litters were separated from their mothers for a period of 3 h (9:30 A.M.–12:30 P.M.) each day between P2 and P14. During the separation period, dams were individually placed in a fresh cage in the home room, whereas pups were housed with littermates in a cage maintained at a temperature of 34–38°C (declining temperature with age) in a neighboring colony room. No food or water was available to the pups during the separation period. After the separation, the pups were returned to their home cage, rolled in the soiled home-cage bedding, and then reunited with their mother. Control litters were left undisturbed. Before weaning on P21, all litters were briefly handled at 4 d intervals to allow for cage cleaning. Once weaned, all pups were housed in same-sex sibling groups of 2–3 rats. For the electrophysiological recordings, we recorded from a total of 34 pups from 6 dams in the control group and 53 pups from 6 dams in the early life stress group.

Adult social isolation. In adulthood (>P55), both control and early life stress animals were randomly assigned to one of two experimental groups: adult social isolation or adult control group. There were a total of four experimental groups: Control (C) (male: 3 pups from 2 dams; female: 3 pups from 3 dams), early life stress (ES) (male: 3 pups from 3 dams; female: 3 pups from 3 dams), controls exposed to social isolation (SI) (male: 3 pups from 3 dams; female: 3 pups from 2 dams), and early life stress and social isolation (ES+SI) (male: 3 pups from 3 dams; female: 2 pups from 2 dams). Animals in the social isolation groups, SI group and ES+SI group, were singly housed for a period of 4–6 d and killed immediately after social isolation. The two remaining groups, control group and ES group, were left undisturbed in their same-sex sibling groups of 2–3 rats except to allow for regular cage changes.

Adult anxiety behavior: novel object

In adulthood (>P55), anxiety behavior was tested in all four experimental groups: C (male: 4 pups from 2 dams; female: 4 pups from 3 dams), ES (male: 4 pups from 2 dams; female: 4 pups from 2 dams), SI (male: 4 pups from 2 dams; female: 4 pups from 3 dams), and ES+SI (male: 4 pups from 2 dams; female: 4 pups from 2 dams). Animals in the social isolation groups (SI and ES+SI) were tested after a 5 d period of social isolation, whereas the two remaining groups (C and ES) remained in grouped housing until testing day. In this experiment, all animals were exposed for the first time to the experimental room (room lighting with 60 W bulbs), the apparatus (clear plastic box: length \times width \times height = 45 cm \times 38 cm \times 26 cm) and test object (brightly colored weight; 12 cm \times 5 cm). During the light cycle (3:00–5:00 P.M.), animals were placed in the open box facing away from the novel test object located in the corner of the apparatus. Each rat was allowed free exploration for 10 min. An observer recorded the number of contacts with the object and number of entries into the arena containing the novel object (defined as a 17 \times 13 cm region bounded by the corner of the box). Contact with object was defined as sniffing of the object or contact with the object with the paws, nose, or mouth.

In situ hybridization

The *in situ* experiments were performed at the Tata Institute of Fundamental Research (TIFR). All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the TIFR Institutional Animal Ethics Committee. Sprague Dawley rats were bred in the animal

facility at the Tata Institute. The animals were kept under constant temperature ($25 \pm 2^\circ\text{C}$) and a 12 h light/dark cycle. To examine the influence of early life stress on the gene expression of the 5-HT_{1A} receptor during postnatal development, adolescence (P40) and adulthood (P50), animals underwent the behavioral paradigm of maternal separation, as described above. The time points for killing of pups were P6, P9, P14, P40, and P50. In the case of the P6, P9, and P14 time points, animals were killed at the end of the period of maternal separation on the respective days. All time points had their own age-matched control groups. Animals were taken from across 4 litters each for the control group and the maternal separation group ($n = 3\text{--}4$ group/time point). At P6, P9, and P14, the groups consisted of males and females pooled. At P50, the males and females were examined as separate groups. At P40, only males were examined. All animals were killed by rapid decapitation, the brains removed and frozen on dry ice, then stored at -70°C . Sections ($14 \mu\text{m}$ thick) were cut on a cryostat and thaw mounted onto RNase free Probe-on (+) slides (Electron Microscopy Services). Tissue sections were fixed in 4% paraformaldehyde, acetylated, dried and stored at -70°C . Rat 5-HT_{1A} riboprobes were generated as described by Newton et al. (2003). In brief, 5-HT_{1A} gene-specific primers (forward: ATTAACCTCAC-TAAAGAGCTTAGGAACCTTCGTCGGCA; reverse: TAATACGACTCACTATAGGGCA-GAGGAAGGTGCTCTTGG) were used to PCR amplify a 150 bp fragment from rat genomic DNA. The reverse primer contained the T7 template sequence and the forward primer contained a T3 template sequence. The PCR product was column purified and used in an *in vitro* transcription reaction with ³⁵S-labeled UTPs. Slides were incubated with hybridization buffer containing the radiolabeled antisense probe (1 million cpm/slide) for 20 h at 60°C . The slides were then treated with ribonuclease A (RNase A, 40 mg/ml), followed by stringent washes in decreasing concentrations of saline sodium citrate (SSC) buffer. Slides were air dried and exposed to Hyperfilm β -max (GE Healthcare UK) for 6–7 d. Hybridization with a sense riboprobe (transcribed with T3 RNA polymerase) did not yield significant hybridization (data not shown), confirming the specificity of the signal. For the *in situ* experiments, there were a total of 14 pups from 4 dams in the control group and also in the early life stress group.

Quantitative analysis of *in situ* hybridization data

Levels of 5-HT_{1A} transcripts were determined using the ScionImage Software (Scion). To correct for nonlinearity, ¹⁴C standards were used for calibration. Equivalent areas of prefrontal cortex were outlined and optical density measurements taken. Prefrontal cortex layer II/III regions from both hemispheres of 3–4 sections from each animal were analyzed to obtain a mean value. Results were subjected to statistical analysis using a two-way ANOVA and tested *post hoc* with Bonferroni test at a significance level of 0.05.

Results

Serotonin elicits an outward current mediated by 5-HT_{1A} receptors on layer II/III pyramidal neurons of the prefrontal cortex

To characterize the responses of layer II/III neurons to serotonin (5-HT), we performed whole-cell recordings in the rat medial prefrontal cortex, as illustrated in the schematic in Figure 1A.

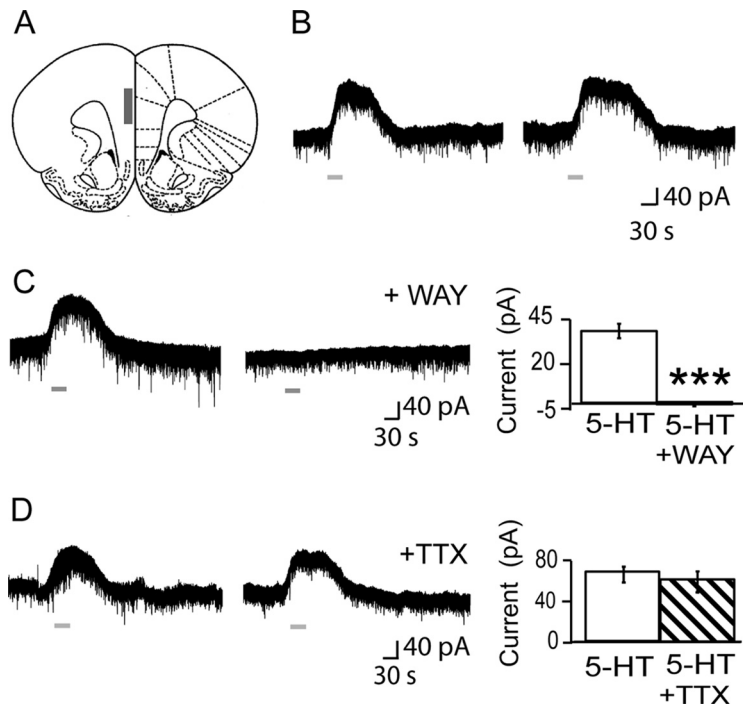


Figure 1. Serotonin elicits a direct 5-HT_{1A}-mediated outward current in layer II/III pyramidal neurons of the rat prefrontal cortex. **A**, Schematic of the prefrontal cortex indicating the region in which whole-cell recordings of layer II/III pyramidal neurons were performed. **B**, An example of a voltage-clamp trace showing that bath application of serotonin ($10 \mu\text{M}$; 30 s) elicited an outward current and that a similar response could be elicited with reapplication of serotonin after a 5 min washout period. The horizontal gray line denotes the application of serotonin. **C**, Voltage-clamp trace showing that the serotonin-elicited outward current recorded in layer II/III pyramidal neuron (left) is completely blocked in the presence of the 5-HT_{1A} antagonist, WAY-100635 (30 nM ; 10 min; center). The bar chart summarizes the amplitude of the peak serotonin current in layer II/III neurons at baseline and after application of WAY ($n = 9$; paired *t* test, $***p < 0.00005$; right). **D**, The 5-HT_{1A} outward currents are directly mediated by the 5-HT_{1A} receptors on the recorded layer II/III neurons. Example of a voltage-clamp trace showing that serotonin elicits an outward current (left) that persists in the presence of action potential blockade by TTX ($2 \mu\text{M}$; 10 min; center). The bar chart summarizes the mean peak amplitude of the 5-HT_{1A} currents in layer II/III neurons at baseline and in the presence of TTX ($n = 10$; paired *t* test, $p = \text{NS}$; right).

Bath application of serotonin ($10 \mu\text{M}$; 30 s) elicited an outward inhibitory current in layer II/III pyramidal neurons. A similar magnitude of response could be elicited after a 5 min washout period (Fig. 1B), suggesting that this application protocol did not alter the response to serotonin.

As illustrated in Figure 1C, the 5-HT_{1A} antagonist WAY-100635 (30 nM ; 10 min) completely blocked the serotonin-elicited outward currents: $43.0 \pm 4.3 \text{ pA}$ baseline versus $-1.1 \pm 1.2 \text{ pA}$ in the presence of WAY ($n = 9$; paired *t* test, $p < 0.00005$). Conversely, the 5-HT_{1A} agonist 8-OH-DPAT ($10 \mu\text{M}$; 10 min) mimicked the serotonin-elicited outward current: $86.0 \pm 26.2 \text{ pA}$ with serotonin versus $58.2 \pm 11.2 \text{ pA}$ with 8-OH-DPAT ($n = 6$; paired *t* test, $p = \text{NS}$). Consistent with these observations, the G-protein-coupled potassium channel (GIRK) blocker BaCl₂ (1 mM ; 10 min) also inhibited the 5-HT_{1A}-mediated outward current: $80.9 \pm 12.7 \text{ pA}$ baseline versus $12.7 \pm 4.1 \text{ pA}$ in the presence of BaCl₂ ($n = 6$; paired *t* test, $p < 0.005$). We observed a partial reversal of the BaCl₂ blockade after 30 min washout: $12.7 \pm 4.1 \text{ pA}$ in the presence of BaCl₂ versus $35.3 \pm 2.9 \text{ pA}$ after 30 min washout ($n = 6$; paired *t* test, $p < 0.05$). These results suggest that the 5-HT_{1A} receptor mediates the serotonin-elicited outward current through activation of GIRK channels in layer II/III pyramidal neurons of the prefrontal cortex.

Figure 1D shows that the 5-HT_{1A} currents were resistant to action potential blockade with TTX ($2 \mu\text{M}$; 10 min): $66.3 \pm 7.5 \text{ pA}$ baseline versus $59.4 \pm 10.0 \text{ pA}$ in the presence of TTX ($n = 10$;

paired *t* test, *p* = NS). Similarly, the 5-HT_{1A} outward current (46.9 ± 6.9 pA baseline) was not suppressed by blocking ionotropic GABAergic and glutamate receptors with the combination of bicuculline ($10 \mu\text{M}$), CNQX ($20 \mu\text{M}$) and AP-5 ($50 \mu\text{M}$) for 10 min (45.6 ± 14.4 pA; *n* = 5; paired *t* test, *p* = NS). Together, these results demonstrate that serotonin activates 5-HT_{1A} receptors directly on layer II/III pyramidal neurons of the prefrontal cortex.

5-HT_{1A} currents in layer II/III pyramidal neurons of the prefrontal cortex are present early in development and persist into adolescence

During postnatal development, we examined changes to these 5-HT_{1A} currents as well as intrinsic membrane properties of the layer II/III pyramidal neurons of the rat prefrontal cortex (age range: P6–P96; *n* = 130 cells). Although all layer II/III pyramidal neurons displayed 5-HT_{1A} currents, we found significant differences in the adult 5-HT_{1A} currents, and have thus divided the results into two sections: developmental (P6–P42) and adult (>P55; see following section in Results).

Throughout the developmental period (age range: P6–P42), layer II/III pyramidal neurons displayed a 5-HT_{1A}-mediated outward current in response to serotonin. In Figure 2A, an example of a voltage-clamp trace from a P7 rat illustrates that bath application of serotonin ($10 \mu\text{M}$; 30 s) elicited a 5-HT_{1A}-mediated outward current. This figure also shows that this current is suppressed in the presence of the 5-HT_{1A} antagonist WAY-100635 (30 nM ; 10 min). Averaging by postnatal week, we observed that the mean peak amplitude of the 5-HT_{1A} current remained constant throughout the postnatal period and into early adolescence (postnatal week 6; main effect of postnatal week *p* = NS; Fig. 2B): 44.0 ± 3.7 pA at postnatal week 2 (*n* = 32); 57.3 ± 6.0 pA at postnatal week 3 (*n* = 38); 57.0 ± 5.6 pA at postnatal week 4 (*n* = 18); 51.0 ± 6.0 pA at postnatal week 5 (*n* = 18); 57.7 ± 8.0 pA at postnatal week 6 (*n* = 13). Throughout the postnatal developmental period, no significant difference was observed between the mean peak amplitude of the 5-HT_{1A}-mediated currents in males and females (Fig. 3); therefore, data from both genders were combined. Previous studies in layer V of the prefrontal cortex, however, have shown that early in postnatal development serotonin elicits an inward current resulting in an increase in neuronal excitability (Zhang, 2003; Béique et al., 2004). To assess whether the response to serotonin was different in layer II/III and layer V during development, we recorded neurons in both layers in the same prefrontal cortical slices. As illustrated in supplemental Figure 1, available at www.jneurosci.org as supplemental material, early in postnatal development (<P15; *n* = 27) bath application of serotonin ($10 \mu\text{M}$; 30 s) elicited an excitatory inward current (-10 ± 3.5 pA, *n* = 35) which was significantly different from the inhibitory outward currents elicited in layer II/III neurons in the same brain slices (39 ± 2.9 pA, *n* = 49; unpaired *t* test, *p* < 0.0001). By early adolescence, however, the net response to serotonin was similar in both layers (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

Having assessed the outward currents elicited by 5-HT_{1A} receptors across development, we asked whether there were developmental changes in the ability of these receptors to lower the input resistance of the layer II/III pyramidal neurons. Figure 2C demonstrates that the 5-HT_{1A} currents in layer II/III pyramidal neurons reduce the input resistance to a larger extent during the first postnatal month and decline significantly thereafter (main effect of postnatal week *p* < 0.01). These findings suggest that serotonin has a greater ability to shunt the excitability of the layer II/III pyramidal neurons during the first postnatal month.

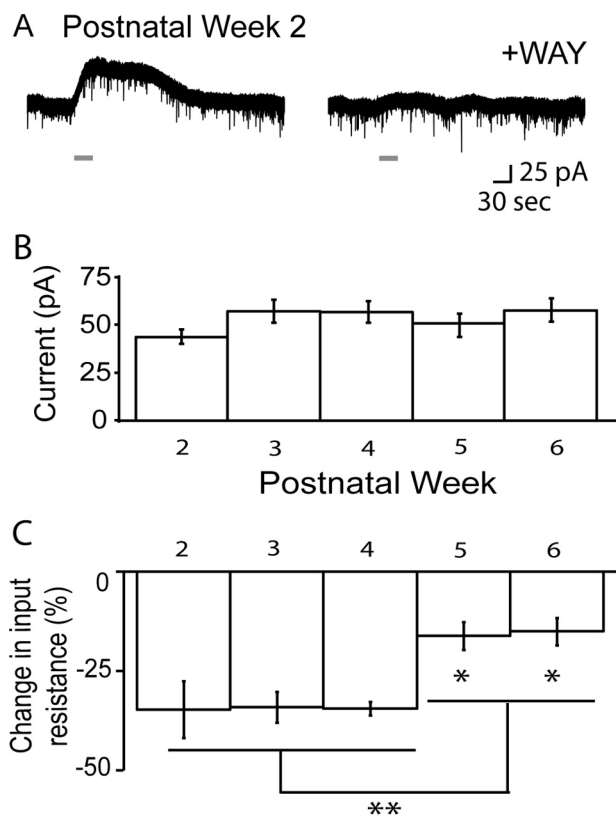


Figure 2. The 5-HT_{1A}-mediated outward currents in layer II/III pyramidal neurons of the rat prefrontal cortex are present early in development and persist into adolescence. **A**, An example of a voltage-clamp trace showing that a 5-HT_{1A} outward current is elicited by serotonin ($10 \mu\text{M}$; 30 s; left) from P7, and this response is blocked in the presence of the 5-HT_{1A} antagonist WAY-100635 (30 nM ; 10 min; right). The horizontal gray line denotes the application of serotonin. **B**, The bar chart summarizes the mean peak amplitude of the 5-HT_{1A} outward current elicited by serotonin in layer II/III pyramidal neurons by postnatal week. These 5-HT_{1A}-mediated outward currents are present during the second postnatal week, and analysis with one-way ANOVA reveals that the magnitude of the peak serotonin current remains constant into early adolescence (postnatal week 6; main effect of postnatal week *p* = NS). Note: The 5-HT_{1A} currents appear to be present almost 2 weeks earlier in development in layer II/III than previously reported for 5-HT_{1A} currents in layer V of the prefrontal cortex (Béique et al., 2004). **C**, In the first postnatal month, stimulation of 5-HT_{1A} receptors has a larger shunting effect on the input resistance. The bar chart summarizes the serotonin-elicited percentage change in input resistance in layer II/III pyramidal neurons by postnatal week. Analysis with one-way ANOVA revealed a highly significant developmental effect (main effect of postnatal week ***p* < 0.01) in which the mean serotonin-induced decrease in input resistance is significantly higher during the first postnatal month (**p* < 0.05), as shown by Newman–Keuls tests.

The 5-HT_{1A} current in layer II/III neurons occurs against a backdrop of many changes to the neurons' size and excitability. During the postnatal development period (P6–P42), pyramidal neurons in layer II/III of the prefrontal cortex display developmental changes in the intrinsic resting membrane properties including decreased resting membrane potential (main effect of postnatal week *p* < 0.0001), increased spike amplitude (main effect of postnatal week *p* < 0.0001) and decreased input resistance (main effect of postnatal week *p* < 0.0001; supplemental Fig. 2, available at www.jneurosci.org as supplemental material).

In adulthood, a sex difference emerges in the 5-HT_{1A} outward current in layer II/III pyramidal neurons of the prefrontal cortex

In adult rats (P55–P96), we observed a sex difference in the 5-HT_{1A}-mediated outward currents in layer II/III pyramidal neurons of the prefrontal cortex (Fig. 3) but no difference in

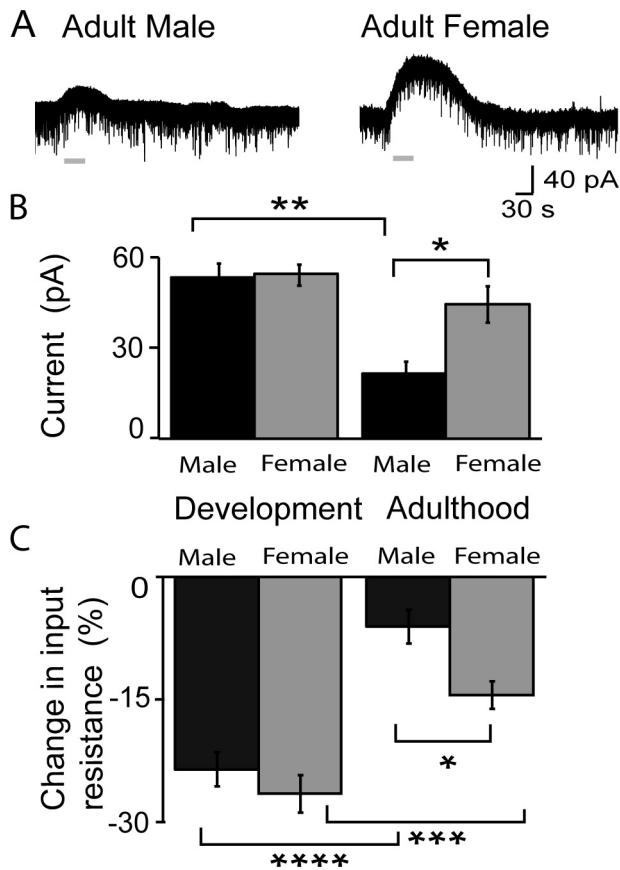


Figure 3. In adulthood, a sex difference is observed in the 5-HT_{1A}-elicited outward current in layer II/III neurons of the prefrontal cortex. **A**, Example voltage-clamp traces of layer II/III pyramidal neurons from an adult male (top) and an adult female (bottom) rat after bath application of serotonin (10 μ M; 30 s). The horizontal gray line denotes the application of serotonin. **B**, The bar chart illustrates that the mean peak amplitude of the 5-HT_{1A} current in layer II/III pyramidal neurons from male (dark gray) and female (light gray) rats in development (P11–P37) and adulthood (>P55). Whereas adult female rats ($n = 14$) display 5-HT_{1A} currents similar to those observed during development ($n = 67$; unpaired t test, $p = \text{NS}$), adult male rats ($n = 12$) showed a substantial drop in the 5-HT_{1A}-mediated currents in layer II/III relative to prepubertal male rats ($n = 62$; unpaired t test, $^{***}p < 0.002$). As a result, adult male rats displayed significantly smaller mean peak amplitude of the 5-HT_{1A} outward current in layer II/III pyramidal neurons compared with the adult female rats (unpaired t test, $^{*}p < 0.01$). **C**, The bar chart illustrates that the 5-HT-elicited percentage change in input resistance in layer II/III pyramidal neurons from male (dark gray) and female (light gray) rats in development (P11–P37) and adulthood (>P55). During development, there is no significant difference between the 5-HT-elicited percentage change in input resistance between males ($n = 33$) and females ($n = 19$; unpaired t test, $p = \text{NS}$). In adulthood, both males ($n = 10$) and females ($n = 10$) display a reduction in the shunting effects of serotonin; however, the drop in males (unpaired t test, $^{****}p < 0.000001$) is significantly larger than in their female counterparts (unpaired t test, $^{***}p < 0.001$). As a result, adult males display a significantly smaller 5-HT-elicited percentage change in input resistance (unpaired t test, $^{*}p < 0.01$).

intrinsic membrane properties (data not shown). Whereas adult female rats (>P55; $n = 14$) display 5-HT_{1A} currents similar to those observed during development (P11–P37; $n = 67$; unpaired t test, $p = \text{NS}$), adult male rats (>P55; $n = 12$) show a substantial drop in the 5-HT_{1A}-mediated currents in layer II/III relative to prepubertal male rats (P11–P37; $n = 62$; unpaired t test, $p < 0.002$). As a result, adult male rats displayed significantly smaller mean peak amplitude of the 5-HT_{1A} outward current in layer II/III pyramidal neurons (21.5 ± 3.8 pA [$n = 12$]) compared with the adult female rats (44.4 ± 6.0 pA [$n = 14$]; unpaired t test, $p < 0.01$; Fig. 3B). Similarly, in adulthood we observed a significantly smaller 5-HT-elicited percentage change in input resistance in

adult male rats ($n = 10$) relative to adult female rats ($n = 10$; unpaired t test, $p < 0.01$), an effect not observed during development (Fig. 3C). To examine whether the gender difference in 5-HT_{1A}-mediated outward current and input resistance was the result of differences in transcription of the 5-HT_{1A} receptors, we measured 5-HT_{1A} receptor expression using *in situ* hybridization in adult (P50) male ($n = 3$) and female ($n = 4$) rats. Autoradiograms revealed no significant difference in 5-HT_{1A} mRNA expression between adult males and females, however, there was a nonsignificant trend for a decrease in adult males (unpaired t test, $p = 0.09$; supplemental Fig. 3, available at www.jneurosci.org as supplemental material).

Early life stress significantly increases the 5-HT_{1A}-mediated outward current during early postnatal development

We examined whether early life stress altered 5-HT_{1A}-mediated outward currents in layer II/III of the prefrontal cortex during development. In these experiments, a total of 12 litters were randomly assigned to either the early life stress group or the control group. In this second developmental study, we measured the 5-HT_{1A} current in layer II/III of the prefrontal cortex in both early life stress and control animals. Since the resting membrane properties (supplemental Fig. 2, available at www.jneurosci.org as supplemental material) and serotonin responses (data not shown) from the control animals used in the early life stress paradigm did not significantly differ from control animals used in the developmental study, data from both groups was pooled together (total number of controls: 76 pups from 11 dams).

As illustrated in Figure 4A, animals exposed to early life stress displayed significantly larger 5-HT_{1A}-mediated outward current in layer II/III pyramidal neurons during the third and fourth postnatal week (main effect of postnatal week $p < 0.001$) but no change in intrinsic membrane properties (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). In Figure 4B, an example voltage-clamp trace illustrates that exposure to early life stress resulted in a doubling of the peak amplitude of the 5-HT_{1A} outward current during the third postnatal week. In animals exposed to early life stress, we tested whether this increase in serotonin-elicited outward currents observed during the third and fourth postnatal weeks was also mediated directly by 5-HT_{1A} receptors on the recorded layer II/III pyramidal neurons. Similar to recordings made from control animals, these larger serotonin-elicited outward currents were resistant to action potential blockade with TTX (2 μ M; 10 min) and were completely abolished in the presence of the 5-HT_{1A} antagonist WAY-100635 (30 nM; 10 min): 92.7 ± 13.3 pA baseline versus 87.1 ± 12.8 pA in the presence of TTX ($p = \text{NS}$) and dropped to 0 ± 0 pA in the presence of TTX and WAY-100635 ($n = 7$; paired t test, $p < 0.0005$). These findings indicate that early life stress increases the 5-HT_{1A}-mediated outward currents directly in layer II/III pyramidal neurons of the prefrontal cortex during the early postnatal developmental period.

Early life stress increases the developmental expression of 5-HT_{1A} receptor mRNA in layer II/III of the rat prefrontal cortex

In another group of early life stressed animals and aged-matched controls, we examined the effects of early life stress on 5-HT_{1A} receptor expression by *in situ* hybridization analysis. Consistent with our electrophysiological findings (see supplemental Fig. 1, available at www.jneurosci.org as supplemental material), the autoradiograms show prominent expression of 5-HT_{1A} receptor mRNA in layer II/III at P6 and P9, whereas expression in layer V

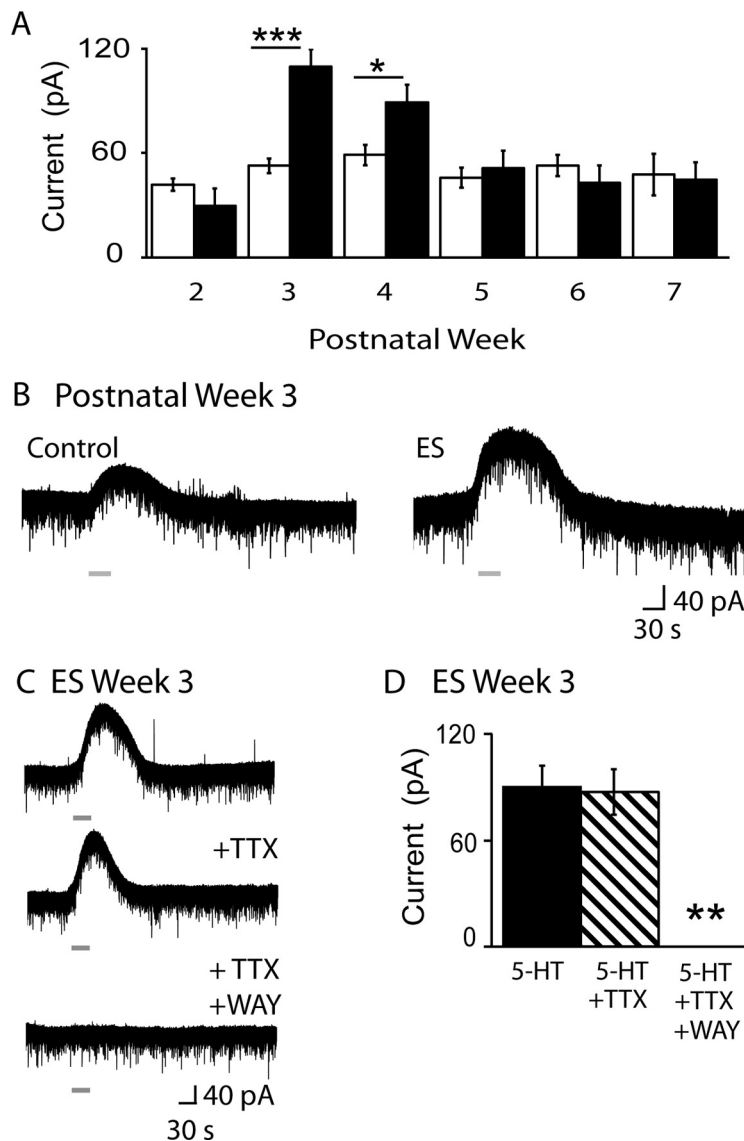


Figure 4. Early life stress significantly increases 5-HT_{1A}-mediated outward currents during postnatal development in layer II/III pyramidal neurons of the rat prefrontal cortex. **A**, The bar chart summarizes the serotonin-elicited mean peak outward current in control (open bars) and early life stress animals (closed bars) by postnatal week. Analysis with two-way ANOVA reveals a significant effect of postnatal week on the mean outward 5-HT_{1A} current in early life stress animals (main effect of postnatal week $p < 0.001$) but no significant effect in control animals (main effect of postnatal week $p = \text{NS}$). A Bonferroni *post hoc* test revealed that early life stress significantly increased the serotonin-elicited outward current during the third ($***p < 0.0001$) and fourth ($*p < 0.05$) postnatal week. **B**, An example of a voltage-clamp trace of a serotonin-elicited outward current in layer II/III from a control animal (left) and an ES animal (right). The horizontal gray line denotes the application of serotonin. **C**, **D**, In early life stress animals, the larger serotonin-elicited currents during the third and fourth postnatal weeks are mediated by 5-HT_{1A} receptors on the recorded layer II/III neurons. **C**, Voltage-clamp traces showing that during the third postnatal week, the serotonin-induced outward current (top) in early life stress animal persists in the presence of TTX ($2 \mu\text{M}$; 10 min; center), and is completely blocked in the presence of the 5-HT_{1A} antagonist, WAY-100635 (30 nM ; 10 min; bottom). **D**, The bar chart summarizes the mean peak amplitude of the 5-HT_{1A} current elicited by serotonin under baseline conditions (closed bar), in the presence of TTX (hatched bar) and in the presence of both TTX and WAY-100635 (open bar; $n = 7$; paired t test, $**p < 0.0005$).

of the prefrontal cortex at these time points is relatively low (Fig. 5A). Early life stress significantly increased 5-HT_{1A} mRNA expression in layer II/III by postnatal day 9 relative to the age matched control (early life stress $n = 3$; control $n = 3$; unpaired t test, $p < 0.05$; Fig. 5B). Previous studies have demonstrated that for cortical 5-HT_{1A} receptors the time course from mRNA to receptor insertion in the membrane is on the order of 5–7 d (Gozlan et al., 1994; Pinto and Battaglia, 1993, 1994; Raghupathi et al., 1996; Keck and Lakoski, 1997). These repopulation studies sug-

gest that the elevated 5-HT_{1A}-mediated outward current during the third and fourth postnatal week is likely the result of the increase in 5-HT_{1A} mRNA expression we observe earlier in postnatal development.

Exposure to early life stress does not alter the adult 5-HT_{1A} outward currents in layer II/III pyramidal neurons of the prefrontal cortex

We examined the effects of early life stress on adult 5-HT_{1A} outward currents in layer II/III of the prefrontal cortex (Fig. 6). Since a significant sex difference in layer II/III currents was observed in adulthood (Fig. 3), for this analysis we considered data from males and females separately. Yet, no statistically significant difference was observed in adulthood ($>P55$) between the mean peak amplitude of the outward current between C and ES animals in either males ($21.5 \pm 3.8 \text{ pA}$ [$n = 12$] controls versus $27.3 \pm 6.8 \text{ pA}$ [$n = 11$] ES; unpaired t test, $p = \text{NS}$) or females ($44.4 \pm 6.0 \text{ pA}$ [$n = 14$] control versus $54.2 \pm 11.1 \text{ pA}$ [$n = 13$] ES; unpaired t test, $p = \text{NS}$). Therefore, under normal conditions (grouped housing: 2–3 rats/cage) early life stress did not significantly alter the 5-HT_{1A} outward current in adulthood. This result is consistent with a previous study of prefrontal 5-HT_{1A} binding in adulthood after early life stress (Vicentic et al., 2006).

In adult rats with a history of early life stress, mild social isolation almost completely attenuates the 5-HT_{1A}-mediated outward current in layer II/III pyramidal neurons of the prefrontal cortex

To evaluate further the effects of early life stress on the 5-HT_{1A} currents in layer II/III of the adult prefrontal cortex, we exposed animals to a brief period of social isolation in adulthood (4–6 d of single housing). As illustrated in Figure 6, A and B, we found that in adult rats ($>P55$) social isolation did not alter the mean peak amplitude of the 5-HT_{1A}-mediated outward current in either males or females. However, we found a significant interaction between a history of early life stress and adult exposure to social isolation

(main effect of interaction in males $p < 0.05$, and in females $p < 0.005$). Only adult rats exposed to the combination of early life stress followed by adult social isolation displayed a significant loss of 5-HT_{1A} outward currents. This decrease in 5-HT_{1A} outward currents in layer II/III pyramidal neurons was observed both in males ($27.3 \pm 6.8 \text{ pA}$ [$n = 11$] in ES animals versus $8.9 \pm 3.6 \text{ pA}$ [$n = 14$] in ES+SI animals; unpaired t test, $p = 0.06$) and females ($54.2 \pm 11.1 \text{ pA}$ [$n = 13$] ES versus $10.6 \pm 4.6 \text{ pA}$ [$n = 8$] in ES+SI animals; unpaired t test, $p < 0.01$).

In another group of early life stressed rats, we examined whether brief social isolation (5 d) altered anxiety-like behavior in control and early life stressed rats. For this purpose, we measured interactions with a novel object, since this test has been shown to be sensitive to cortical 5-HT_{1A} receptor manipulations (Heisler et al., 1998). In females, there was an interaction between early life stress and adult social isolation on anxiety-like behaviors resulting in a decrease in the number of contacts with the novel object (main effect of interaction $p < 0.001$) and a decrease in the number of entries into the arena containing the novel object (main effect of interaction $p < 0.005$; supplemental Fig. 4, available at www.jneurosci.org as supplemental material). In males, there was no significant interaction between a history of early life stress and adult experience on this novelty-object behavioral task (supplemental Fig. 4, available at www.jneurosci.org as supplemental material).

Discussion

In the present study, we examined the effects of serotonin on layer II/III pyramidal neurons in the prefrontal cortex during development and after early life stress. We found that serotonin elicited an outward current directly mediated by 5-HT_{1A} receptors located on layer II/III pyramidal neurons. These 5-HT_{1A}-mediated currents were present early in development and can be seen ~2 weeks earlier than previously reported for 5-HT_{1A} currents in layer V of the prefrontal cortex (Béique et al., 2004). In adulthood, a substantial sex difference emerged in 5-HT_{1A} currents in layer II/III. Early life stress significantly increased the 5-HT_{1A} outward currents in layer II/III pyramidal neurons during the early postnatal period. This increased current likely results from an increase in the 5-HT_{1A} mRNA observed during early life stress. By adulthood, there appeared to be no effect of early life stress on 5-HT_{1A} currents in layer II/III at baseline. Yet, subsequent exposure to brief social isolation resulted in a precipitous drop of the 5-HT_{1A}-mediated currents in prefrontal cortex. These experiments suggest that layer II/III 5-HT_{1A} currents are the target of a powerful interaction between early life and adult stress.

Prefrontal 5-HT_{1A} receptors in layer II/III throughout postnatal development

Studies in humans and rodents suggest that the 5-HT_{1A} receptor subtype is present early in the development of the cortex (Barpeled et al., 1991; del Olmo et al., 1998; Bonnin et al., 2006). Here, we have demonstrated for the first time that the 5-HT_{1A} receptors located on layer II/III pyramidal neurons of the prefrontal cortex are functional throughout postnatal maturation (P6–P96) and have a larger overall inhibitory effect during the early postnatal period. Interestingly, the greatest serotonin-mediated inhibition in layer II/III neurons occurs during a developmental window in which serotonin elicits predominantly excitatory responses in layer V neurons (Zhang, 2003; Béique et al., 2004). Together, these results suggest that during early postnatal development serotonin tips the balance of prefrontal output toward activation of

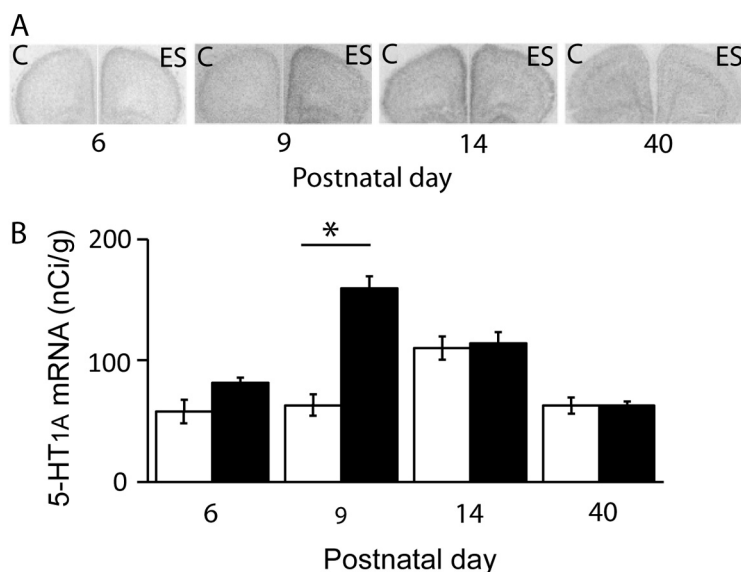


Figure 5. Early life stress increases the expression of 5-HT_{1A} mRNA during postnatal development in layer II/III of the rat prefrontal cortex. **A**, Autoradiograms of 5-HT_{1A} receptor expression in C and ES animals at P6, P9, P14, and P40. **B**, The bar graph summarizes the quantitation of the level of 5-HT_{1A} mRNA using *in situ* hybridization followed by densitometric analysis from control (open bars) and early life stress animals (closed bars) at P6, P9, P14, and P40. Early life stress ($n = 3$) significantly increased 5-HT_{1A} mRNA expression at postnatal day 9 relative to the age-matched control ($n = 3$; unpaired *t* test, $*p < 0.05$). Note that, consistent with our electrophysiological findings (see supplemental Fig. 1, available at www.jneurosci.org as supplemental material), at postnatal days 6, 9, and 14, the expression of the 5-HT_{1A} receptor appears more prominently in layer II/III than in layer V of the prefrontal cortex.

the layer V subcortical projections and away from the layer II/III corticocortical and corticoamygdalar projections.

In adult layer II/III neurons, prominent levels of 5-HT_{1A} mRNA (Burnet et al., 1995) and 5-HT_{1A} receptor binding (Hall et al., 1997) have been reported in postmortem studies of human prefrontal cortex. Here, we show that adult layer II/III pyramidal neurons display 5-HT_{1A}-mediated currents, but that these currents are significantly smaller in males compared with females. These findings are consistent with human imaging studies showing that males have lower 5-HT_{1A} binding in the prefrontal cortex (Parsey et al., 2002; Jovanovic et al., 2008). Work in rodents demonstrates that gonadectomy in male rats significantly increases 5-HT_{1A} expression in the cerebral cortex (Zhang et al., 1999), suggesting that 5-HT_{1A} receptors may be under tonic inhibition by testosterone. In layer II/III of rat prefrontal cortex, we found a trend toward decreased 5-HT_{1A} mRNA in male rats at P50, and significantly reduced 5-HT_{1A} currents in male rats after puberty. The mechanisms mediating the sex difference in the adult prefrontal 5-HT_{1A} currents, however, require further investigation.

Implications of altering 5-HT_{1A} receptors during development

Altering 5-HT_{1A} receptors can have a profound impact on emotional responses. In healthy individuals, a single nucleotide polymorphism in the 5-HT_{1A} promoter has been associated with individual differences in anxiety and depressive personality traits (Strobel et al., 2003). In addition, clinical studies indicate that alterations to prefrontal 5-HT_{1A} receptor levels are associated with psychiatric disorders including suicide, depression and anxiety (Arango et al., 1995; Bhagwager et al., 2004; Neumeister et al., 2004; Lanzenberger et al., 2007). Consistent with human studies, genetic manipulation in rodents has shown that deletion of 5-HT_{1A} receptors results in an increase in depressive and anxiety-like behaviors (Parks et al., 1998; Ramboz et al., 1998).

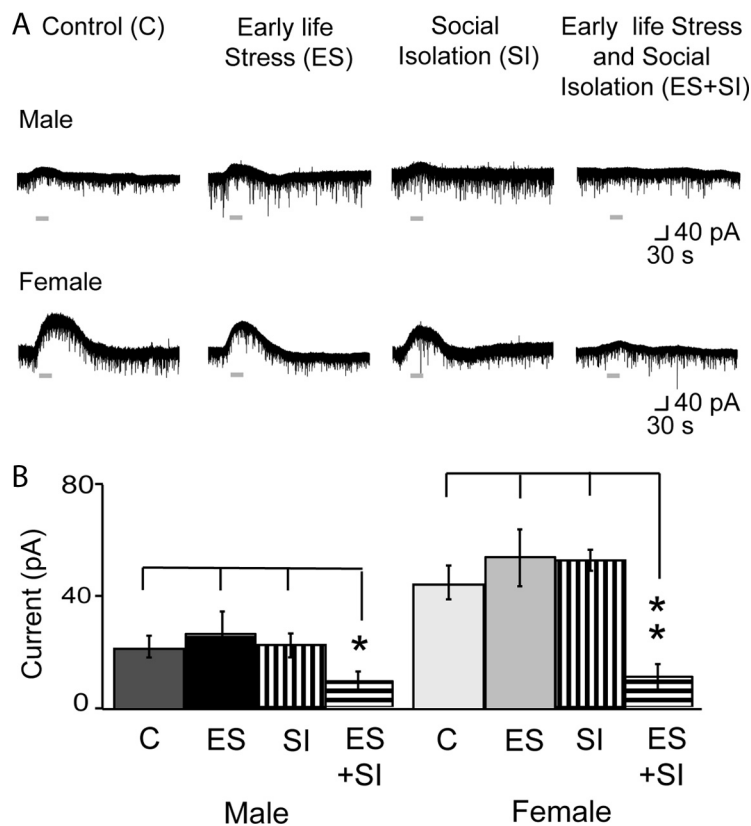


Figure 6. In adulthood, exposure to a brief period of social isolation dramatically reduces the 5-HT_{1A}-elicited outward currents in layer II/III pyramidal neurons. **A**, Examples of voltage-clamp traces of serotonin-mediated outward current from adult male (top) and female (bottom) animals exposed to no stress paradigm (C), ES, SI, or ES+SI. The horizontal gray line denotes the application of serotonin. Note that the combination of early life stress and social isolation (ES+SI) almost completely eliminates the 5-HT_{1A} outward current in layer II/III pyramidal neurons. **B**, The bar chart shows the mean peak amplitude of the 5-HT_{1A}-mediated outward current in C, ES, SI, and ES+SI from both adult male (left) and female (right) rats. Only adult rats exposed to the combination of early life stress and social isolation displayed a striking decrease in 5-HT_{1A}-mediated outward current in layer II/III pyramidal neurons (main effect of interaction: male, * $p < 0.05$; female, ** $p < 0.005$).

Interestingly, the absence of 5-HT_{1A} receptors in the forebrain during early postnatal development is sufficient to produce an anxiety-like phenotype in adulthood (Gross et al., 2002). The same behavioral phenotype is also seen with pharmacological blockade of 5-HT_{1A} receptors during postnatal development (Lo Iacono and Gross, 2008). These results suggest that the maturing cortical circuitry may be particularly sensitive to changes of the 5-HT_{1A} receptors.

Our results demonstrate that exposure to early life stress increases 5-HT_{1A} mRNA during the early postnatal period and subsequently increases 5-HT_{1A}-mediated currents in layer II/III neurons of the prefrontal cortex. The time course we observed between the increase in 5-HT_{1A} expression (P9) and the elevated 5-HT_{1A} currents (P14–26) appears to be in line with the kinetics previously observed for cortical 5-HT_{1A} receptors in repopulation experiments (Gozlan et al., 1994; Pinto and Battaglia, 1993, 1994; Raghupathi et al., 1996; Keck and Lakoski, 1997). Consistent with our electrophysiological findings, previous work has shown that early life stress upregulates 5-HT_{1A} receptor expression and binding in the hippocampus during early postnatal development (Vázquez et al., 2000, 2002; Ziabreva et al., 2003). Similarly, exposure to synthetic glucocorticoid can also increase 5-HT_{1A} receptor expression in the hippocampus during the early developmental period (Andrews et al., 2004).

Early life stress significantly increases corticosterone levels during early postnatal development (Mesquita et al., 2007), leading to the speculation that maturing prefrontal and hippocampal neurons may attempt to compensate for an adverse neuronal environment by increasing 5-HT_{1A} receptor levels. Neuronal cells under threat of degeneration and apoptosis are known to upregulate 5-HT_{1A} receptor mRNA in an attempt to survive (Singh et al., 1996). Consistent with these reports, animals exposed to early life stress appear to have increased apoptosis in the hippocampus (Fabricius et al., 2008) and in the cortex (Zhang et al., 2002), whereas the surviving prefrontal layer II/III neurons have reduced spine density in adulthood (Bock et al., 2005; Pascual and Zamora-Leon, 2007). A body of work suggests that during development 5-HT_{1A} activation is important for maintaining normal synaptic density (Yan et al., 1997; Faber and Haring, 1999). Recently, it has been suggested that 5-HT_{1A} receptors play an important role in the refinement of forebrain circuits by preventing the activation of α -CaMKII (Lo Iacono and Gross, 2008). Interestingly, the increase in 5-HT_{1A} currents we observe in the early life-stressed animals occurs during a time period that is critical for synaptogenesis and the development of the neuronal circuitry of the prefrontal cortex (Bourgeois, 1997; Zhang, 2004). Since layer II/III is the main layer for intracortical communication and also innervates the amygdala (Gabbott et al., 2005), a failure to establish normal neuronal connectivity during

this critical period would have long-term ramifications on the cellular substrates of cognitive flexibility and, potentially, the ability to cope with stress in adulthood (for review, see Holmes and Wellman, 2009).

Adult 5-HT_{1A} receptors as a target in the interaction between early life stress and later life experience

In humans, early life events are thought to influence the susceptibility toward the development of an anxiety disorder in adulthood (for review, see Heim and Nemeroff, 2001; Gross and Hen, 2004). It has been suggested that stress in early life acts on the maturing circuitry to predispose an individual to be vulnerable to mood disorders, whereas stress in adulthood activates or amplifies the induction of symptoms (Leonardo and Hen, 2008). Our results show that early life stress in combination with adult social isolation dramatically decreases the 5-HT_{1A}-mediated inhibition of layer II/III pyramidal neurons. This decrease in layer II/III 5-HT_{1A} currents likely changes the communication between the prefrontal cortex and other limbic structures (Gabbott et al., 2005; Holmes, 2008). The inhibitory 5-HT_{1A} receptors in layer II/III of the prefrontal cortex are located on dendrites (Kia et al., 1996; Riad et al., 2000) and on the axon initial segment (Czyrak et al., 2003) and, thus, are poised to regulate the excitability of neurons and their ability to communicate with other cortical regions

such as the amygdala. Disinhibition of the layer II/III projections to the amygdala is consistent with the hyperexcitability of the amygdala seen in patients with anxiety disorders (Etkin and Wager, 2007).

Previous work has demonstrated that corticosterone can decrease 5-HT_{1A} expression in the hippocampus of adult rodents (Meijer and de Kloet, 1995; Neumaier et al., 2000). In cell systems, expression of the 5-HT_{1A} receptor appears to be regulated by direct corticosteroid-elicited transrepression of the 5-HT_{1A} promoter by glucocorticoid and mineralocorticoid receptors (Ou et al., 2001). However, adrenalectomy in adult rats does not appear to change 5-HT_{1A} receptor binding in the superficial layers of cerebral cortex (Kuroda et al., 1994). In preliminary work, we found no significant change in 5-HT_{1A} receptor mRNA levels in adult animals exposed to early life stress even after exposure to severe stress in adulthood (see supplemental Results). Alternatively, corticosterone may regulate the 5-HT_{1A} receptor via non-transcriptional processes. Work by Okuhara and Beck (1998) shows that corticosterone decreases 5-HT_{1A} receptor responses in adult rats by uncoupling these receptors from their G-protein effectors.

Conclusions

A number of striking parallels can be drawn between the human 5-HT_{1A} receptors in the prefrontal cortex and those in layer II/III of the rat prefrontal cortex that we have characterized in the present study. In both the human and rat prefrontal cortex, the 5-HT_{1A} receptors are present early in development, show a large sex difference in adulthood and are sensitive to stress. The dramatic reduction we observed in 5-HT_{1A} currents from the interaction of early and adult experiences is likely not detectable by conventional ligand binding methods in human imaging work and urges further study using pharmacological functional imaging approaches.

References

Andrews MH, Kostaki A, Setiawan E, McCabe L, Matthews SG (2004) Developmental regulation of 5-HT_{1A} mRNA in the fetal limbic system: response to antenatal glucocorticoid. *Brain Res Dev Brain Res* 149:39–44.

Anisman H, Zaharia MD, Meaney MJ, Merali Z (1998) Do early life events permanently alter behavioral and hormonal responses to stressors? *Int J Dev Neurosci* 16:149–164.

Arango V, Underwood MD, Gubbi AV, Mann JJ (1995) Localized alterations in pre- and postsynaptic serotonin binding sites in the ventrolateral prefrontal cortex of suicide victims. *Brain Res* 688:121–133.

Bar-Peled O, Gross-Isseroff R, Ben-Hur H, Hoskins I, Groner Y, Biegon A (1991) Fetal human brain exhibits a prenatal peak in the density of serotonin 5-HT_{1A} receptors. *Neurosci Lett* 127:173–176.

Béique JC, Campbell B, Perring P, Hamblin MW, Walker P, Mladenovic L, Andrade R (2004) Serotonergic regulation of membrane potential in developing rat prefrontal cortex: coordinated expression of 5-hydroxytryptamine (5-HT)_{1A}, 5-HT_{2A}, and 5-HT₇ receptors. *J Neurosci* 24:4807–4817.

Bhagwagar Z, Rabiner EA, Sargent PA, Grasby PM, Cowen PJ (2004) Persistent reduction in brain serotonin_{1A} receptor binding in recovered depressed men measured by positron emission tomography with [¹¹C]WAY-100635. *Mol Psychiatry* 9:386–392.

Bock J, Gruss M, Becker S, Braun K (2005) Experience-induced changes of dendritic spine densities in the prefrontal and sensory cortex: correlation with developmental time windows. *Cereb Cortex* 15:802–808.

Bonnin A, Peng W, Hewlett W, Levitt P (2006) Expression mapping of 5-HT₁ serotonin receptor subtypes during fetal and early postnatal mouse forebrain development. *Neuroscience* 141:781–794.

Bourgeois JP (1997) Synaptogenesis, heterochrony and epigenesis in the mammalian neocortex. *Acta Paediatr Suppl* 422:27–33.

Bouwmeester H, Smits K, Van Ree JM (2002) Neonatal development of projections to the basolateral amygdala from prefrontal and thalamic structures in rat. *J Comp Neurol* 450:241–255.

Burnet PW, Eastwood SL, Lacey K, Harrison PJ (1995) The distribution of 5-HT_{1A} and 5-HT_{2A} receptor mRNA in human brain. *Brain Res* 676:157–168.

Clancy B, Darlington RB, Finlay BL (2001) Translating developmental time across mammalian species. *Neuroscience* 105:7–17.

Czyrak A, Czepiel K, Mačkowiak M, Chocyk A, Wedzony K (2003) Serotonin 5-HT_{1A} receptors might control the output of cortical glutamatergic neurons in rat cingulate cortex. *Brain Res* 989:42–51.

del Olmo E, López-Giménez JF, Vilaró MT, Mengod G, Palacios JM, Pazos A (1998) Early localization of mRNA coding for 5-HT_{1A} receptors in human brain during development. *Brain Res Mol Brain Res* 60:123–126.

Drevets WC, Frank E, Price JC, Kupfer DJ, Holt D, Greer PJ, Huang Y, Gautier C, Mathis C (1999) PET imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry* 46:1375–1387.

Etkin A, Wager TD (2007) Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry* 164:1476–1488.

Faber KM, Haring JH (1999) Synaptogenesis in the postnatal rat fascia dentata is influenced by 5-HT_{1A} receptor activation. *Brain Res Dev Brain Res* 114:245–252.

Fabricius K, Wörtwein G, Pakkenberg B (2008) The impact of maternal separation on adult mouse behaviour and on the total neuron number in the mouse hippocampus. *Brain Struct Funct* 212:403–416.

Gabbott PL, Warner TA, Jays PR, Salway P, Busby SJ (2005) Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. *J Comp Neurol* 492:145–177.

Gozlan H, Laporte AM, Thibault S, Schechter LE, Bolaños F, Hamon M (1994) Differential effects of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) on various 5-HT binding sites in the rat brain. *Neuropharmacology* 33:423–431.

Gross C, Hen R (2004) The developmental origins of anxiety. *Nat Rev Neurosci* 5:545–552.

Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R (2002) Serotonin_{1A} receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* 416:396–400.

Hall H, Lundkvist C, Halldin C, Farde L, Pike VW, McCarron JA, Fletcher A, Cliffe IA, Barf T, Wikström H, Sedvall G (1997) Autoradiographic localization of 5-HT_{1A} receptors in the post-mortem human brain using [³H]WAY-100635 and [¹¹C]way-100635. *Brain Res* 745:96–108.

Heim C, Nemeroff CB (2001) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* 49:1023–1039.

Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH, Tecott LH (1998) Elevated anxiety and antidepressant-like responses in serotonin 5-HT_{1A} receptor mutant mice. *Proc Natl Acad Sci U S A* 95:15049–15054.

Holmes A (2008) Genetic variation in cortico-amygdala serotonin function and risk for stress-related disease. *Neurosci Biobehav Rev* 32:1293–1314.

Holmes A, Wellman CL (2009) Stress-induced prefrontal reorganization and executive dysfunction in rodent. *Neurosci Biobehav Rev* 33:773–783.

Jovanovic H, Lundberg J, Karlsson P, Cerin A, Saijo T, Varrone A, Halldin C, Nordström AL (2008) Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. *Neuroimage* 39:1408–1419.

Kagan J, Snidman N (1999) Early childhood predictors of adult anxiety disorders. *Biol Psychiatry* 46:1536–1541.

Keck BJ, Lakoski JM (1997) *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) administration for studies of 5-HT_{1A} receptor binding site inactivation and turnover. *Brain Res Brain Res Protoc* 1:364–370.

Kia HK, Brisorgueil MJ, Hamon M, Calas A, Vergé D (1996) Ultrastructural localization of 5-hydroxytryptamine_{1A} receptors in the rat brain. *J Neurosci Res* 46:697–708.

Kuroda Y, Watanabe Y, Albeck DS, Hastings NB, McEwen BS (1994) Effects of adrenalectomy and type I and type II glucocorticoid receptor activation on 5-HT_{1A} and 5-HT₂ receptor binding and 5-HT transporter mRNA expression in rat brain. *Brain Res* 648:157–161.

Lanzenberger RR, Mitterhauser M, Spindelegger C, Wadsak W, Klein N, Mien LK, Holik A, Attarbaschi T, Mossaheb N, Sacher J, Geiss-Granadia T, Kletter K, Kasper S, Tauscher J (2007) Reduced serotonin-1A receptor binding in social anxiety disorder. *Biol Psychiatry* 61:1081–1089.

Leonardo ED, Hen R (2008) Anxiety as a developmental disorder. *Neuropsychopharmacology* 33:134–140.

Lidov HG, Molliver ME (1982) An immunohistochemical study of seroto-

- nin neuron development in the rat: ascending pathways and terminal fields. *Brain Res Bull* 8:389–430.
- Lidov HG, Grzanna R, Molliver ME (1980) The serotonin innervation of the cerebral cortex in the rat—an immunohistochemical analysis. *Neuroscience* 5:207–227.
- Lo Iacono L, Gross C (2008) Alpha-Ca₂+/*calmodulin-dependent protein kinase II* contributes to the developmental programming of anxiety in serotonin receptor 1A knock-out mice. *J Neurosci* 28:6250–6257.
- Meijer OC, de Kloet ER (1995) A role for mineralocorticoid receptor in a rapid and transient suppression of hippocampal 5-HT_{1A} receptor mRNA by corticosterone. *J Neuroendocrinol* 7:653–657.
- Mesquita AR, Pêgo JM, Summavielle T, Maciel P, Almeida OF, Sousa N (2007) Neurodevelopment milestone abnormalities in rats exposed to stress in early life. *Neuroscience* 147:1022–1033.
- Nair A, Vadodaria KC, Banerjee SB, Benekareddy M, Dias BG, Duman RS, Vaidya VA (2007) Stressor-specific regulation of distinct brain-derived neurotrophic factor transcripts and cyclic AMP response element-binding protein expression in the postnatal and adult rat hippocampus. *Neuropsychopharmacology* 32:1504–1519.
- Neumaier JF, Sexton TJ, Hamblin MW, Beck SG (2000) Corticosteroids regulate 5-HT(1A) but not 5-HT(1B) receptor mRNA in rat hippocampus. *Brain Res Mol Brain Res* 82:65–73.
- Neumeister A, Bain E, Nugent AC, Carson RE, Bonne O, Luckenbaugh DA, Eckelman W, Herscovitch P, Charney DS, Drevets WC (2004) Reduced serotonin type 1A receptor binding in panic disorder. *J Neurosci* 24:589–591.
- Newton SS, Collier EF, Hunsberger J, Adams D, Terwilliger R, Selvanayagam E, Duman RS (2003) Gene profile of electroconvulsive seizures: induction of neurotrophic and angiogenic factors. *J Neurosci* 23:10841–10851.
- Okuhara DY, Beck SG (1998) Corticosteroids alter 5-hydroxytryptamine 1A receptor-effector pathway in hippocampal subfield CA3 pyramidal cells. *J Pharmacol Exp Ther* 284:1227–1233.
- Ou XM, Storrington JM, Kushwaha N, Albert PR (2001) Heterodimerization of mineralocorticoid and glucocorticoid receptors at a novel negative response element of the 5-HT_{1A} receptor gene. *J Bio Chem* 276:14299–14307.
- Parks CL, Robinson PS, Sibille E, Shenk T, Toth M (1998) Increased anxiety of mice lacking the serotonin 1A receptor. *Proc Natl Acad Sci U S A* 95:10734–10739.
- Parsey RV, Oquendo MA, Simpson NR, Ogden RT, Van Heertum R, Arango V, Mann JJ (2002) Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT_{1A} receptor binding potential measured by PET using [C-11]WAY-100635. *Brain Res* 954:173–182.
- Pascual R, Zamora-León SP (2007) Effects of neonatal maternal deprivation and postweaning environmental complexity on dendritic morphology of prefrontal pyramidal neurons in the rat. *Acta Neurobiol Exp (Wars)* 67:471–479.
- Pinto W, Battaglia G (1993) In vivo EEDQ dose-dependently inactivates rat brain 5-HT receptors but not 5-HT uptake sites. *Neuroreport* 5:61–64.
- Pinto W, Battaglia G (1994) Comparative recovery kinetics of 5-hydroxytryptamine 1A, 1B, and 2A receptor subtypes in rat cortex after receptor inactivation: evidence for differences in receptor production and degradation. *Mol Pharmacol* 46:1111–1119.
- Raghupathi RK, Brousseau DA, McGonigle P (1996) Time-course of recovery of 5-HT_{1A} receptors and changes in 5-HT_{1A} receptor mRNA after irreversible inactivation with EEDQ. *Brain Res Mol Brain Res* 38:233–242.
- Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci U S A* 95:14476–14481.
- Riad M, Garcia S, Watkins KC, Jodoin N, Doucet E, Langlois X, el Mestikawy S, Hamon M, Descarries L (2000) Somatodendritic localization of 5-HT_{1A} and preterminal axonal localization of 5-HT_{1B} serotonin receptors in adult rat brain. *J Comp Neurol* 417:181–194.
- Romijn HJ, Hofman MA, Gramsbergen A (1991) At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby? *Early Hum Dev* 26:61–67.
- Singh JK, Chromy BA, Boyers MJ, Dawson G, Banerjee P (1996) Induction of the serotonin 1A receptor in neuronal cells during prolonged stress and degeneration. *J Neurochem* 66:2361–2372.
- Strobel A, Gutknecht L, Rothe C, Reif A, Mössner R, Zeng Y, Brocke B, Lesch KP (2003) Allelic variation in 5-HT_{1A} receptor expression is associated with anxiety- and depression-related personality traits. *J Neural Transm* 110:1445–1453.
- Tauscher J, Bagby RM, Javanmard M, Christensen BK, Kasper S, Kapur S (2001) Inverse relationship between serotonin 5-HT(1A) receptor binding and anxiety: a [(11)C]WAY-100635 PET investigation in healthy volunteers. *Am J Psychiatry* 158:1326–1328.
- Vázquez DM, López JF, Van Hoers H, Watson SJ, Levine S (2000) Maternal deprivation regulates serotonin 1A and 2A receptors in the infant rat. *Brain Res* 855:76–82.
- Vázquez DM, Eskandari R, Zimmer CA, Levine S, López JF (2002) Brain 5-HT receptor system in the stressed infant rat: implications for vulnerability to substance abuse. *Psychoneuroendocrinology* 27:245–272.
- Vicentic A, Francis D, Moffett M, Lakatos A, Rogge G, Hubert GW, Harley J, Kuhar MJ (2006) Maternal separation alters serotonergic transporter densities and serotonergic 1A receptors in rat brain. *Neuroscience* 140:355–365.
- Watson RE, Desesso JM, Hurtt ME, Cappon GD (2006) Postnatal growth and morphological development of the brain: a species comparison. *Birth Defects Res B Dev Reprod Toxicol* 77:471–484.
- Yan W, Wilson CC, Haring JH (1997) 5-HT_{1A} receptors mediate the neurotrophic effect of serotonin on developing dentate granule cells. *Brain Res Dev Brain Res* 98:177–184.
- Zhang L, Ma W, Barker JL, Rubinow DR (1999) Sex differences in expression of serotonin receptors (subtypes 1A and 2A) in rat brain: a possible role of testosterone. *Neuroscience* 94:251–259.
- Zhang LX, Levine S, Dent G, Zhan Y, Xing G, Okimoto D, Kathleen Gordon M, Post RM, Smith MA (2002) Maternal deprivation increases cell death in the infant rat brain. *Brain Res Dev Brain Res* 133:1–11.
- Zhang ZW (2003) Serotonin induces tonic firing in layer V pyramidal neurons of rat prefrontal cortex during postnatal development. *J Neurosci* 23:3373–3384.
- Zhang ZW (2004) Maturation of layer V pyramidal neurons in the rat prefrontal cortex: intrinsic properties and synaptic function. *J Neurophysiol* 91:1171–1182.
- Ziabreva I, Poeggel G, Schnabel R, Braun K (2003) Separation-induced receptor changes in the hippocampus and amygdala of *Octodon degus*: influence of maternal vocalizations. *J Neurosci* 23:5329–5336.