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The Effects of Fluoxetine on Sensory-Evoked Responses in the Neonatal Rat Barrel Cortex

Daria Vinokurova[1] · **Andrey Zakharov**[1,2] · **Dinara Akhmetshina**[1] · **Azat Nasretdinov**[1] · **Guzel Valeeva**[1] · **Roustem Khazipov**[1,3,4]

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Abstract Inhibition of serotonin uptake disrupts the development of thalamocortical barrel maps in neonatal rodents. Previous studies, using the selective serotonin reuptake inhibitor citalopram, have suggested that this may involve a suppression of the early activity in the developing cortex. Here, we addressed the acute effects of another frequently used serotonin uptake inhibitor, fluoxetine (10–120 mg/kg, intraperitoneally), on the sensory-evoked electrical responses in the neonatal (postnatal days P2-6) rat barrel cortex. We found that the administration of fluoxetine minimally affected the sensory-evoked responses in the rat pups. Two hours after the fluoxetine administration, there was a slight increase in the sensory-evoked potential (SEP) onset latency. There also was a tendency of SEP's amplitude to decrease, but this was not significant. Fluoxetine also had no significant effect on the multiple unit activity during the SEP and sensory-evoked bursts and neither did it affect the spontaneous multiple unit activity. We suggest that the inhibitory effects of fluoxetine on the activity in the neonatal rat barrel cortex are much weaker, or that they develop over a slower time scale, than those evoked by citalopram, probably reflecting a lower potency of fluoxetine to inhibit the serotonin uptake.

Daria Vinokurova and Andrey Zakharov contributed equally to this work.

✉ Guzel Valeeva
gurvaleeva@kpfu.ru

[1] Laboratory of Neurobiology, Kazan Federal University, 18 Kremlevskaya st, Kazan 420008, Russia

[2] Department of Physiology, Kazan State Medical University, 420012 Kazan, Russia

[3] INMED - INSERM U901, 163 avenue de Luminy, Marseille 13273, France

[4] Aix-Marseille University, Marseille 13273, France

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1 Introduction

Development of the somatosensory cortical body maps is an activity-dependent process controlled by serotonin. Elevation of extracellular serotonin levels through the inhibition of serotonin uptake with the selective serotonin uptake inhibitors (SSRIs) and genetic blockade of serotonin transporters or serotonin degrading enzyme monoamine oxidase A disturbs the formation of the cortical maps during the neonatal period in rodents (for review, see [1]). Studies using thalamocortical slices revealed that exogenously applied serotonin strongly inhibits the thalamocortical transmission in neonatal rodents [2, 3]. Along with these findings, a highly selective and potent SSRI citalopram has been shown to inhibit spontaneous and sensory-evoked responses, to prolong the delays of the sensory-evoked potentials (SEPs) and to reduce the frequency and power of the early gamma oscillations in the neonatal rat barrel cortex [4]. In the present study, we addressed the effects of another widely used SSRI antidepressant, fluoxetine, on the activity in the neonatal rat barrel cortex.

2 Material and Methods

This work has been carried out in accordance with EU Directive 2010/63/EU for animal experiments and all protocols were approved by INSERM (N007.08.01) and KSMU (N9-2013). Wistar rats of both sexes from postnatal days (P) 2–6 were used. Preparation of the

animals for the head-restrained recordings and the recording setup were as described previously [4]. The recordings of the local field potential (LFP) and multiple unit activity (MUA) were performed from a barrel column with an identified principal whisker (PW) using linear silicone probes (16 channels, 50 or 100 μm separation distance (Neuronexus Technologies, USA)) under urethane anesthesia (1–1.5 g/kg, i.p.). The whiskers were trimmed to a length of 0.8–1.5 mm and were stimulated (2 ms pulse durations) using piezo actuator at 10–20 s intervals through the entire experiment. A needle (22G) was glued to the end of piezo actuator (Noliac, Denmark) and the tip of the whisker was inserted 0.5 mm into the blunt tip of the needle, so that the whisker rested snugly inside. Spontaneous (8 s period before the stimulus) and sensory-evoked activity was recorded for 1 h before and 2–3 h after the injection of 50-μl fluoxetine solution in normal saline prepared from a 1 % stock solution of fluoxetine hydrochloride (Sigma) at a dose of 10–120 mg/kg. Sensory-evoked responses within 15 min recording periods before and 2 h after fluoxetine administration were analyzed in each animal. The signals were amplified and filtered (×10,000; 0.1 Hz–10 kHz) using a Digital Lynx (Neuralynx, USA) amplifier, digitized at 32 kHz and saved on a PC for a post hoc analysis using custom-written functions in Matlab (MathWorks, USA) as described previously [4, 5]. The statistical analysis was performed using the Matlab Statistics toolbox. The statistical comparisons between the groups were performed using the paired sample one tailed Wilcoxon signed-rank test. The significance level was set at $p < 0.05$. The group data in the text are expressed as mean ± SEM, and in the figure as mean ± SEM, median and 25–75 % quartile range.

3 Results and Discussion

Under the control conditions, a brief mechanical PW deflection evoked complex responses in the corresponding cortical barrel column of P2-6 rats, consisting of the initial SEP, followed by gamma- and spindle-burst oscillations (Fig. 1a, left panels). The maximal LFP signals, current sinks, oscillation power, and MUA during the sensory-evoked responses were located in cortical layer 4 (L4) (Fig. 1a) that is in keeping with the results of previous studies [4, 5].

After the fluoxetine administration (10–120 mg/kg, i.p.), both the sensory-evoked and spontaneous activity remained essentially unchanged within the recording period of 2 h (Fig. 1a, right panels). Moreover, we did not find any difference in the effects of fluoxetine at doses of 10–20 mg/kg ($n = 5$ rats) and 60–120 mg/kg ($n = 3$ rats), and therefore, we pooled together the data obtained at all (10–120 mg/kg) doses used in the present study. In order to quantify the effects of fluoxetine, we analyzed the following parameters: SEP amplitude and onset latency, MUA during SEP and MUA during 500 ms time window after SEP, and spontaneous MUA that was calculated within 8 s long time window before the stimulus. Overall, there was a tendency towards suppressive effects of fluoxetine (that had been previously reported using another SSRI citalopram [4]), but most of these changes were not significant, except for the effect on the SEP onset latency. The parameters of the sensory-evoked and spontaneous activity were as follows: (i) SEP amplitude, 511 ± 101 μV and 454 ± 120 μV ($p > 0.05$); (ii) SEP latency, 39 ± 4 ms and 45 ± 6 ms ($p < 0.05$); (iii) MUA during SEP, 6 ± 1 units/20 ms and 5 ± 1 units/20 ms ($p > 0.05$); (iv) MUA after SEP, 58 ± 24 units/500 ms and 48 ± 22 units/500 ms ($p > 0.05$); and (v) spontaneous MUA, 2 ± 1 units/s and 2 ± 1 units/s ($p > 0.05$), before and 2 h after fluoxetine administration, respectively ($n = 8$ P2-6 rats). The values of these parameters obtained 2 h after the fluoxetine administration and normalized to the control values are presented on Fig. 1b, revealing an overall tendency to the suppressive effects of fluoxetine on spontaneous and sensory-evoked activity in barrel cortex of neonatal rats yet with only SEP onset latency showing a significant increase by 13 ± 6 % ($p < 0.05$; $n = 8$).

Thus, we observed only minimal inhibitory effects of fluoxetine on spontaneous and sensory-evoked activity in the barrel cortex of neonatal rats within a time window of 2 h after the drug administration. This is different from the robust suppression of the activity by another SSRI citalopram that has been reported previously [4]. Although the reasons for this difference are unknown, it could be suggested that they involve a difference in the potency of these two SSRIs to bind to the serotonin transporter. Indeed, citalopram is more selective and more potent than fluoxetine [6, 7]. Also, while we did not find any major acute effect of fluoxetine on the brain activity within a few hours after the administration, it remains possible that these effects develop over a longer time scale along with an accumulation of fluoxetine during chronic treatment.

4 Conclusions

Thus, the administration of fluoxetine exerts minimal acute effects on the spontaneous and sensory-evoked activity in

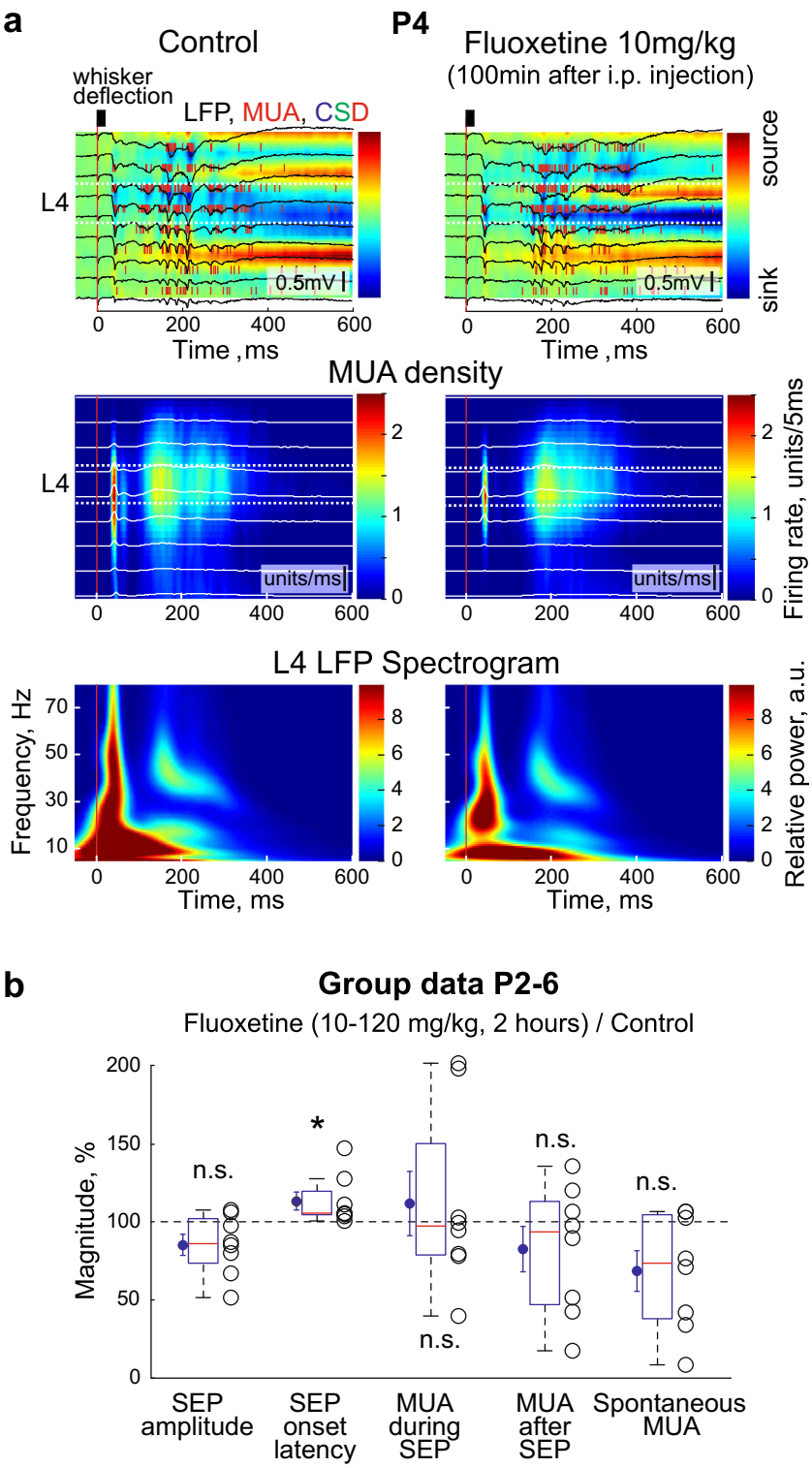

Fig. 1 Effects of fluoxetine on the activity in the neonatal rat barrel cortex. **a** Electrical responses evoked by the principal whisker deflection at a different depth of the corresponding cortical barrel column (50 μm distance between the adjacent recording sites) of P4 rat before (*left*) and 100 min after the fluoxetine administration (*right*). Cortical layer 4 (L4) is marked by *dashed lines*. Shown below are the corresponding stimulus-triggered averages for MUA across layers and L4 LFP spectra. **b** The parameters of the sensory-evoked responses and the spontaneous activity in the neonatal rat barrel cortex 2 h after the administration of fluoxetine (10–120 mg/kg, i.p.) normalized to the control values. Each *open circle* corresponds to an individual rat. The medians of the *box plots* (25–75 % quartile range) are shown by the *red lines*, and the mean values are shown by the *closed circles* with standard error bars. The data pooled from eight P2-6 rats. (*$p < 0.05$; *n.s.* non-significant)

the rat pups' barrel cortex, which is different from the powerful inhibitory actions of another serotonin reuptake inhibitor, citalopram. We suggest that the inhibitory effects of fluoxetine are much weaker, or that they develop over a slower time scale, than those evoked by citalopram, probably reflecting a lower potency of fluoxetine to inhibit the serotonin uptake.

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References

1. van Kleef, E. S., Gaspar, P., Bonnin, A. (2012). Insights into the complex influence of 5-HT signaling on thalamocortical axonal system development. *European Journal of Neuroscience, 35*, 1563–1572.
2. Rhoades, R. W., Bennett-Clarke, C. A., Shi, M. Y., Mooney, R. D. (1994). Effects of 5-HT on thalamocortical synaptic transmission in the developing rat. *Journal of Neurophysiology, 72*, 2438–2450.
3. Laurent, A., Goaillard, J. M., Cases, O., Lebrand, C., Gaspar, P., Ropert, N. (2002). Activity-dependent presynaptic effect of serotonin 1B receptors on the somatosensory thalamocortical transmission in neonatal mice. *Journal of Neuroscience, 22*, 886–900.
4. Akhmetshina, D., Zakharov, A., Vinokurova, D., Nasretdinov, A., Valeeva, G., Khazipov, R. (2016). The serotonin reuptake inhibitor citalopram suppresses activity in the neonatal rat barrel cortex in vivo. *Brain Research Bulletin, 124*, 48–54.
5. Minlebaev, M., Colonnese, M., Tsintsadze, T., Sirota, A., Khazipov, R. (2011). Early gamma oscillations synchronize developing thalamus and cortex. *Science, 334*, 226–229.
6. Thomas, D. R., Nelson, D. R., Johnson, A. M. (1987). Biochemical effects of the antidepressant paroxetine, a specific 5-hydroxytryptamine uptake inhibitor. *Psychopharmacology, 93*, 193–200.
7. Shank, R. P., Vaught, J. L., Pelley, K. A., Setler, P. E., McComsey, D. F., Maryanoff, B. E. (1988). McN-5652: a highly potent inhibitor of serotonin uptake. *Journal of Pharmacology and Experimental Therapeutics, 247*, 1032–1038.