DOI 10.1007/s11055-020-00918-x
Neuroscience and Behavioral Physiology, Vol. 50, No. 4, May, 2020

Inhibition of Serotonin Reuptake in the Medial Prefrontal Cortex during Acquisition of a Condition Reflex Fear Reaction Promotes Formation of Generalized Fear

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UDC 612.821 + 612.826

Translated from Zhurnal Vysshei Nervnoi Deyatel'nosti imeni I. P. Pavlova, Vol. 69, No. 3, pp. 343–353, May–June, 2019. Original article submitted February 27, 2018. Revised version received March 16, 2018. Accepted May 14, 2018.

Intracerebral microdialysis studies of Sprague–Dawley rats showed that acquisition of a conditioned reflex fear reaction (combination of a conditioned sound signal CS+ and an unavoidable electrocutaneous stimulus) was accompanied by an increase in the level of extracellular serotonin in the medial prefrontal cortex. Administration of the selective serotonin transporter inhibitor fluoxetine (1 μM) into the medial prefrontal cortex during acquisition of the conditioned reflex fear reaction magnified the increase in extracellular serotonin in these structures seen during this test and, secondly, strengthened freezing of the animals at 1 day (the measure of fear) in response to the differential signal CS–, not associated with pain stimulation (test for generalization of fear), but had no effect on freezing in the same animals in response to the conditioned stimulus CS+, previously combined with pain stimulation (test for expression of the fear reaction). These data provide the first evidence that stimulation of the serotoninergic system of the medial prefrontal cortex during acquisition of a condition reflex fear reaction promotes subsequent generalization of this conditioned reflex reaction without affecting its formation.

Keywords: medial prefrontal cortex, intracerebral microdialysis, serotonin release, conditioned reflex fear reaction, generalization of fear.

The medial prefrontal cortex (mPFC) is involved in regulating a number of important physiological processes, supporting the integrity of the body's responses. These include attention, assessment, prioritization, inhibition of irrelevant behavioral programs and signs of impulsivity, and monitoring of visceral functions and emotional reactions [Kuleshova et al., 2008; Arnsten et al., 2015]. Impairments to the normal functioning of the mPFC have been identified in several forms of psychopathology, particularly posttraumatic [Pitman et al., 2012] and anxious-depressive stress disorders [Cha et al., 2014]. One of the central characteristics of these disorders is stable generalization of fear, expressed as manifestations of fear in response to safe stimuli [Greenberg et al., 2013; Jovanovic et al., 2012; Kaczkurkin et al., 2017]. Studies in recent years have demonstrated that the mPFC may be involved in controlling the generalization of fear in health [Xu and Sudhof, 2013; Zelikowsky et al., 2013] and may mediate impairments of such control in pathology [Cha et al., 2014]. In particular, use of a conditioned reflex fear reaction (CFR – a model of fear) has shown that inactivation of the mPFC leads to enhanced freezing (a measure of fear in rodents) in response to contextual signals not associated with pain stimulation without any change in freezing in response to sound and pain stimuli previously combined with pain [Xu et al., 2012; Xu and Sudhof, 2013]. Studies of the neurochemical mechanisms of this phenomenon identified important roles for NMDA receptors [Vieira et al., 2015] and transcription factor CREB [Vieira et al., 2014] in the mPFC in the formation of differential memory, which improves the precision of the CFR, and we recently demonstrated the involvement of nitrergic system of the mPFC in repressing generalization of this conditioned reflex reaction [Saulskaya and Sudorgina,

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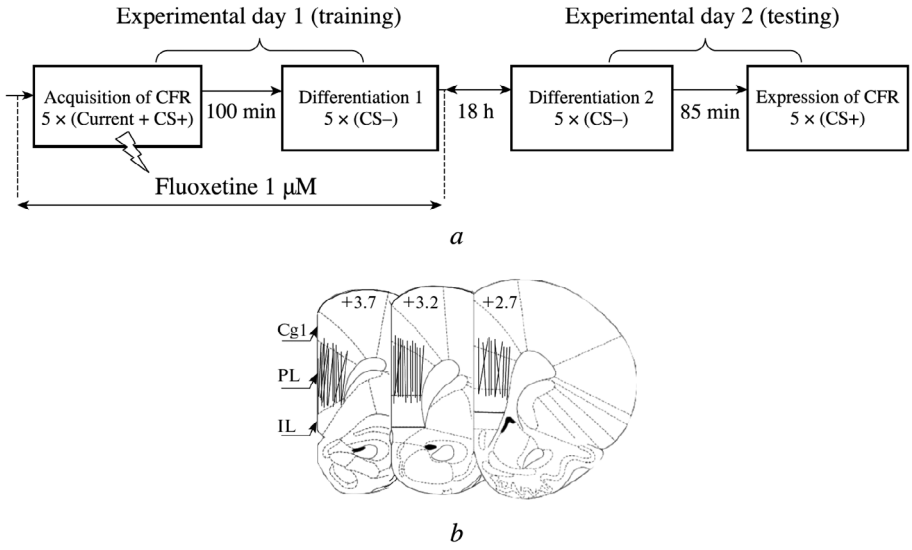

Fig. 1. *a*) Experimental scheme; *b*) position of dialysis zones of cannulas in the medial prefrontal cortex. Numbers show distances (mm) from the bregma [Paxinos and Watson, 1997]. Cg1 – cingulate cortex, field 1; PL – prelimbic cortex; IL – infralimbic cortex.

2015]. However, the contributions of other neurotransmitter systems of the mPFC, particularly its serotoninergic system, to controlling fear generalization have not previously been studied. Serotonin is known to have a significant influence on the functioning of the mPFC, acting on neural excitability, spike activity, and the plasticity of neurons in this area of the cortex [Puig et al., 2005; Meunier et al., 2013].

The aim of the present work was to study the involvement of the serotoninergic system of the mPFC in the generalization of fear induced by acquisition of a CFR. Vital intracerebral microdialysis studies were performed to investigate the effects of administration of the selective serotonin transporter inhibitor fluoxetine into the mPFC during acquisition of the CFR on serotonin release in the mPFC during the test and on the formation of the CFR and subsequent generalization of this conditioned reflex reaction. The literature contains no reports on this point.

Methods. Studies were carried out on male Sprague–Dawley rats weighing 260–350 g from the biological collection of the Pavlov Institute of Physiology, Russian Academy of Sciences, Collection of Laboratory Mammals of Different Taxonomic Groups, supported by the bioresource collections program of the Russian Federal Agency of Scientific Organizations. Experiments were run in compliance with international norms for the humane treatment of experimental animals (European Union Directive No. 86/609/EEC).

Rats were anesthetized (Rometar 1.4 μg/100 g and Zoletil 5 mg/100 g, i.m.) and underwent unilateral implantation of concentric dialysis cannulas into the mPFC as described previously [Saulskaya and Sudorgina, 2015]. On the following day (experimental day 1 – training stage), each rat was placed in the daytime home cage and dialysis perfusion of the mPFC with artificial cerebrospinal fluid (iCSF) was started [Saulskaya and Fofonova, 2006] at a rate of 1 μl/min. An SP-100 dialysis pump (Next Advance, USA) was used. After a stabilization period (1.5 h), five portions of dialysate (each of 15 min) were collected, after which the animals were divided into three groups: two experimental and one control. The experimental scheme is shown in Fig. 1, *a*. Animals of experimental group 1 (n = 14) were trained to the CFR: each rat was placed in the conditioned reflex chamber with a grid floor covering (30 × 30 × 35 cm) for 5 min and the conditioned signal (CS+) – a continuous tone (1000 Hz, 51 dB, 5 × 10 sec, 1-min intervals) was presented in combination with electrocutaneous stimulation of the paws (1.5 mA, 1 sec) during the last 1 sec of sounding. Without interrupting dialysate collection, the animal was then returned to the daytime home cage. After 100 min, differential session 1 was run, which had the aim of familiarizing the animal with the safe signals: the rat was placed in the differential chamber (30 × 30 × 35 cm, white floor, white walls) for 5 min, where it was presented with the differential signal (CS–) – a discontinuous tone (1000 Hz, 51 dB, 0.06 sec sound/0.06 sec pause, 5 × 10 sec with 1-min intervals) without use of pain stimulation. After 5 min, the rat was returned to the daytime home cage for 70 min, after which the experiment was complete. Training was terminated at this stage. Animals of the control group (n = 7) underwent the same procedures on the first day of the experiments, though without electrocutaneous stimulation. In experimental group 2 (n = 8), collection of baseline portions of dialysate was followed by supplementation of the iCSF used for perfusion of the mPFC with the selective serotonin transporter inhibitor fluoxetine (1 μM, Sigma, USA) and a further four portions of dialysate were collected, each of 15 min. Animals of experimental group 2 were then trained to the CFR and, after a further 100 min, took part in differentiation session 1 as described for experimental group 1.

After 70 min, perfusion liquid containing fluoxetine was replaced by iCSF and additional perfusion was continued for 30 min to remove fluoxetine from the cannula and brain tissue. The experiment was then complete. Dialysate was collected every 15 min throughout the experiment.

On the next day (experimental day 2 – the test stage), each animal was placed in a plus maze for 5 min (open arms 50 × 14 cm; closed arms 50 × 14 × 40 cm; central platform 14 × 14 cm) for determination of the index of anxiety (duration of time spent in the open arms of the maze as a percentage of test duration) and the level of exploratory activity (crossing sectors in the closed arms of the maze). Animals were then connected to the dialysis pump delivering iCSF and, after a stabilization period (1 h), five portions of dialysate were collected (each of 15 min) for determination of the baseline level of extracellular serotonin in the mPFC in animals subjected (experimental group 2) and not subjected (experimental group 1 and control group) to fluoxetine administration the day before. Animals of experimental groups 1 and 2 were then included in differentiation session 2 (test for generalization of the CFR) as in differentiation session 1; the CFR was implemented after 85 min (test for CFR acquisition) – the animal was placed in the conditioned reflex chamber for 5 min, where it was presented with the conditioned stimulus (CS+) (5 × 10 sec, 1-min intervals) but without electrocutaneous stimulation. The animal was then returned to the daytime home cage and the experiment was complete. On experimental day 2, the animal underwent the same procedures as rats of the experimental groups. During behavioral tests, recordings were made of freezing time (sec) in response to the conditioned (CS+) or differential (CS–) signals – parameters reflecting the extent of CFR acquisition and the level of CFR generalization, respectively. Video recordings of behavior were made on a personal computer and a webcam (Logitec, China) during the tests.

A separate group of animals (n = 12) with dialysis cannulas implanted into the mPFC was used to study the effects of administration of fluoxetine into the mPFC on sensitivity to electrocutaneous stimulation. In these experiments, half the animals were given 1 μM fluoxetine for 1 h by addition to the perfusion liquid. The second half of the group continued perfusion with unaltered perfusion fluid. Each rat was then placed in a chamber with a grid floor covering and the threshold of sensitivity to the current was determined (μA) in terms of the occurrence of a reaction as the current strength was increased and the disappearance of this reaction as the current was decreased. The sensitivity of each animal was computed as the mean of these two values. A power supply was used (B5, 120/0.75, Akip, Russia) with the direct current function.

mPFC dialysate serotonin levels were assayed by high-performance liquid chromatography with electrochemical detection. Studies used a chromatography system (Shimazdu, Germany) as described previously [Saulskaya and Fofonova, 2006] but with a Kinetex (Phenomenex, USA) chromatography column (150 × 2.1 mm, 2.6 μm, C18). The voltage on the working electrode was +0.61 V. The mobile phase contained 0.1 M $NaH_2PO_4 \cdot 2H_2O$, 2 mM KCl, 0.5 mM EDTA, 0.26 mM sodium octylsulfonate, 11% carbinol, pH 5. The flow rate was 0.135 ml/min at a pressure of about 170 bar. Chromatograms were recorded and processed in real time (MultiChrom 1.72, Ampersend, Russia). The serotonin content in each dialysate sample was expressed in nM and as a percentage of the individual baseline level. Morphological examination of cannula sites was conducted when the experiments were complete. Rats with cannulas located in the mPFC were included in processing (Fig. 1, *b*; mainly the prelimbic cortex).

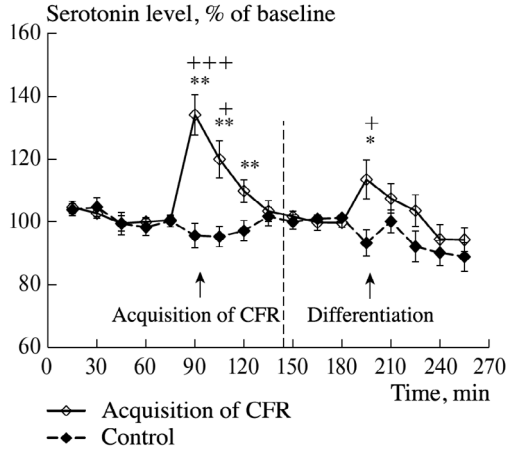

Fig. 2. Changes in the extracellular serotonin level in the mPFC during acquisition of the CFR and differentiation session 1 (Acquisition of CFR) and control tests (Control). The abscissa shows time, min; the ordinate shows the serotonin level, % of baseline; spreads on plots show errors of the mean; black arrows show the beginning of tests; $^{*}p < 0.01$; $^{**}p < 0.001$, comparison with individual baseline (t test); $^{+}p < 0.05$, $^{++}p < 0.01$ – for between-group comparison (t test).

Statistical processing was run using SigmaStat (3.5). Changes in the extracellular serotonin level relative to baseline were compared by unifactorial analysis of variance (factor – time; F test). This was followed by comparison of changes at individual time points relative to baseline using Student's t test. Between-group and between-test comparisons were made by two-factor analysis of variance (first factor – group or test; second factor – time; F test) with further comparison of groups using Student's t test. Behavioral indicators were compared using the Mann–Whitney test.

Results. The baseline serotonin level in mPFC dialysate in these experiments was 0.20 ± 0.01 nM (n = 29), which was comparable with data from other researchers [Mork et al., 2017; Yoshitake et al., 2014].

Acquisition of the CFR (combination of CS+ with unavoidable pain stimulation) in animals of experimental group 1 was accompanied by an increase in the extracellular serotonin level in the mPFC relative to individual pre-test baseline levels (Fig. 2, $F_{9,117} = 6.4$, $p < 0.001$). This increase

TABLE 1. Duration of Freezing (sec) in Response to the Differential (CS–) and Conditioned (CS+) Signals during Testing for the Manifestation of Fear in Rats Given (Experimental group 2) and not Given (Experimental group 1) 1 μM Fluoxetine into the mPFC during Acquisition of the CFR; Level of Immobility in Rats of the Control Group (Control group)

Group	Experimental group 1	Experimental group 2	Control group
CS–	$26 \pm 3^{*++\#}$	$36 \pm 2^{+\#\#\#}$	12 ± 5
CS+	$40 \pm 2^{\#\#}$	$43 \pm 2^{\#\#\#}$	19 ± 2
Number of rats	14	8	7

$^{*}p < 0.05$, comparison with experimental group 2 (Mann–Whitney test); $^{+}p < 0.05$, $^{++}p < 0.001$, comparison with freezing in response to the CS+ (Mann–Whitney test); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, comparison with control group (Mann–Whitney test).

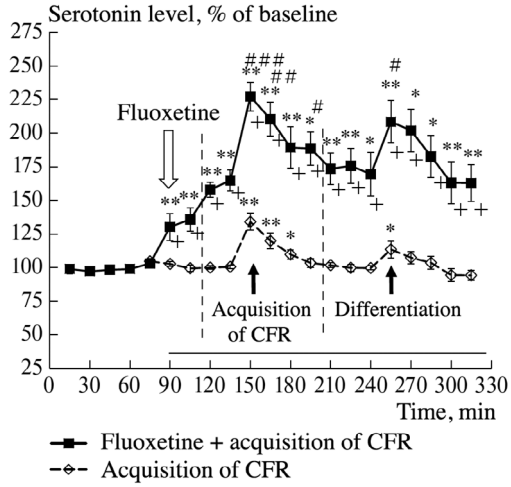

Fig. 3. Changes in the extracellular serotonin level in the mPFC during acquisition of the CFR and differentiation session 1 in animals given 1 μM fluoxetine into the mPFC (Fluoxetine + Acquisition of CFR) and rats not given drug (Acquisition of CFR). $^{*}p < 0.01$, $^{**}p < 0.001$ – comparison with baseline (*t* test); $^{+}p < 0.001$ – between-group comparison (*t* test); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ – comparison with pre-test serotonin level (*t* test). The white arrow shows the start of fluoxetine administration. The horizontal line shows the period of fluoxetine administration. For further details see caption to Fig. 2.

did not occur in animals of the control group during the control test for acquisition (Fig. 2, $F_{9,54} = 1.8$, $p = 0.08$). Between-group comparison showed that changes in the serotonin level in the mPFC during CFR acquisition in animals of experimental group 1 were significantly greater than in rats of the control group ($F_{9,190} = 3.4$, $p < 0.001$).

Presentation of the differential signal CS– without pain stimulation to animals of experimental group 1 during differentiation session 1 induced a small increase in the extracellular serotonin level in the mPFC relative to pre-test baseline (Fig. 2; $F_{7,91} = 3.2$, $p = 0.005$). However, this increase was not statistically significantly different from the serotonin level in the mPFC of animals of the control group during the control test for differentiation 1 (Fig. 2; $F_{7,152} = 1.4$, $p = 0.22$). This control test was not accompanied by changes in the serotonin level in the mPFC (Fig. 2; $F_{7,42} = 2.2$, $p = 0.06$).

Administration of the selective serotonin transporter inhibitor fluoxetine (1 μM) into the mPFC of rats of experimental group 2 increased the extracellular serotonin level in this region to 165 ± 8% relative to the pretreatment baseline (Fig. 3; $F_{8,56} = 24.7$, $p < 0.001$), which is evidence of gentle stimulation of the serotonin-reactive system of the mPFC by this fluoxetine dose. This treatment also increased the rise in the extracellular serotonin level in the mPFC induced by acquisition of the CFR (Fig. 3). Acquisition of the CFR on the background of fluoxetine treatment was accompanied by an increase in the extracellular serotonin level in the mPFC (by a maximum of 227 ± 8%) relative to the individual baseline before treatment ($F_{13,91} = 37.5$, $p < 0.001$). This increase was also significant relative to the extracellular serotonin level before this behavioral test (increased by administration of fluoxetine) ($F_{6,42} = 11.7$, $p < 0.001$), and two-factor analysis of variance showed it to be significant relative to changes in the extracellular serotonin level in the mPFC during acquisition of the CFR in animals of experimental group 1, not given fluoxetine (Fig. 3, maximum 134 ± 6%; $F_{9,200} = 10.2$, $p < 0.001$).

Administration of 1 μM fluoxetine into the mPFC of animals of experimental group 2 increased the rise in the extracellular serotonin level in this area during differentiation session 1 from 114 ± 6% (maximal increase in animals of experimental group 1, not given drug) to 209 ± 16% (maximum in rats of experimental group 2, given fluoxetine) (Fig. 3; $F_{9,200} = 10.2$, $p < 0.001$). This increase occurring on the background of fluoxetine administration was significant relative to the serotonin level before the behavioral test (increased by administration of fluoxetine) ($F_{7,49} = 2.5$, $p = 0.03$) and also relative to the baseline extracellular serotonin level in the mPFC before drug administration ($F_{13,91} = 15.9$, $p < 0.001$).

Administration of fluoxetine into the mPFC of rats of experimental group 2 during acquisition of the CFR and differentiation session 1 (training stage) had no effect on the baseline extracellular serotonin level in this part of the cortex at one day, at the testing stage (0.18 ± 0.01 nM in rats of experimental group 1; 0.18 ± 0.02 nM in rats of experimental group 2; $t = 0.07$, $p = 0.94$).

Testing of the animals for fear reactions one day after acquisition of the CFR showed that rats of experimental group 1 were characterized by significant levels of freezing in response to the conditioned signal CS+ (a measure

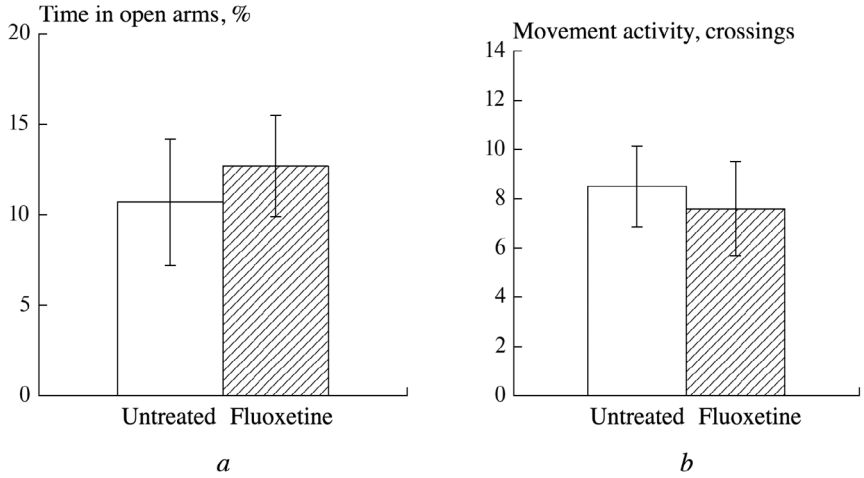

Fig. 4. *a*) Time spent (% of test duration) in the open arms of the plus maze and *b*) horizontal movement activity (crossings) in the closed arms of the plus maze in animals given (Fluoxetine) and not given (Untreated) 1 μM fluoxetine into the mPFC one day before testing.

of acquisition of the CFR) during realization of the CFR (Table 1) and a lower level of freezing in response to the differential signal CS– (a measure of generalization of the CFR) during differentiation session 2 ($p < 0.001$), which is evidence that these animals discriminated the potentially dangerous and safe sound stimuli. Freezing of these animals in response to the CS+ and CS– stimuli was greater than the level of immobility of rats of the control group during the corresponding control tests (Table 1; $p = 0.002$ for CS+ and $p = 0.014$ for CS–).

Administration of fluoxetine (1 μM) into the mPFC of animals of experimental group 2 during acquisition of the CFR and differentiation session 1 (training stage) led to an increase in the duration of freezing in response to the differentiation signal (CS–) not associated with pain stimulation one day after training (testing stage), as compared with the duration in rats of experimental group 1, not given fluoxetine (Table 1, $p = 0.03$), but had no effect on freezing in these same animals in response to the conditioned signal (CS+), previously combined with pain stimulation (Table 1, $p = 0.36$).

Administration of 1 μM fluoxetine into the mPFC during acquisition of the CFR had no effect on anxiety levels one day after administration in animals in the plus maze (time spent in the open arms) (Fig. 4, *a*; $p = 0.45$) or motor activity (crossings) (Fig. 4, *b*; $p = 0.35$) during this test.

Administration of 1 μM fluoxetine into the mPFC did not alter the threshold of current sensitivity on testing 1 h after the start of administration, which corresponds in time to the beginning of CFR acquisition in animals of experimental group 2, which were given fluoxetine (Fig. 5; $p = 0.6$).

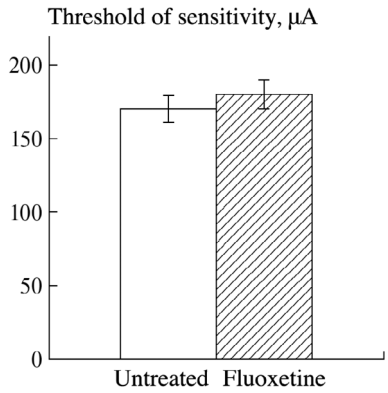

Fig. 5. Threshold of sensitivity to electric current (μA) in animals given (Fluoxetine) and not given (Untreated) 1 μM fluoxetine into the mPFC.

Discussion. The last two decades have seen significant progress in our understanding of the neurophysiological mechanisms of the formation, expression, and extinction of fear. The central role in these processes is played by three interacting structures – the amygdala, the hippocampal formation, and the mPFC, which together form the higher component of the intracerebral fear system (see [Orsini and Maren, 2012]). The mPFC occupies an important position in this system, maintaining the state of fear (the prelimbic part of the mPFC) and its extinction (the infralimbic part of the mPFC) [Burgos-Robles et al., 2009; Sortes-Bayon and Quirk, 2010]. Studies in recent years using CFR in rodents (to model fear) have shown that the mPFC (prelimbic and infralimbic parts) also takes part in the inhibitory control of CFR generalization [Xu et al., 2012; Xu and Sudhof, 2013; Zelikowsky et al., 2013], this involvement occurring by means of an interaction with the amygdala and hippocampal formation [Rozeske et al., 2015].

Serotoninergic signals from the raphe nuclei have significant influences on the functioning of the structures of the fear system (see [Bauer, 2015]). The importance of serotoninergic neurotransmission in the mPFC, amygdala, hippocampal formation, and the raphe nuclei themselves for regulating the manifestations of fear on expression of the CFR has been demonstrated [Almada et al., 2009; Almadaetal

2015; Ferreira and Nobre, 2014; Leon et al., 2017], as has the contribution of serotonin-dependent processes in the lateral amygdala to forming this conditioned reflex reaction (see [Bocchio et al., 2016]). At the same time, there are few reports on the role of CNS serotoninergic mechanisms in CFR generalization. We found only one report, which showed that mice with knockout of the serotonin 5-HT_{1A} receptor gene displayed increased acquisition and generalization of a CFR to contextual stimuli (increased freezing in response to conditioned and differential contextual stimuli) [Klemenhagen et al., 2006]. These data provide evidence that overall, the serotoninergic system of the brain takes part in controlling generalization of the CFR, though this involvement is clearly not selective. The results obtained here provide the first demonstration that the serotoninergic system of the mPFC may contribute to this process, enhancing generalization of the developing fear. This is supported by data showing that stimulation of serotoninergic neurotransmission in the mPFC by administration of the serotonin reuptake blocker fluoxetine into this area during acquisition of the CFR leads to increased manifestation of fear (freezing) in response to the safe differential signal (CS–) but has no effect on freezing in the same animals in response to the potentially dangerous conditioned signal (CS+). This selectivity of the action of fluoxetine in relation to the manifestations of fear in response to the differential stimulus in the same animals provides a reliable control demonstrating that the effects of the drug may not be linked with changes in the animals' ability to freeze when signs of danger appear or with other factors which can be indiscriminately expressed in terms of the level of freezing. This is supported by the behavioral tests conducted here, showing that administration of fluoxetine into the mPFC had no effect on current sensitivity or the animals' mobility or anxiety at the corresponding stages of the experiments. These points also lead to the conclusion that the effects of fluoxetine seen here are probably linked with its action on generalization of the CFR.

It is unlikely that the absence of any fluoxetine effect on acquisition of the CFR, apparent as the lack of any change in freezing in response to the CS+ one day after training, is associated with a limiting level of acquisition of the CFR, as our previous studies using the same procedure for CFR acquisition [Saulskaya et al., 2008] showed that when a large number of combinations of the CS+ with pain stimulation was used (10 instead of five) during acquisition, freezing of the animals in response to the CS+ during expression of the CFR increased to 93 ± 2% (compared with 80 ± 4% in the present study). Freezing in response to the CS+ could reach 98% in some animals.

Published data obtained by inactivation and lesioning indicate that the mPFC overall restrains generalization of the CFR [Xu et al., 2012; Xu and Sudhof, 2013; Zelikowsky et al., 2013; Rozeske et al., 2015]. Furthermore, our recent studies showed that this restraining influence of the mPFC on generalization involves the nitrergic system of this area of the cortex [Saulskaya and Sudorgina, 2015]. The results of the present studies demonstrate that the mPFC can have oppositely directed influences controlled by the serotoninergic input, enhancing generalization of fear. These points lead to the conclusion that the mPFC has neurochemical mechanisms with bidirectional influences on the generalization of fear at the formation stage, which provides a flexible balance between the generalization and specialization of fear.

One important question is that of which information is transmitted by serotoninergic signals arriving in the mPFC during acquisition of the CFR and stimulating generalization of this conditioned reflex reaction. Data have been reported showing that these signals, running from the raphe nuclei, may reflect the uncontrollability of stress [Maswood et al., 1998; Bland et al., 2003; Amat et al., 2005] (in the case of acquisition of the CFR, this is the unavoidability of the pain stimulation), which makes the situation more aversive for the animals. It can be suggested that the serotonin release in the mPFC triggered by uncontrollable stress leads to weakening of the inhibitory influence of this area on the serotoninergic neurons of the raphe nuclei [Puglisi-Allegra and Andolina, 2015], and this initiates a change in the strategy for overcoming stress from active to passive (freezing instead of avoiding) [Amat et al., 2005]. This rearrangement of behavior is justified in the situation of unavoidable punishment and, as believed by [Puglisi-Allegra and Andolina, 2015], is associated with strengthening of the serotoninergic influences of the raphe nuclei (controlled by the mPFC) on the amygdala. The results of this work suggest that another consequence of uncontrollable stress mediated by the raphe nuclei/mPFC system is serotonin-dependent enhancement of fear generalization, which in our view reflects stress-induced inhibition of one of the central functions of the mPFC – a restraining influence on inappropriate behavioral or emotional responses [Arnsten et al., 2015].

Overall, the data on the involvement of serotoninergic neurotransmission in the mPFC in forming fear generalization contribute to our understanding of the neurochemical processes underlying fear generalization in health and may be useful in developing approaches to treating fear generalization in pathology.

Conclusions

1. Acquisition of a conditioned reflex fear reaction (combination of conditioned sound signal and an unavoidable pain stimulus) is accompanied by release of serotonin in the medial prefrontal cortex, which is evidence of activation of its serotoninergic input.

2. Administration of the serotonin transporter inhibitor fluoxetine (1 μM) into the medial prefrontal cortex during acquisition of the conditioned reflex fear reaction increased serotonin release during this test and, secondly, increased freezing of the animals (a measure of fear) in response to the differential sound signal not associated with pain stimulation, at one day, but did not alter freezing in these animals in response to the potentially dangerous conditioned sound signal.

3. These data provide the first evidence that activation of the serotoninergic input of the medial prefrontal cortex during acquisition of a conditioned reflex fear reaction (a model of the formation of fear) promoted generalization of this conditioned reflex reaction without affecting its formation.

This study was supported financially by the Russian Foundation for Basic Science (Project No. 16-04-00449).

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