学位論文

The neurobiological basis of the antidepressant-like effect of exercise

（運動の抗うつ様効果の神経生物学的基盤に関する研究）

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Contents

List of publications

1. **Chen, C.**, Nakagawa, S., Kitaichi, Y., An, Y., Omiya, Y., Song, N., Koga, M., Kato, A., Inoue, T., Kusumi, I. The role of medial prefrontal corticosterone and dopamine in the antidepressant-like effect of exercise. *Psychoneuroendocrinology*. Under review

2. **Chen, C.**, Takahashi, T., Nakagawa, S., Inoue, T. & Kusumi, I. Reinforcement learning in depression: A review of computational research. *Neuroscience & Biobehavioral Reviews* **55**, 247-267 (2015).

List of presentations

1. **Chen, C.**, Nakagawa, S., Kitaichi, Y., An, Y., Omiya, Y., Song, N., Koga, M., Inoue, T., Kusumi, I. Exercise improves stress coping through a dopamine D2 receptor pathway in the medial prefrontal cortex. Presented at 9th Annual Scientific Meeting of the Hong Kong Society of Biological Psychiatry "Brain and the Environment", Kowloon, Hong Kong, November 21-22, 2015

2. **Chen, C.**, Nakagawa, S., Kitaichi, Y., An, Y., Omiya, Y., Song, N., Koga, M., Inoue, T., Kusumi, I. How exercise improves stress coping despite increasing corticosterone: by upregulating dopamine in the medial prefrontal cortex? Presented at 6th International Regional (Asia) International Stress and Behavior Society Neuroscience Conference "Stress and Behavior", Kobe, Japan, June 26-27, 2015

3. **Chen, C.**, Nakagawa, S., Kitaichi, Y., Omiya, Y., An, Y., Song, N., Inoue, T., Kusumi, I. Exercise improves stress coping despite increasing circadian peak of corticosterone: a microdialysis study. Presented at 36th meeting of Japanese Society of Biological Psychiatry, Nara, Japan, September 29-October 1, 2014

Introduction

The beneficial effects of exercise, or physical activity, on stress coping and mental health have been well documented. For instance, several meta-analyses of exercise interventions, including randomized controlled trials, have shown that exercise can significantly reduce depressive mood in both healthy subjects and clinical patients [1-4]. In parallel with these findings in humans, animal studies also found that exercise, such as wheel and treadmill running, improves stress coping, and exerts antidepressant-like effects [5-7]. However, despite these well replicated beneficial outcomes, the precise neurobiological mechanism underlying such beneficial outcomes remains to be completely elucidated [8].

Available evidence suggests that exercise may affect angiogenesis (thus increase blood flow), neurogenesis in the hippocampus (thus increase neuron proliferation and survival), synaptogenesis (thus increase spine density, etc), stimulate such neurotrophins as brain-derived neurotrophic factor (BDNF), and insulin-like growth factor-1 (IGF-1), and change the neurotransmitter system (both basal neurotransmitter release and that in response to stress) [8-10], which are believed to account for the above beneficial outcomes of exercise. However, to our knowledge, rarely has any research examined the causality between the observed neurobiological changes by exercise and the beneficial outcomes.

On the other hand, this lack of knowledge on the neurobiological mechanism of exercise is further complicated by a 'side effect' of exercise: it increases basal glucocorticoid (cortisol, so called in humans and corticosterone in rodents, CORT) (e.g., [11-13] in rodents; [14-15] in humans), the final product of the hypothalamic–pituitary–adrenal axis (HPA axis) in response to stressful events [16, 17]. CORT, well known as 'the stress hormone', has been shown to be a mediator of the detrimental effects of stress on memory, cognitive functions, and mental health [16-21]. For instance, rodent research has consistently found that, various models of stress and depression, such as chronic mild stress [22-27], chronic immobilization [28], chronic social defeat [29-30], and repeated electric shock [31], lead to increased basal CORT, which is reversed by antidepressant treatment [22, 26, 32]. Further, repeated exogenous administration of CORT in rodents induces such depression-like

behaviors as ‘learned helpless’ and anhedonia, which resembles human depressive states [33, 34]. In the meantime, human studies also reported elevated basal CORT in plasma [35, 36], and saliva [37-39] in clinically depressed patients, which is normalized by antidepressant or psychological treatment (for a review and meta-analysis, see [40-43]). Longitudinal observations have found that higher basal plasma CORT (esp. morning) predicts the onset of depression [44, 45].

Yet, surprisingly, exercise, with so many beneficial outcomes on stress coping and exerts powerful antidepressant-like effects, has also been reported to increase basal CORT (see references above). Further, the amount of increase in basal CORT by exercise is actually comparable to that observed by various chronic stress, which typically ranges from 50% (e.g. [13] for exercise, [26] for chronic mild stress, [28] for chronic immobilization) to 150% (e.g. [12] for exercise, [25] for chronic mild stress) of control animals. Thus this is apparently a paradox.

In the present study we aimed to investigate this paradox with regard to stress coping and antidepressant-like effect using rats, for animal research allows extensive cellular and molecular investigation, as well as subsequent pioneering pharmacological intervention to establish causality. Thus we aimed to identify the underlying neurobiological mechanism by which exercise improves stress coping and exerts antidepressant-like effect from the perspective of neurotransmitter system, as neurotransmission underlies the final information processing of the central nervous system.

List of abbreviations

ACC	anterior cingulate cortex
ACTH	adrenocorticotropic hormone
AUC	area under the curve
aCSF	artificial cerebrospinal fluid
BDNF	brain-derived neurotrophic factor
CON	control
CORT	corticosterone/glucocorticoid
DA	dopamine
DAT	dopamine transporter
D1R	D1 receptor
D2R	D2 receptor
DRN	dorsal raphe nucleus
EX	exercise
FST	Forced Swim Test
GR	glucocorticoid receptor
HPA axis	hypothalamic-pituitary-adrenal axis
HPLC	high-performance liquid chromatography system
IGF-1	insulin-like growth factor-1
MR	mineralocorticoid receptor
mPFC	medial prefrontal cortex
NA	noradrenaline
Nacc	nucleus accumbens

PFC	prefrontal cortex
SSRIs	selective serotonin re-uptake inhibitors
5-HT	serotonin
5-HT1AR	serotonin 1A receptor
VTA	ventral tegmental area

Chapter 1 The behavioral and neurobiological effects of exercise

1.1 Introduction

The purpose of the first experiment was to find a duration of exercise long enough to induce antidepressant-like effect in rats, and explore the concurrent neurobiological effects of exercise. First, we chose the commonly employed voluntary wheel running as a model of exercise, for it has been frequently reported to improve stress coping and reduce depression-like behavior [7].

Second, we chose the Forced Swim Test (FST) to measure the antidepressant-like effect. The FST was first developed by Porsolt et al [46] and later modified by Lucki and co-workers [47, 48]. It is based on the observation that when exposed to water, rats initially show intense active escape behavior, such as climbing and swimming, and then eventually stop active escaping and instead show passive immobile behavior (i.e. immobility). The immobility is thought to reflect behavioral despair and learned helplessness [49-51]. Indeed, a large amount of research has found that various stress treatments intended to induce depression-like behavior, increasing immobility and/or decreasing climbing and swimming behavior, which is typically reversed by various antidepressant treatments [49-51]. Thus the FST has been a most widely employed model for assessing antidepressant-like activity in rats.

Third, we employed the microdialysis technique to examine the neurobiological effects of exercise. Microdialysis is a minimally-invasive technique of continuously monitoring analyte (e.g. various neurotransmitters and CORT) concentrations in the extracellular fluid [52], which has been frequently used in our previous research [53-55].

Fourth, to investigate the neurobiological effect of exercise, we chose the medial prefrontal cortex (mPFC) as the brain area for microdialysis, for it is generally believed to be the final brain center for exerting behavioral control and coping [56, 57].

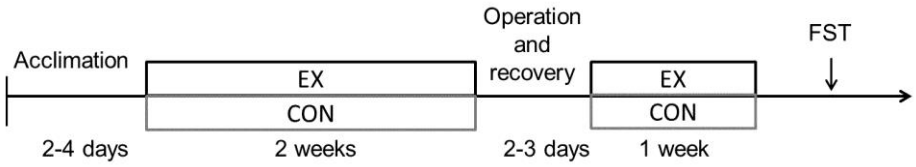
1.2 Methods

Animals and procedure

Six weeks old male Sprague-Dawley rats were obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan). Upon arriving, rats were housed in polypropylene cages (2-3 animals per cage) with wood shavings on the floor in a temperature-controlled environment (22 ± 2 °C) with unlimited access to food and water. They were maintained on a 12-hour light/dark cycle (light phase: 07:00-19:00). All experiments were approved by the Hokkaido University School of Medicine Animal Care and Use Committee and were in compliance with the Guide for the Care and Use of Laboratory Animals.

As shown in Figure 1a, after 2-4 days of acclimation, rats were randomly allocated to exercise (EX) or control (CON) group, both raised in the same cage box (W 300 x D 300 x H 400 mm) while only EX rats had free access to a running wheel attached on the side of the box (diameter 300 mm, ASTEC, Japan). Running cycles were memoed every day and later transformed into running distances (m).

a



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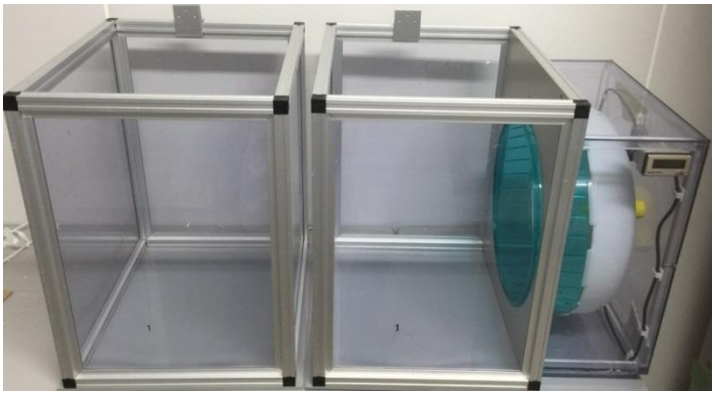


Figure 1-1 The procedure of the experiment (a) and the photo of the cage box with (b right) or without (b left) a running wheel used in the experiment

Two weeks after the allocation and treatment, all rats underwent microdialysis surgery. Stereotaxically and under pentobarbital anesthesia (30 mg/kg i.p.), AG-4 guide cannulae (Eicom Corp., Kyoto, Japan) were implanted into the rat brain, directing toward the mPFC at the following coordinates relative to the bregma from the stereotaxic atlas of Paxinos and Watson [58]: A +3.2, ML +0.6, DV +1.8 mm (See Figure 1-2). Dummy probes were inserted into the guide cannulae. Then, rats were kept in polypropylene cages independently for 2-3 days to recover, after which they were returned to their previous cage boxes with (EX) or without (CON) running wheels for another week. All rats were handled two or three time a week before surgery, and daily after surgery.

FST

A subset of rats underwent a typical FST [49-51, 59]. The test was conducted at 19:00 straight in dark (the onset of the dark phase, previous research has shown that rats subjected to the FST at this time perform similarly to that in the light phase [60]) on two consecutive days. On day-1, rats were placed individually in water in an opaque cylindrical water tank (20 cm diameter; water depth 30 cm, water temperature 25 °C) for 15 min. Then they were kept in polypropylene cages individually. Twenty-four hours later (day-2) subjects were placed again in the water tank for 5 min. After the test, rats were dried with Kim towel and returned to the polypropylene cage. Rats' behaviors in the water tank were all video recorded with an infrared camera and later analyzed as climbing, swimming or immobility by another researcher in our lab who was blind to the experimental manipulation. Climbing was defined as upward directed movements of the forepaws usually along the side of the swim chamber in order to escape. Swimming was defined as movement (usually horizontal) throughout the water tank which includes crossing across quadrants of the cylinder, diving, and rigorous padding with all four legs in order to escape. Immobility was considered when no additional behaviors were observed other than those necessary to keep the rat's head above the water to breathe, such as floating. The scoring measures the frequency of each behavior over 5-s intervals during the day-2 5-min test session.

In vivo microdialysis

Another subset of rats underwent the FST while under microdialysis. After day-1 test of the standard FST, under pentobarbital anesthesia (30 mg/kg i.p.), they were implanted with a dialysis probe with an outer diameter of 0.22 mm (A-I-4-03; Eicom). The probe was inserted into the guide cannulae so that 3.0 mm of the probe was exposed to the tissue of the mPFC. The next day, perfusion was started using artificial cerebrospinal fluid (aCSF, 145mMNaCl, 3.0mMKCl, 1.3mMCaCl2, 1.0mMMgCl2) at a flow rate of 2 μl/min at 15:00. Following the initial perfusion for 2 hours, dialysate samples were collected in sample vials every 30 min. At 19:00 straight (the onset of the dark phase), rats were placed into the water tank for 5 min, with the dialysis probe in their brains. Later they were dried with Kim towel and returned to the dialysis box. The dislysis perfusion and sampling lasted 3 hours following the onset of the test till 22:00. The exact placement of the probe tips was verified the next day during dissection using a microscope (Figure 1-2).

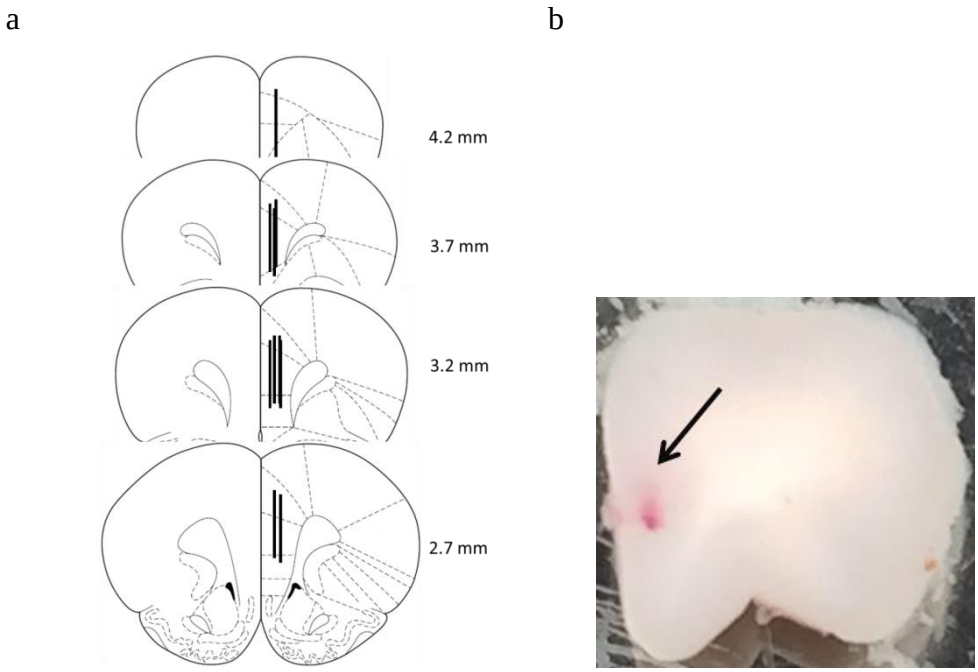


Figure 1-2 The placement of the microdialysis probe tips. Schematic representation of placement of the probe tips (a); Actual photo taken from rats' brain during dissection showing the placement of probe tips (b).

Dialysate analysis

CORT was measured using an enzymatic immunoassay (Elisa, Cayman, MI, USA). Previous research has demonstrated that CORT in the extracellular fluids of the brain can be reliably detected [61-65]. The calibration curve corresponds to the plot % bound/maximum bound for standards vs concentration of corticosterone from 8.2 to 5000 pg/ml using linear (y axis) and Log (x axis) (Figure 1-3). The calibration curve was linear with a regression coefficient (Pearson) of 0.9728, P<0.001. The dotted line corresponds to the detection limit and is equivalent to 80% bound/maximum bound. This value was approximately 25.59 pg/ml. Areas under the curve (AUCs) were calculated using the standard trapezoidal method and are expressed as arbitrary units (pg*hour /ml).

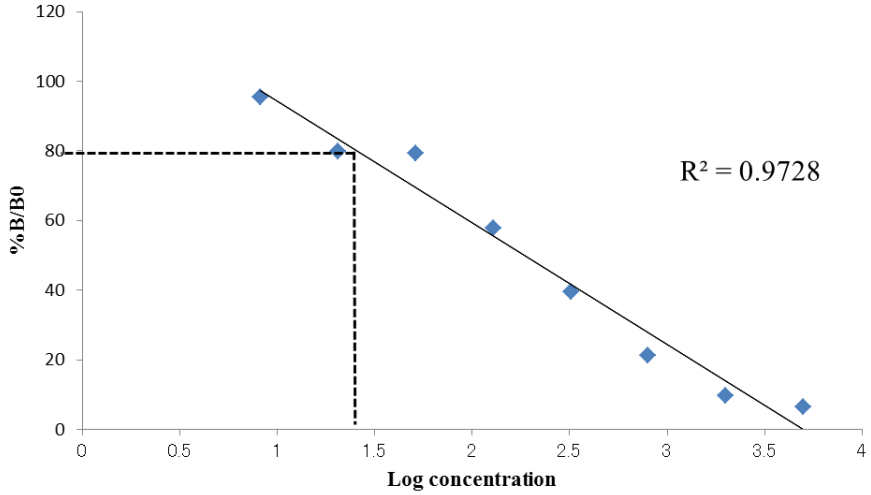


Figure 1-3 The calibration curve for quantification of CORT by Elisa. The calibration curve corresponds to the plot % bound/maximum bound for standards versus concentration of CORT from 8.2 to 5000 pg/ml using linear (y axis) and Log (x axis). The calibration curve was linear with a regression coefficient (Pearson) of 0.9728, P<0.001. The dotted line corresponds to the detection limit and is equivalent to 80% bound/maximum bound. This value was approximately 25.59 pg/ml.

For noradrenaline (NA), DA and 5-HT determination, the dialysate was mixed with the same volume of 0.05 M acetic acid. NA levels were determined using high-performance liquid chromatography system (HPLC) with electrochemical detection (Eicom) as described previously [53, 54]. The system consisted of a liquid chromatograph pump (EP-300; Eicom), a degasser (DG-300; Eicom), a reverse phase ODS column (Eicompak CA-5ODS 150 2.1 mm; Eicom), an ECD-300 electrochemical detector (Eicom), and a data acquisition system (PowerChrom; AD Instruments Pty. Ltd., Sydney, Australia). For the NA analysis, 30 µl of dialysate was injected into the HPLC system that used a 0.1 M phosphate buffer (pH 6.0) mobile phase containing 5% (v/v) methanol, 50 mg/l Na2EDTA and 500 mg/l L-octanesulfonic acid. Separations were conducted at 25 °C with a flow rate of 0.23 ml/min. The electrochemical detector was set at an oxidation potential of 550 mV. NA standard solutions were injected every working day and the peak heights for the standard were used for comparison to determine the amount of NA in the samples.

DA and 5-HT levels were determined using the same equipment with the exception of a different reverse phase ODS column, an Eicompak PP-ODS 30 4.6 mm (Eicom) was used. For DA and 5-HT analysis, 20 µl of dialysate was injected into the HPLC system that used a 0.1 M phosphate buffer (pH 6.0) mobile phase containing 1% (v/v) methanol, 50 mg/l Na2EDTA and 500 mg/l sodium L-decanesulfonate. Separations were conducted at 25 °C with a flow rate of 0.5 ml/min. In the electrochemical detector, an oxidation potential was set at 400mV. Standard solutions for 5-HT and DA were injected every working day, and the peak heights for the standards were used for comparison to determine the amount of DA and 5-HT in the samples.

Amino acids, including glycine, alanine, taurine, glutamine, and glutamate, were also determined by the HPLC systems similar to that described previously [55]. The system consisted of a liquid chromatograph pump (EP-300), a degasser (DG-300; Eicom), a fluorometric detector (FLD-370; Eicom), a reverse phase ODS column (Eicompak SC-5 ODS 2.1× 150 mm, Eicom), and a column oven (ATC-300; Eicom). The derivatization reagent was prepared by dissolving 54 mg o-phthalaldehyde (OPA) in 1 ml 99.9%

methanol and 9 ml 0.1 M Na2CO3 (pH 9.5). This solution (2.5 ml) was diluted 1:1 with 0.1 M Na2CO3, and 10 μl of β-mercaptoethanol was added. A 10 μl aliquot of OPA derivatization reagent was added to 30 μl dialysate, and, after a 2.5 min reaction period, 30 μl of the reactant was injected into the HPLC system coupled with a fluorometric detector with excitation and emission wavelengths of 340 nm and 445 nm, respectively. The mobile phase consisted of 0.06 M NaH2PO4, 0.01 M Na2HPO4, 5 mg/l Na2-EDTA (pH 6.0) and 30% (v/v) methanol. Flow rate was 0.3 ml/min. Separation was conducted isocratically at 30°C. The above amino acids standard solutions were injected every working day and the peak heights for the standard were used for comparison to determine the amount of each amino acid in the samples.

Western blot

The day following day-2 test of the FST, rats were satisfied under pentobarbital anesthesia (30 mg/kg i.p.). The brains were removed and washed with ice-cold phosphate-buffered saline (PBS; pH 7.4). Coronal sections with a thickness of 1 mm were cut using a Brain Slicer (Muromachi, Tokyo, Japan) and immersed into dishes containing ice-cold PBS. The regions containing mPFC were dissected carefully with a blade. The mPFC brain tissue was then homogenized in a lysis buffer T-PER Tissue Protein Extraction Reagent (Thermo Scientific, IL, USA) and cOmplete Protease Inhibitor Cocktail Tablets (Roche, IN, USA), followed by centrifugation at 10,000 g for 5 min at 4 C. The supernatant solutions were used as cytosolic fraction samples to detect protein levels. The subsequent procedure was essentially the same as as previously described [66]. Protein concentration was measured by the Protein Assay Kit (Pierce, IL, USA), and an equal amount of proteins (20 ug per well) was loaded onto a 10% SDS gel. The gel was transferred onto a nitrocellulose membrane (GE Healthcare, UK) and incubated with a primary antibody (Table 1-1). After washing, the membrane was incubated with a secondary antibody (Table 1-1). Protein expression was detected with the Amersham ECL Plus Western Blotting Detection System (GE Healthcare) and ImageQuant LAS 4000 (GE Healthcare). The pictures were converted to digital files and the intensity of each band was analyzed with ImageQuant TL.

	Antibody	Obtained from	Concentration
Primary antibodies	mouse monoclonal mineralocorticoid receptor (MR) antibody [H10E4C9F] ab2774	Abcam, UK	1:250
	mouse monoclonal glucocorticoid receptor (GR) antibody [BuGR2]-CHIP Grade ab2768	Abcam	1:250
	guinea pig DA D1 receptor (D1R) antibody	Narushima et al [67]	1:500
	rabbit DA D2 receptor (D2R) antibody	Narushima et al [67]	1:500
	rabbit polyclonal 5-HT1A receptor antibody (H-119: sc-10801)	Santa Cruz Biotechnology, CA, USA	1:500
	mouse monoclonal anti-GAPDH antibody (sc-32233)	Santa Cruz Biotechnology	1:1000
Secondary antibodies	horseradish peroxidaseconjugated secondary anti-mouse IgG antibody	GE Healthcare	1:10000 for GAPDH; 1:2000 for MR and GR
	horseradish peroxidase-conjugated secondary anti-rabbit IgG antibody	GE Healthcare	1:10000
	horseradish peroxidase-conjugated secondary anti guinea pig IgG antibody (SC-2438)	Santa Cruz Biotechnology	1:10000

Table 1-1 Primary and secondary antibodies used for the western blot

Statistical analysis

Statistical analyses of the FST behavior (total), dialysate AUC and western blot data employed the Student's t-test, while those of the FST behavior (across the 5-min duration) and the dialysate time series data employed two-way ANOVA with repeated measures. When the time*group interaction was significant, the Student's t-test was used as the post hoc test after two-way ANOVA with repeated measures. Significance was defined as $p < 0.05$. Data are expressed as the means ±SEM.

1.3 Results

Behaviors

Running distance of those rats that only underwent the FST is shown below in Figure 1-4 (red). This amount of wheel running significantly reduced immobility in the FST (t=2.638, p=0.021), and increased swimming with a trend towards significance (t=1.810, p=0.094), without affecting climbing (t=1.184, p=0.258), as shown in Figure 1-4. We then analyzed the FST data across the 5 minutes duration, and the results are shown in Table 1-2. Again, EX rats showed reduced immobility across the 5 minutes duration and increased swimming with a trend towards significance (Figure 1-5). As it can be seen clearly from the figure, EX rats had more swimming behavior at the end of the test, especially at 5 min, thus we analyzed the swimming data using a Student's t-test. The Student's t-test showed that at 5 min, EX rats had significantly more swimming than CON rats (t=-3.0185, p=0.010). Lastly, there was no correlation between running distance, whether pre- or post- operation or total, and immobility, swimming or climbing (Table 1-3).

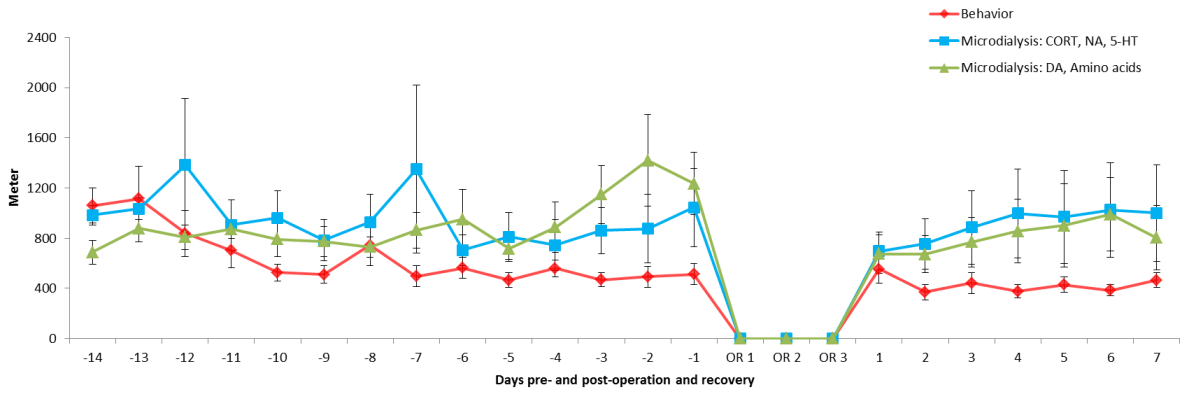


Figure 1-4 Mean running distance per day pre- and post- operation and recovery (OR) of EX rats. Red for rats that underwent the FST (n=7), blue for rats that underwent microdialysis for CORT, NA, and 5-HT (n=6), and green for rats that underwent microdialysis for DA and amino acids (n=7). Mean±SEM.

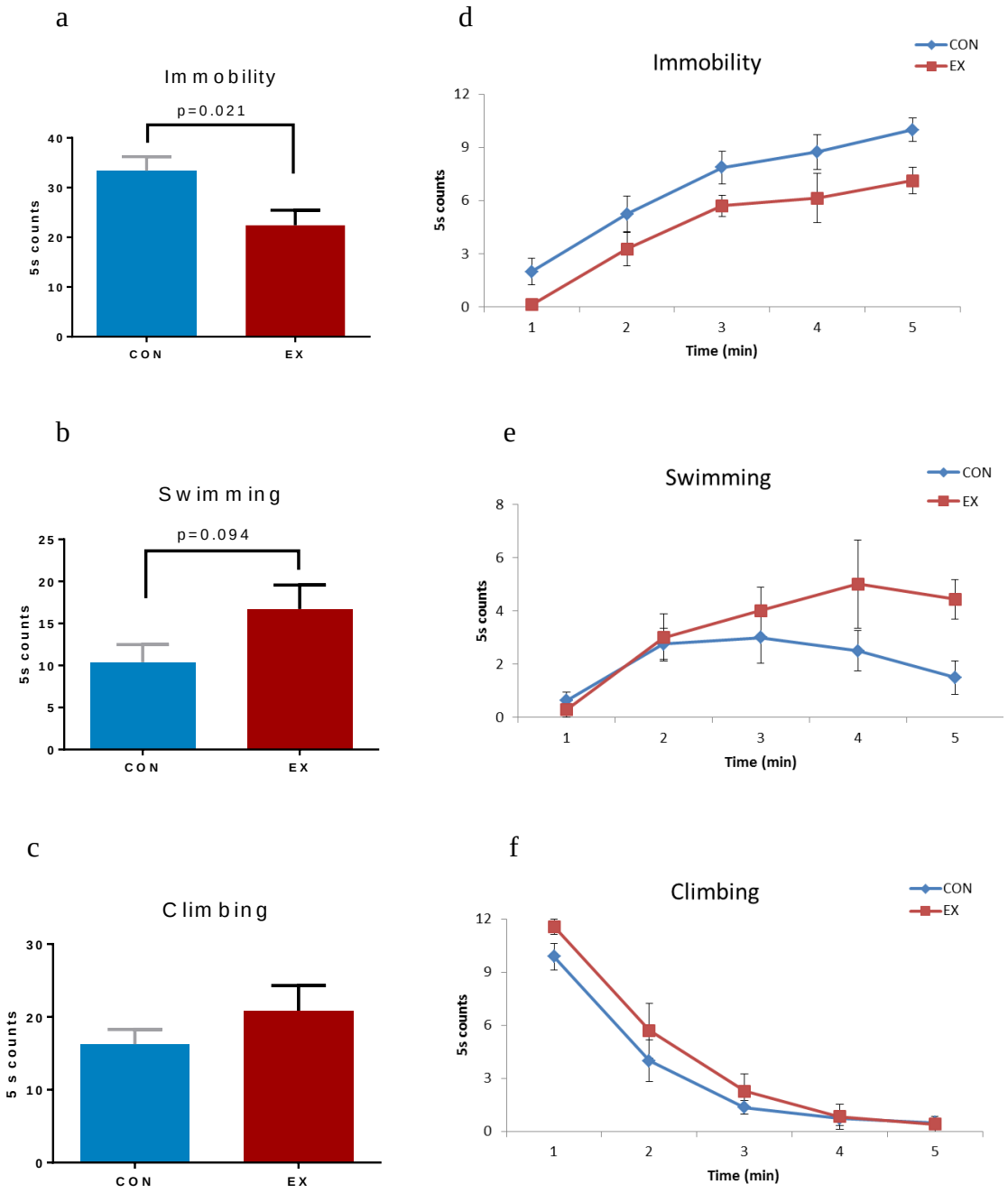


Figure 1-5 The antidepressant-like effect of exercise. (a-f) The FST for assessing antidepressant-like behavior. The duration of immobility, swimming, and climbing in the 5 min totally (a, b, c, in order) and across the 5 min (d, e, f, in order). (EX n=7; CON n=8) Mean±SEM.

	Time	Group	Time*Group
Immobility	F=32.979, p<0.001	F=7.583, p=0.016	F=0.168, p=0.954
Swimming	F=6.143, p=0.006	F=3.276, p=0.093	F=1.778, p=0.187
Climbing	F=85.376, p<0.001	F=1.285, p=0.277	F=0.838, p=0.444

Table 1-2 Two-way ANOVA with repeated measures of the FST data across the 5 min duration.

	Pre-operation	Post-operation	Total
Immobility	r=-0.192, p=0.679	r=0.469, p=0.289	r=-0.032, p=0.945
Swimming	r=0.139, p=0.767	r=-0.557, p=0.194	r=-0.037, p=0.937
Climbing	r=0.054, p=0.908	r=0.050, p=0.915	r= 0.059, p=0.900

Table 1-3 Statistical results of the correlation between running distance and performance in the FST of exercise rats (n=7)

Dialysate

The running distance of those EX rats that underwent microdialysis is shown in Figure 1-4 (blue and green).

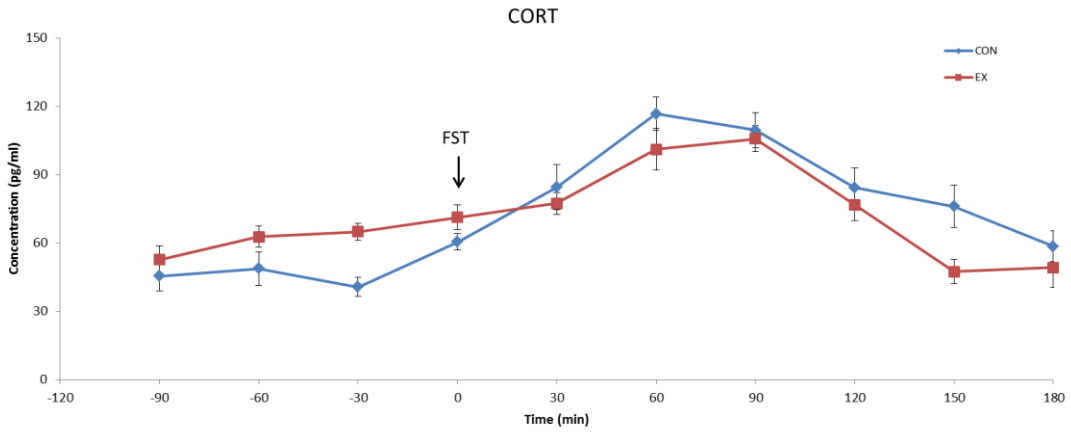
The results of CORT at baseline and after FST are shown in Figure 1-6a. Two-way ANOVA with repeated measures showed a main effect of time (F=27.341, p<0.001), time*group interaction (F=3.396, p=0.001), but not group (F=0.083, p=0.779). The time*group interaction suggests that the two groups may have responded differently to the FST (a brief look at the figure confirms this suggestion). Thus we re-analyzed the CORT data at baseline (from -90 min to 0 min) and following FST (from 0 min to 180 min). Two-way ANOVA with repeated measures analysis of CORT at baseline revealed a main effect of time (F=5.041, p=0.006), group (F=8.005, p=0.018), and time*group interaction (F=3.396, p=0.001), suggesting that EX group had significantly higher basal CORT. Further, two-way ANOVA with repeated measures analysis of CORT after FST revealed a main effect of time (F=27.041, p<0.001) and a trend toward significant effect of time*group interaction (F=2.050, p=0.073), but not group (F=1.600, p=0.235). Post hoc

analysis demonstrated a significant effect at 150 min (t=2.680, p=0.023). That is to say, after the FST, although the absolute peak concentration of CORT was similar between EX and CON groups, CORT in EX rats returned to a lower level earlier than CON rats.

To gain a better image of the difference between groups regarding the FST-responsive CORT, we then transformed the absolute concentration data to percentage changes from the baseline (0 min, Figure 1-6b) and performed the statistical analysis again. Two-way ANOVA with repeated measures analysis of % CORT following FST revealed a main effect of time ($F=23.351$, $p<0.001$) and group ($F=12.177$, $p=0.006$) but not time*group interaction ($F=1.803$, $p=0.114$), suggesting that EX group had significantly lower CORT response following FST than CON group.

In the meantime, replicating the above results, comparison of the AUC showed that EX group had higher AUC at baseline ($t=3.116$, $p=0.011$) but lower AUC after the FST ($t=2.484$, $p= 0.032$) than CON group (Figure 1-6c). That is, EX group showed higher baseline level of CORT, but lower FST-responsive CORT compared to CON.

a



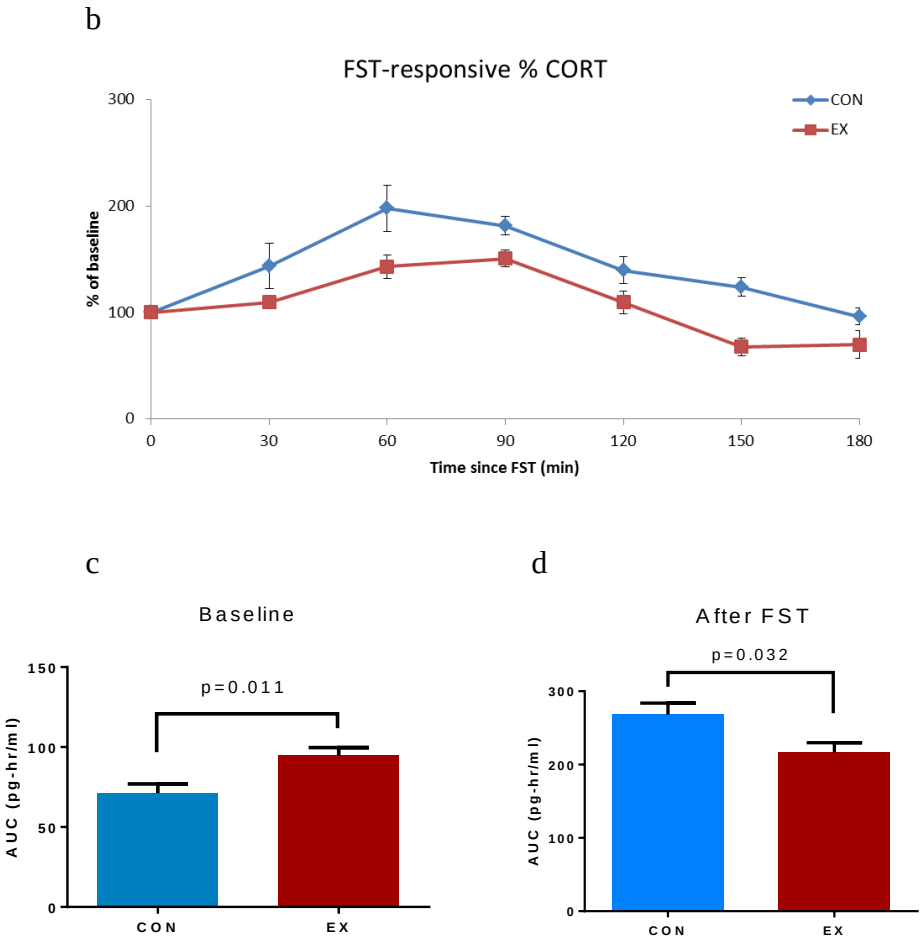
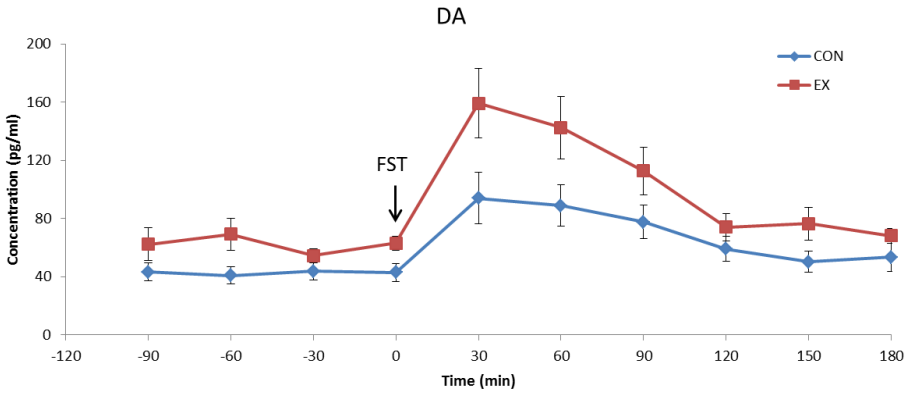


Figure 1-6 The effect of exercise on CORT in the mPFC. The effect of exercise on the concentrations of CORT (pg/ml) at baseline and following the FST (a). The effect of exercise on the FST-responsive CORT, as shown in % of baseline (b). Baseline was defined as the concentration of the last sample obtained before the FST (0 min). The effect of exercise on the AUC of CORT (pg*hr/ml) at baseline (c) and following the FST (d). (n=6/group) Mean±SEM.

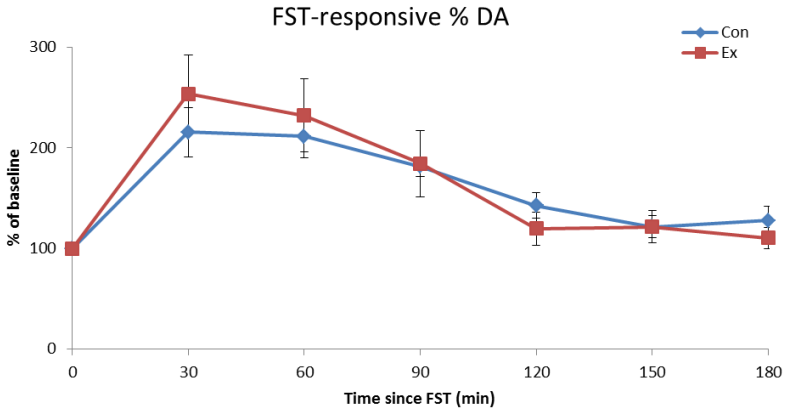
The result of DA is shown in Figure 1-7a. Two-way ANOVA with repeated measures analysis showed a main effect of both time ($F=18.981$, $p<0.001$) and group ($F=6.273$, $p=0.031$). DA was significantly higher after FST than that at baseline, and in EX group than CON group. The effect of time*group interaction was not significant ($F=1.901$, $p=0.149$), suggesting that the two group responded similarly to the FST (as confirmed

analysis of the % DA data, shown in Figure 1-8b). Comparison of AUC again suggests that EX group had higher level of DA both at baseline (t=-2.131, p=0.059; Figure 1-7c) and after FST (t=-2.382, p=0.038; Figure 1-7d) than CON group.

a



b



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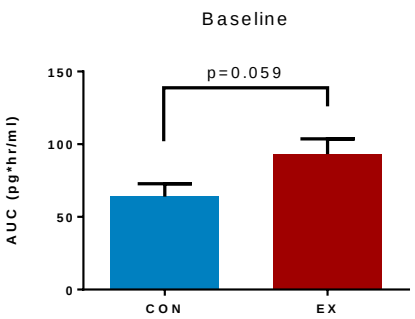

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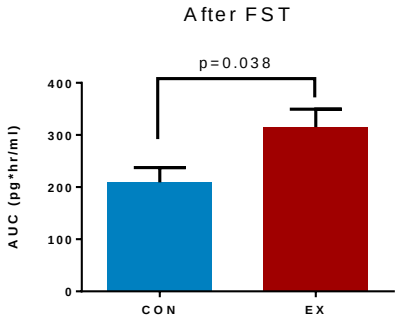
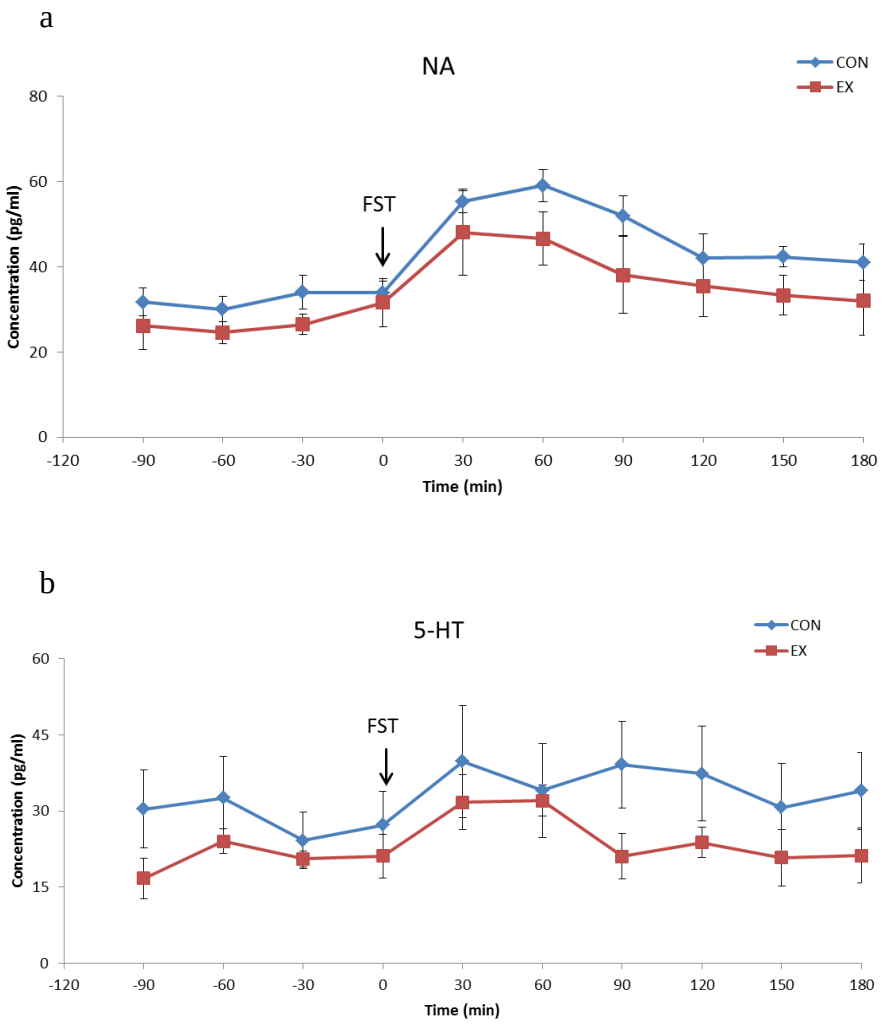


Figure 1-7 The effect of exercise on DA in the mPFC. The effect of exercise on the concentrations of DA (pg/ml) at baseline and following the FST (a). The effect of exercise on the FST-responsive DA, as shown in % of baseline (b). Baseline was defined as the concentration of the last sample obtained before the FST (0 min). The effect of exercise on the AUC of DA (pg*hr/ml) at baseline (c) and following the FST (d). (n=6/group) Mean±SEM.

The results of other neurotransmitters are shown in Figure 1-8. Statistical analysis (as shown in Table 1-4) suggests that there was only significant effect of time with regard to NA, 5-HT, and alanine, without any other effect.



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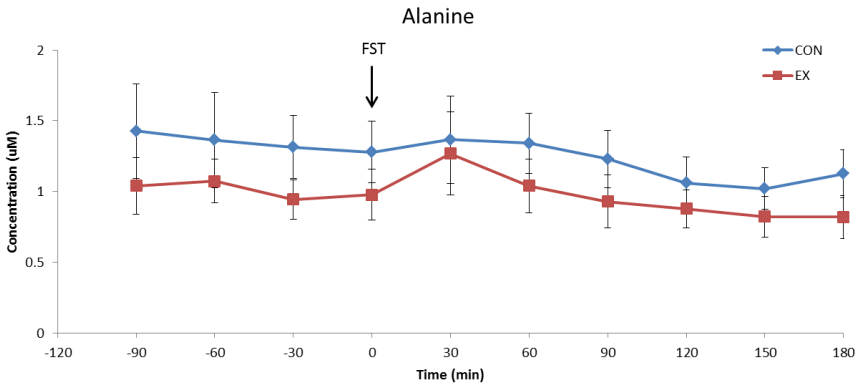
Alanine
FST
CON
EX
Concentration (uM)
Time (min)
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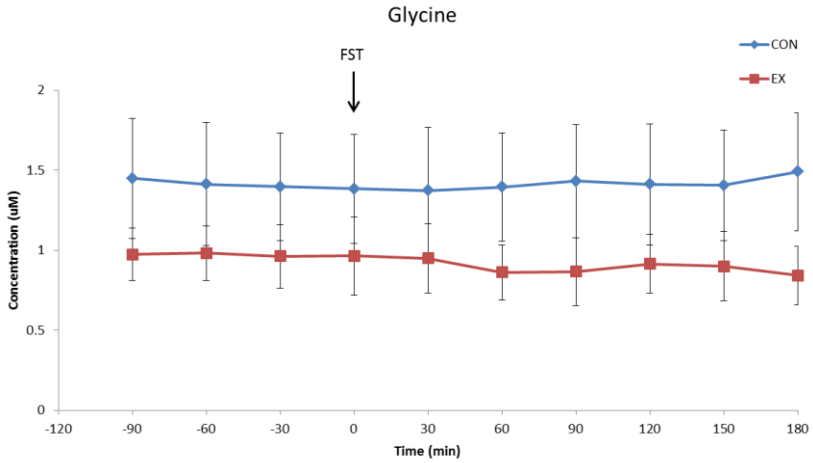
1.5
1
0.5
0
-120
-90
-60
-30
0
30
60
90
120
150
180

d


Glycine
FST
CON
EX
Concentration (uM)
Time (min)
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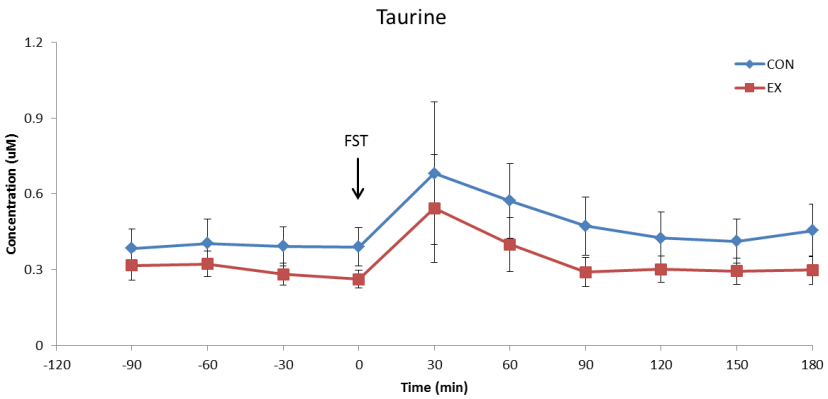
1
0.5
0
-120
-90
-60
-30
0
30
60
90
120
150
180

e


Taurine
FST
CON
EX
Concentration (uM)
Time (min)
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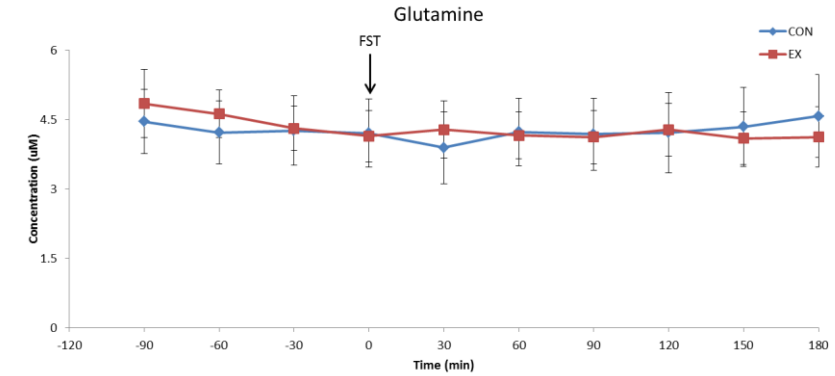
0.9
0.6
0.3
0
-120
-90
-60
-30
0
30
60
90
120
150
180

f



g

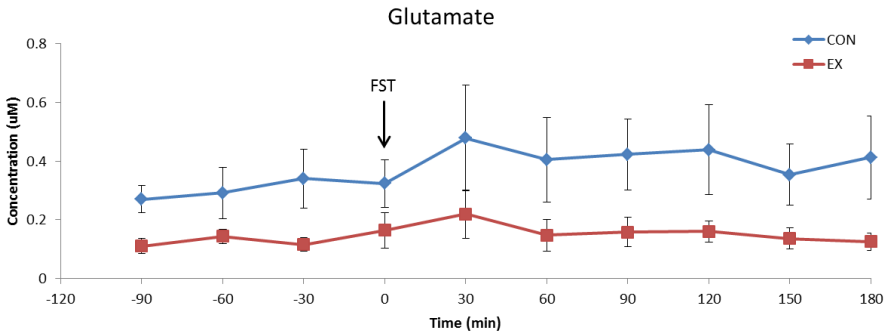


Figure 1-8 The effect of exercise on the concentrations of NA (a), 5-HT (b), alanine (c), glycine (d), taurine (e), glutamine (f), and glutamate (g) at baseline and following the FST. (n=6/group for NA and 5-HT; n=7/group for alanine and glutamine; CON n=7, EX n=6 for glycine and taurine; CON n=6, EX n=7 for glutamate) Mean±SEM.

	Time	Group	Time*Group
NA	F=13.794, p<0.001	F=1.728, p=0.218	F=0.479, p=0.885
5-HT	F=2.789, p=0.006	F=1.463, p=0.254	F=0.931, p=0.503
Alanine	F=3.229, p=0.044	F=1.018, p=0.333	F=0.376, p=0.736
Glycine	F=0.271, p=0.823	F=1.313, p=0.276	F=0.646, p=0.574
Taurine	F=2.937, p=0.110	F=0.886, p=0.367	F=0.126, p=0.753
Glutamine	F=1.398, p=0.226	F=0.002, p=0.968	F=1.066, p=0.363
Glutamate	F=2.273, p=0.118	F=3.656, p=0.082	F=0.789, p=0.464

Table 1-4 Statistical results of other neurotransmitters, including NA, 5-HT, alanine, glycine, taurine, glutamine, and glutamate, by two-way ANOVA with repeated measures. (sample size as reported in Figure 1-8)

Western blot

There was no difference regarding the protein expression density of GR (t=0.241, p=0.816), D1R (t=0.021, p=0.984), D2R (t=0.070, p=0.945), or 5-HT1AR (t=0.152, p=0.884), as shown in Figure 1-10. Due to its low level of expression in the mPFC [68], we failed to reliably detect MR in our samples.

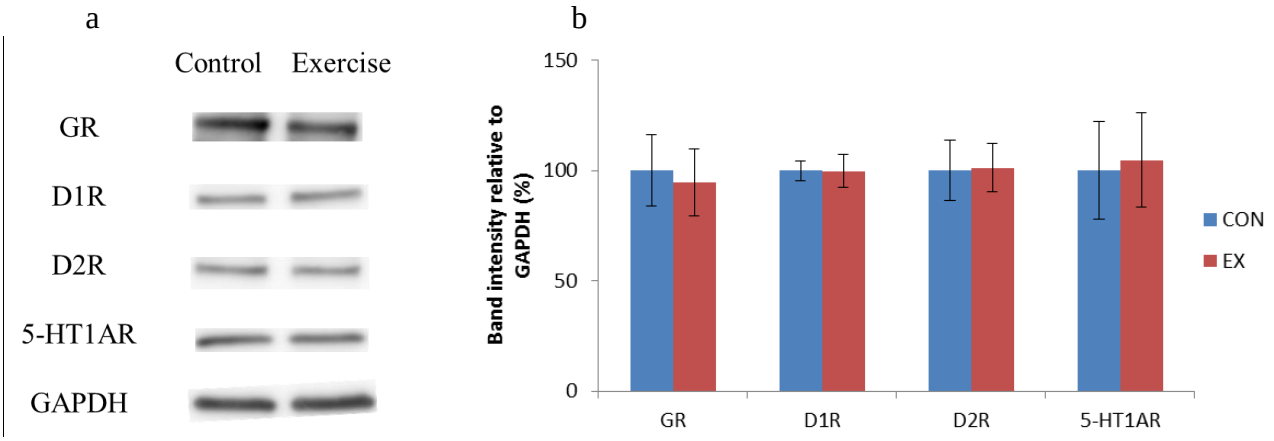


Figure 1-10 The effect of exercise on the protein expression of GR, D1R, D2R, 5-HT1AR, and GAPDH in the mPFC. Representative western blot bands for each protein (a). Relative band intensity was normalized for GAPDH and expressed as a percentage compared with the value of CON (b). (n=5/group for GR; n=6/group for D1R and D2R; n=4/group for 5-HT1AR) Mean±SEM.

1.4 Discussion

In summary, the first experiment showed three weeks of voluntary wheel running reduced immobility in the FST, suggesting stress coping or antidepressant-like effect. This is in line with a dozen previous reports that wheel running exerts antidepressant-like effect as assessed by the FST [69-74], shuttle box escape deficit [72, 75-77], and chronic mild stress [25, 69]. Also consistent with previous studies [6], we observed that the antidepressant-like effect of

wheel running did not seem to depend on running distances. More interestingly, we found that this antidepressant-like effect of wheel running was accompanied by overall upregulated DA in the mPFC. EX rats also showed higher basal CORT but overall lower FST-responsive CORT. The DA and CORT receptors remained unchanged. There was no significant effect of exercise on NA, 5-HT, glutamate, glutamine, glycine, taurine, or alanine.

Exercise, basal CORT, and GR

Several previous studies have reported that chronic wheel running, typically 2-4 weeks, increases basal CORT in plasma, in the morning (around the onset of light phase) [12] and/or evening (around the onset of dark phase) [11, 13, 78], but not midnight (amid the dark phase) [11, 13, 78], although some studies failed to find such difference [79, 80]. It is likely that the duration of wheel running and species may modulate the result. To our knowledge, merely one study has examined the effect of exercise on basal CORT level in the brain, i.e. extracellular fluids [62]. Microdialysis study has shown that CORT within different areas of the brain, hippocampus and striatum, show a similar pattern of change in response to stress, which assembles but is roughly 20 min delayed to that in plasma [61], and that CORT within the amygdala fluctuates in accordance to systematic administration of CORT [64]. Replicating plasma research, Droste et al [62] found that 4 but not 1 week of wheel running significantly increased basal hippocampal CORT (+42%) between 1500 and 2100 h but not between 0900 and 1500 h (0500h lights on, 1900 lights off). In our experiment, we further extended the above results by showing that basal CORT in the mPFC is also upregulated 33% (as calculated from the AUC at baseline) by 3 weeks of wheel running, although we only examined basal CORT between 17:00-19:00, i.e., during the two hours before the onset of dark phase. In the meantime, we found that the upregulated CORT level in the mPFC by wheel running was not accompanied by any change of GR expression.

Although both chronic stress and exercise upregulate basal CORT, their influence on CORT receptors, MR and GR, seems to be different. Accompanying the upregulated basal CORT by chronic stress and in depression, decreased MR and GR have been consistently

reported. For instance, compared to non-depressed subjects postmortem analysis of brains of MDD patients showed a decrease in MR mRNA expression in the hippocampus, inferior frontal gyrus, and cingulate gyrus [81]. Indeed, increasing activity or expression of brain MRs may prevent or reverse symptoms of stress-related depression, and individuals with a relatively low MR functionality may possess an increased stress susceptibility for depression [20, 82]. Equally important, downregulation of GR in the brain, especially the hippocampus by chronic stress has been proposed to be a most important pathology of depression [83, 84]. Mice with selective genetic depletion of forebrain GR receptor show robust depression-like phenotype [85]. The therapeutic effect of antidepressants is also believed to be at least partially mediated by restoring the GR function [83, 84].

In contrast to the downregulated MR and GR by chronic stress, MR and especially GR are not reduced but even increased by exercise. To our knowledge to date only one study measured GR in the mPFC (infralimbic cortex), which failed to find any difference between controls and rats subjected to 4 weeks of wheel running [86]. The same study also found increased GR in dorsal but not ventral hippocampus. Several other studies reported either an increase [87] or no change [88, 89] of GR in the hippocampus of wheel running rats or mice. With regard to MR, Droste et al [11] reported a 38% decrease of MR within hippocampus in exercise mice, which the authors failed to replicate in their later study [86].

Thus based on these observations, it is tempting to conclude that whereas both chronic stress and exercise increases CORT, chronic stress downregulates while exercise either upregulate or does not affect GR. Thus, GR may be one explanation to the exercise-CORT paradox, which deserves future investigation. We will return to this topic later in *General discussion and conclusion*.

Exercise and stress-responsive CORT

We also observed that following the FST, exercise and control rats showed similar absolute concentration of peak CORT but CORT in exercise rats decreased to a lower level earlier than control rats thus resulting in overall less exposure to CORT. When considering

the data from percentage changes compared to baseline, exercise rats showed significantly lower FST-responsive CORT.

Previous studies have suggested that CORT response to stress is somewhat time and intensity specific. For low intensity acute stress, several week of wheel running results in either the same (elevated plus maze [90]) or lower (novel environment [11, 86, 91, 92]) CORT response in plasma immediately following the stress session, but lower CORT 30 min later (saline injection [93]). For moderate to strong acute stress, wheel running may lead to the same (electric shock [92, 93]; predator odour, restraint [92]) or higher (FST [11, 86]; social conflict [94]; restraint [11, 91]) CORT response immediately following the stress session, and the same CORT response 30 min post (electric shock, [92, 93]). The seemingly mixed results can be partly accounted for by the measurement method used by these studies, namely, they all made a single measurement at one time point. The big picture might be different. Indeed, to date two studies have followed the time course change of stress-responsive CORT. Fediuc et al [88] used a 20 min restraint stress and measured CORT at 0, 5, 10, 20, 30, 40, 55, 70, 85, 100, 115 min after the onset of the stress. They found no difference of CORT (absolute concentration) between control and exercise rats that have ran 5 weeks in a running wheel (although a closer look at the time course curve suggests that exercise rats show a trend toward peaked earlier and decayed more rapidly). Using a 30min restraint stress, Hare et al [80] found higher CORT (absolute concentration) 20 min following the onset of the stress but lower CORT 90 min following the end of the stress. Further analysis showed that the higher CORT at 20 min in exercise rats was because CORT peaked earlier in these rats. The peak of CORT following the stress was actually similar between exercise and control rats. This is also consistent with and may well explain the above seemingly contradictory findings. Similar to Hare et al [79], we also found the same absolute peak concentration of CORT following FST but earlier decaying in the mPFC of exercise rats, leading to overall less exposure to CORT. These results suggest that exercise may exert its beneficial effect by buffer the overall CORT exposure to stress. In line with this, studies using chronic stress paradigms consistently found a lower CORT in plasma in exercise rats after the stress,

whether it's 30 days of predictable or unpredictable electrical shocks [94], 4 weeks chronic unpredictable stress [25], or 11 days of 98-dB noise [95].

Let's briefly return to the exercise-CORT paradox for a moment. We've listed evidence showing that both exercise and chronic mild stress increase basal CORT in the Introduction. Here we further discussed that exercise buffers stress-responsive CORT. Interestingly, it has also been found that various forms of chronic or repeated stress increase overall CORT exposure to subsequent novel stress [24, 31, 96] (for a review [21]), which is attenuated by chronic antidepressant treatment [97]. Elevated stress-responsive CORT has been linked to passive coping behavior, such as staying immobile when confronted with inescapable electric shock [98]. On the other hand, patients suffering from depression have hyper-responsive CORT in the dexamethasone/CRH test [42, 99] and demonstrate higher CORT during the recovery period after stress [40], which are also normalized after successful antidepressant treatment [20, 82]. Actually, excessive stress-responsive HPA especially CORT activity has been proposed as a biological endophenotype for depression [100]. These outcomes again are in sharp contrast to that of exercise observed in our current experiment. Thus the effect of buffering overall CORT exposure to novel stress may be another mechanism by which exercise achieves its beneficial outcomes, which will be reviewed again in *General discussion and conclusion.*

DA in the mPFC

Among the neurotransmitters we measured, the only significant changed was DA, which was upregulated by wheel running, consistent with a previous report showing that four weeks of wheel running increases DA in the cortex in mice as measured in the brain tissue [101]. But we found no effect of exercise on the protein expression of D1R or D2R, although previous research has reported increased striatal D2R density by treadmill running [102], reduced D2R mRNA in the nucleus accumbens (Nacc) core by 6 weeks of wheel running [103], increased expression of D2R in the hippocampus [104] or increased D2 autoreceptor mRNA in the midbrain and D2R postsynaptic mRNA in the striatum [105] of

selective bred high running rats or mice. Anyway, the upregulated DA level in the mPFC itself deserves further attention.

Recently the DA system has been attracting more and more attention in the context of stress and depression [106, 107]. At least three lines of research argue for a positive role of mPFC DA in the antidepressant-like effect. First, it has been consistently reported that various models of stress and depression, including chronic mild stress [108-111], chronic restraint [112, 113], chronic water bath [114], chronic cold [115], chronic social isolation [116], chronic unavoidable footshock [113], adolescent social defeat [117-120], and maternal separation [121] all decrease DA in the mPFC. In the meantime, various antidepressant treatment, such as tricyclic antidepressants [122-124], selective 5-HT re-uptake inhibitors (SSRIs, [122, 124-126]), 5-HT agonist [124, 127], 5-HT-NA reuptake inhibitors [128, 129], atypical antidepressant mianserin [130], combination of atypical antipsychotic with SSRIs [131-133], or deep brain stimulation of mPFC (infralimbic) [134] increase DA in the mPFC.

Second, acute treatment with a cannabinoid receptor 1 antagonist [135] or a triple monoamine uptake inhibitor [136], or chronic antidepressant treatment with reboxetine and mirtazapine [137] or a Chinese herbal prescription [110], concurrently increases DA in the mPFC and decreases animal's immobility in the FST. More importantly, several studies found a correlation between DA levels in the anterior cortex [138], prefrontal cortex (PFC) [139] , or mPFC [140], and reduced immobility in the FST [138], increased active escape behavior [139], or the efficacy of antidepressants in reducing immobility in the FST [140].

Third, in the literature of decision making and reinforcement learning, DA in mPFC has been proposed to be associated with effortful behavior. Human PET imaging studies reported a correlation between DA function within ventromedial and ventrolateral PFC and the willingness to expand effort for larger rewards, particularly when the probability of reward receipt was low [141]. In rats, D1 and/or D2 antagonist microinjected into the mPFC (orbitofrontal cortex) [142, 143], D1 antagonist microinjected into the anterior cingulate cortex (ACC) [144], or ACC DA depletion [145], leads to decreased effortful behavior and motivation. Of note, this kind of effortful behavior and motivation is dysregulated in depression [107].

Taken together, the evidence suggests that higher DA in the mPFC may account for the antidepressant-like effect of wheel running in our experiment. A second experiment as described in Chapter two will be devoted to test this hypothesis.

DA and CORT: what is the relationship?

While DA was overall upregulated both at baseline and after FST, CORT was increased at baseline but decreased following FST. What is the relationship between them?

On the one hand, the mPFC is believed to provide negative feedback regulation of the HPA axis [147, 148]. Lesions of the mPFC significantly increase plasma levels of both adrenocorticotropic hormone (ACTH) and CORT in response to a 20 min restraint stress [148], and MDD patients manifest significantly thinned mPFC and higher CORT simultaneously [36]. Interestingly, lesion of the mPFC does not affect basal ACTH and CORT [148, 149], suggesting that the mPFC selectively modulates stress-responsive HPA activity. More specific, DA D1/D2 antagonist injected into the mPFC enhances stress-induced increase of ACTH and CORT [150], suggesting that DA in the mPFC normally acts to suppress the HPA activity. Supporting this, research has indicated that the mPFC-GABAergic interneurons at the anterior bed nucleus of the stria terminalis-paraventricular hypothalamic nucleus may mediate this effect [151-153]. In line with this, we observed higher DA in exercise rats, together with overall reduced FST-responsive CORT. Of note, higher CORT within mPFC itself has also been reported to inhibit stress-responsive HPA activity, through an unclear mechanism. CORT locally delivered in to the mPFC rather than central amygdala of adrenalectomized rats decreases plasma ACTH response to acute restraint [154]. Further, this effect is likely to be GR-dependent, for GR knockdown in the mPFC (infralimbic and prelimbic) leads to hyper-responsive CORT to acute restraint in normal and chronically stressed rats [155]. Our results may provide a potential explanation to these reports.

On the other hand, suppression of CORT by adrenalectomy decreases DA in the mPFC, which is prevented by CORT replacement [156]. Local injection of CORT into the mPFC increases DA in this brain area, which is blocked by a GR antagonist [157]. Whereas blocking

GR locally within the mPFC results in attenuated stress-evoked glutamate in the ventral tegmental area (VTA) and DA in the mPFC, blocking glutamate receptors in the VTA also attenuates stress-evoked DA in the mPFC [158]. These results suggest that CORT may potentiate the mPFC glutamatergic input onto DA neurons in the VTA [158]. Prominently, an electron microscopic tract-tracing study demonstrated that VTA DA neurons that receive afferents from the mPFC preferentially project reciprocally to the PFC [159]. Besides, there is also evidence showing that CORT inhibits the presynaptic reuptake of DA which results in elevated DA level [160]. Thus, it seems that higher basal CORT plays an important role in maintaining high level of DA in the mPFC. To put it differently, the reason why exercise rats had higher DA in the mPFC was because they had higher CORT at the first place, since higher DA itself acts to suppress CORT as discussed above. Thus it leads us to propose a second hypothesis, that higher CORT induces higher DA in exercise rats, possibly through a GR mechanism. This will be tested in a third experiment as described in Chapter three.

To make the argument one step further and much clearer, the stress-responsive CORT itself is adaptive and essential for coping with the situation [16], and it may do so in a DA-dependent way. Namely, upon stress, CORT is released into the blood and eventually reaches the mPFC to potentiate the glutamatergic input onto DA neurons in the VTA [158], which releases DA into the mPFC to achieve subsequent antidepressant-like effect. However, as CORT in the brain is roughly 20 min delayed to that in plasma [61], the animals with higher basal CORT and DA in the mPFC will stand in a beneficial position to cope with stress of short duration, for instance, the 5 min FST used in the present study.

Beyond CORT and DA: other mechanisms responsible for the upregulated DA in the mPFC

There might be other mechanisms by which DA is upregulated in the mPFC by exercise, for instance, increased DA release from the VTA because of increased DA neurons, and/or decreased DA reuptake in the mPFC (for a review of the DA metabolism and neurotransmission [107]). Although to our knowledge to date no study has directly examined these effects, existing evidence suggests that these might be possible.

It has been shown that 5 days of treadmill running [161] and 6 weeks of wheel running [103] increase the mRNA expression of tyrosine hydroxylase (the rate limiting enzyme in DA synthesis, therefore a marker of DA neurons) in the VTA by 80% and 100%, respectively. This has been linked to higher level of serum calcium induced by exercise, which enters into the brain and influences calcium/calmodulin-dependent DA synthesis by activating the tyrosine hydroxylase enzyme [162]. In the meantime, exercise has been consistently demonstrated to increase BDNF, IGF-1 and other neurotrophic factors, which are known to promote the survival of DA neurons [10, 163]. The increased DA synthesis or DA neurons in the VTA may lead to increased DA release in the mPFC.

Further, there are reports that treadmill running [164] or an enriched environment (a combination of wheel running, novel objects and social interactions etc.) [165, 166] reduces the mRNA and/or protein expression of DA transporter (DAT) in the striatum (or midbrain nigrostriatal neurons for the mRNA). In the meantime, a human PET imaging study showed that a 50 min walking exercise reduces DAT availability in the striatum in normal subjects and in the striatum and mPFC of patients with Parkinson's disease [167]. It remains for future study to investigate whether exercise may actually increase DA release and decrease DAT in the mPFC.

Other neurotransmitters

5-HT [5, 6] and NA [168] have also been proposed to mediate the beneficial effect of exercise. Although previous research has reported that chronic wheel running increases basal level of 5-HT in the blood [169], increases basal level of NA in the locus coeruleus and dorsal raphe nucleus (DRN) [74], blunts the release of NA in the PFC in response to electric foot shock [170], and increase 5-HT1A [76] and 5-HT1B [105] autoreceptor mRNA in the DRN, we did not observe any significant effect of exercise on them in the mPFC in our experiment. Another study also failed to find any influence by four weeks of wheel running on the 5-HT and NA level in the cortex as measured in the brain tissue [101]. It is possible that the duration of wheel running (9-12 weeks [74], 6 weeks [76]; 4-5 weeks [170], while 3 weeks in our experiment), type of stress (electric foot shock was used by [170], while FST by us), and

method of measuring (microdissected brain regions were assayed for concentrations in [74], while microdialysis dialysate was used by [170] and us) may contribute to the difference. Indeed, Greenwood et al [171] found that the central 5-HT system is sensitive to exercise in a time-dependent manner: 6 but not 3 weeks of wheel running increases 5-HT1A mRNA in the DRN.

Anyway, it is likely that 5-HT, NA and DA may all be involved in the antidepressant-like effect of exercise, but the precise brain area where they function may be different. For instance, Cunha et al [72] reported that three weeks of wheel running reduces immobility in the FST in mice (without affecting locomotion), which is blocked by pretreatment of an inhibitor of 5-HT synthesis or an inhibitor of NA and DA synthesis. Another recent report found that the antidepressant-like effect of wheel running in the FST is absent in 5-HT3R knockout mice, which also lacks the running induced hippocampal neurogenesis [73]. It remains for future study to determine when exercise exerts beneficial effects through a/several specific neurotransmitter(s) rather than others, and what brain regions does each neurotransmitter actually and precisely target to achieve these effects.

In conclusion, voluntary wheel running exerts antidepressant-like effect in the FST in rats, and upregulates DA in the mPFC, increases basal CORT while blunts FST-responsive CORT, without changing D1R, D2R, or GR. Based on the literature, we hypothesize that elevated DA level in the mPFC may be a mechanism by which exercise achieves the antidepressant-like effect, and that the elevated DA level is due to increased basal CORT. In the following we are going to subject these hypotheses to experimental investigation.

Chapter 2 Exercise exerts antidepressant-like effect in a D2R-dependent manner in the medial prefrontal cortex

2.1 Introduction

As discussed in Chapter one, based on the literature, we hypothesized that upregulated DA in the mPFC may account for the antidepressant-like effect of wheel running. Thus we performed a microinjection experiment to test this hypothesis. Briefly speaking, we subjected another group of rats to the FST, before which DA D1R antagonist (SCH23390) or D2R antagonist (haloperidol) was locally delivered into the mPFC by microinjection. If, for instance, the antidepressant-like effect of wheel running is blocked by a specific receptor antagonist or both antagonists, we can assume that wheel running exerts the antidepressant-like effect through that specific receptor or both receptors. This will support our hypothesis. If, however, both antagonists fail to block the antidepressant-like effect of wheel running, then it suggests that wheel running may exerts its antidepressant-like effect through other mechanism(s) other than DA.

2.2 Methods

Animals and procedure

Another group of rats underwent exactly the same procedure as described in Chapter one except that a guide cannulae for bilateral microinjection rather than for unilateral microdialysis was implanted toward the mPFC after two weeks of wheel running (for EX) or no wheel running (for CON), and that after one more week (thus totally three weeks of wheel running for EX, the same as in chapter one) a 33-gauge injection cannulae, projecting 1.0 mm beyond the tips of the guide cannulae (Eicom) was inserted. The injection cannulae was connected by polyethylene tubing to motor-driven microsyringes. The exact placement of the injector cannulae tips was verified the next day during dissection using a microscope (see Figure 2-1). EX rats were injected a D1R or D2R antagonist while CON rats only the vehicle. The solution (0.5 µl) was infused through each injector at a rate of 0.25 µl/min for 2 min at 25-30 min (27.23±0.33) before the day-2 FST

(one EX and one CON rats were always infused at the same time). The injection cannulae was left in position for an additional 60 s after drug infusion. All drug solutions were freshly prepared immediately before each experiment.

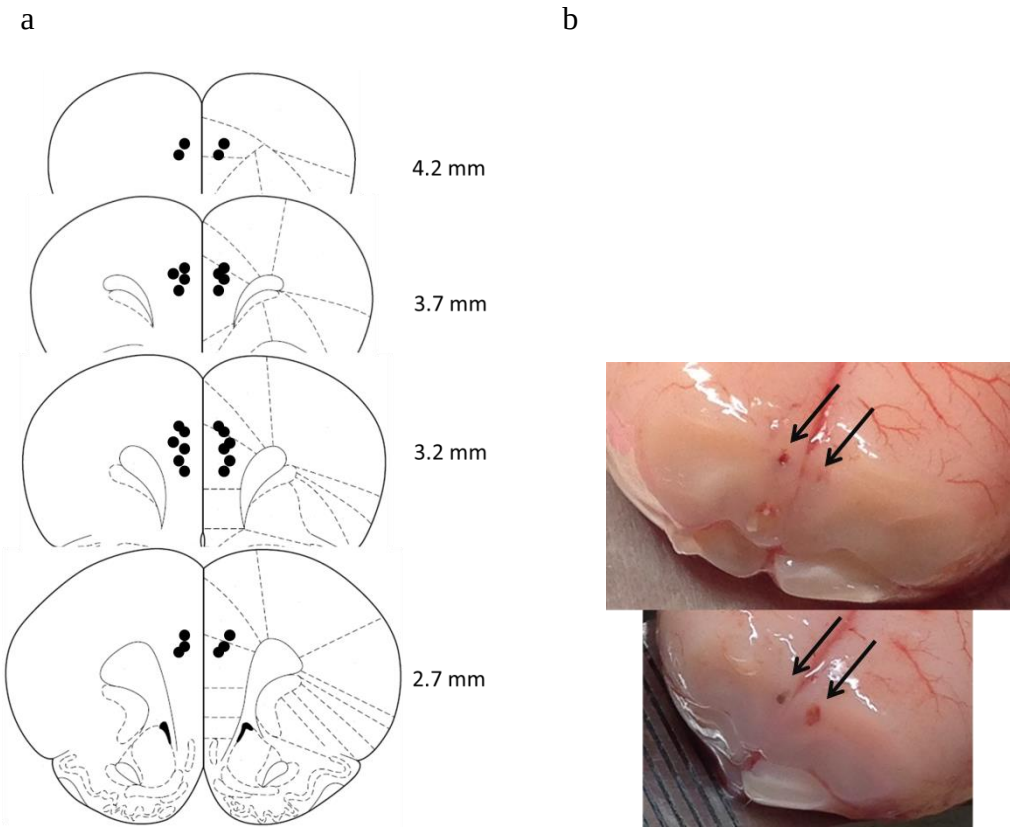


Figure 2-1 The placement of the injector cannulae tips. a, Schematic representation of bilateral placement of the injector cannulae tips; b, Actual photo taken from rats' brain during dissection showing the placement of injector cannulae tips.

Drugs

The D1R antagonist SCH 23390 (dissolved in saline, Cayman) and D2R antagonist haloperidol (dissolved in DMSO, Cayman) were used. The doses for each drug were determined by a pilot experiment below.

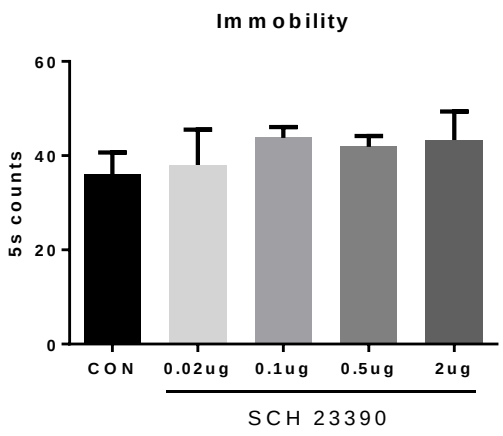
Analysis of the FST results

The procedure for the FST was completely the same as described in Chapter one for the FST. Climbing, swimming and immobility were scored using the frequency of each behavior over 5-s intervals during the day-2 5-min test session.

Pilot experiment

Another group of rats underwent a procedure exactly the same to the control group above. 25-30 min before the day-2 FST, each rat was randomly injected SCH 23390 or haloperidol with the following dose: for SCH 23390, 0 ug (0 uM), 0.02 ug (55.6 uM), 0.1 ug (278 uM), 0.5 ug (1390 uM), 2.0 (5560 uM); for haloperidol, 0 ug (0 uM), 0.02 ug (106.4 uM), 0.05 ug (266 uM), 0.1 ug (532 uM), 0.5 ug (2660 uM). Their performance in the FST was then compared, the results of which are shown below in Figure 2-2 and 2-3, respectively. For SCH 23390, one-way ANOVA analysis showed that there was no difference regarding immobility (F=0.658, p=0.629), swimming (F=0.751, p=0.571) or climbing (F=1.107, p=0.384) between any doses. Since 2 ug (5560 uM) is almost the highest dose used in the literature using microinjection, we chose to use this dose for the experiment.

a



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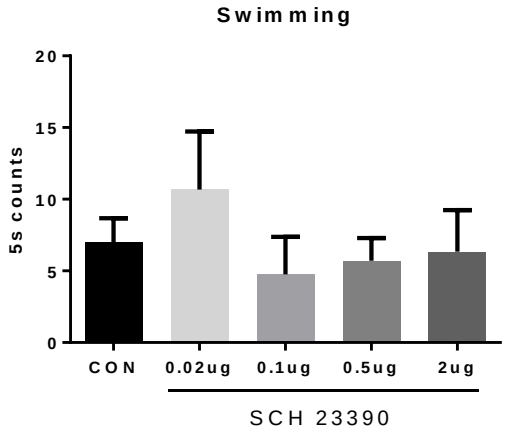

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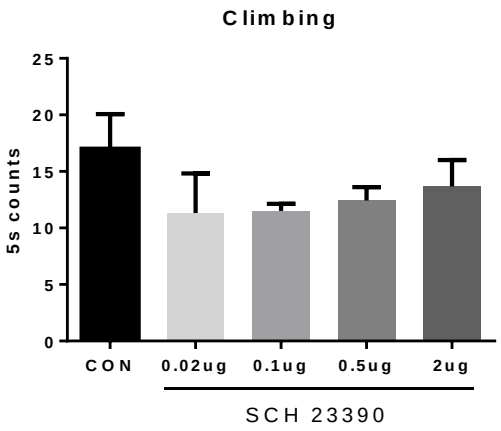


Figure 2-2 The effect of pre-intra-mPFC microinjection of SCH23390 on depression-like behavior. (a-c) The FST for assessing depression-like behavior. The duration of immobility (a), swimming (b), and climbing (c) in the 5 min FST for CON and different doses of SCH23390. (n=3-7/group) Mean±SEM.

As for haloperidol (Figure 2-3), one-way ANOVA analysis showed that there was significant effect of immobility ($F=5.903$, $p=0.004$), swimming ($F=5.417$, $p=0.005$), and climbing ($F=3.473$, $p=0.030$). Post hoc comparison showed that 0.5 ug significantly increased immobility while both 0.1 and 0.5 ug reduced swimming compared to control group. Thus we chose the dose 0.05 ug (266 uM) which does not affect rats' performance in the FST for our experiment.

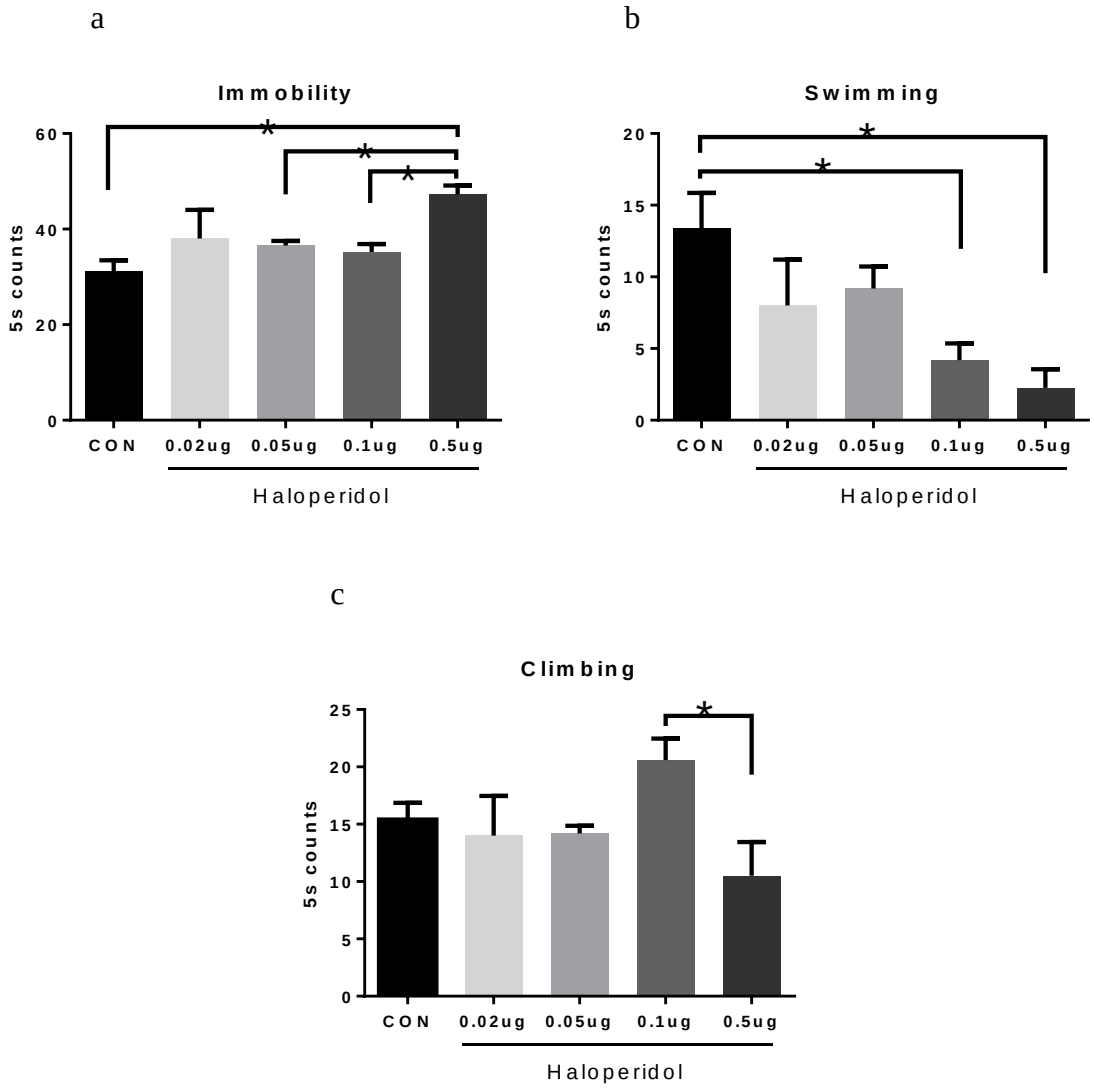


Figure 2-3 The dose-dependent effect of pre-intra-mPFC microinjection of haloperidol on depression-like behavior. (a-c) The FST for assessing depression-like behavior. The duration of immobility (a), swimming (b), and climbing (c) in the 5 min FST for CON and different doses of haloperidol. (n=3-5/group) Mean±SEM.

Locomotion

When we performed the first experiment, our animal facility was under renewal and we were using a new facility where we could not use an apparatus with an infrared sensor that

detects thermal radiation from animals. Here during the second experiment we moved back to our previous animal facility and thus could use the apparatus to measure locomotion.

The polypropylene cage for each rat was placed under a sensor before 14:00 on day-2 of the FST. Measurements of locomotor activity using an apparatus with an infrared sensor that detects thermal radiation from animals (Supermex; Muromachi, Tokyo, Japan) began after a 2-hour habituation period, as described previously [172]. That is, in this experiment, the measurement lasted from 16:00 to 18:30 (-180 min to -30 min of the FST), after which rats went on to undergo the D1R antagonist SCH23390 microinjection procedure. Horizontal movements of the rats were digitized and stored in a computer every 5 min. For analysis we averaged all the data to a 30 min duration and a total sum.

2.3 Results

Running distance

The running distance of EX rats breed for D1R and D2R antagonist experiment is shown in Figure 2-4.

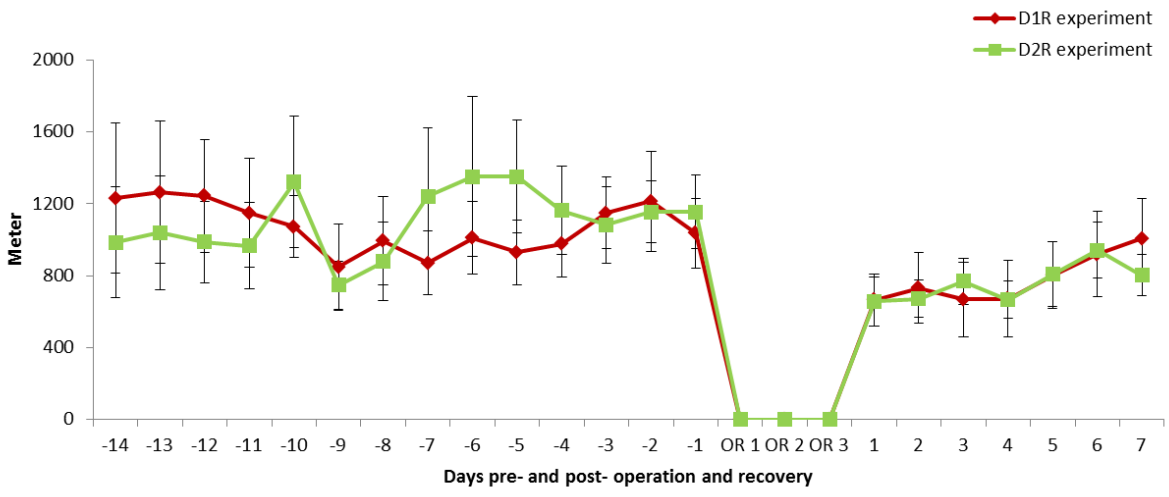
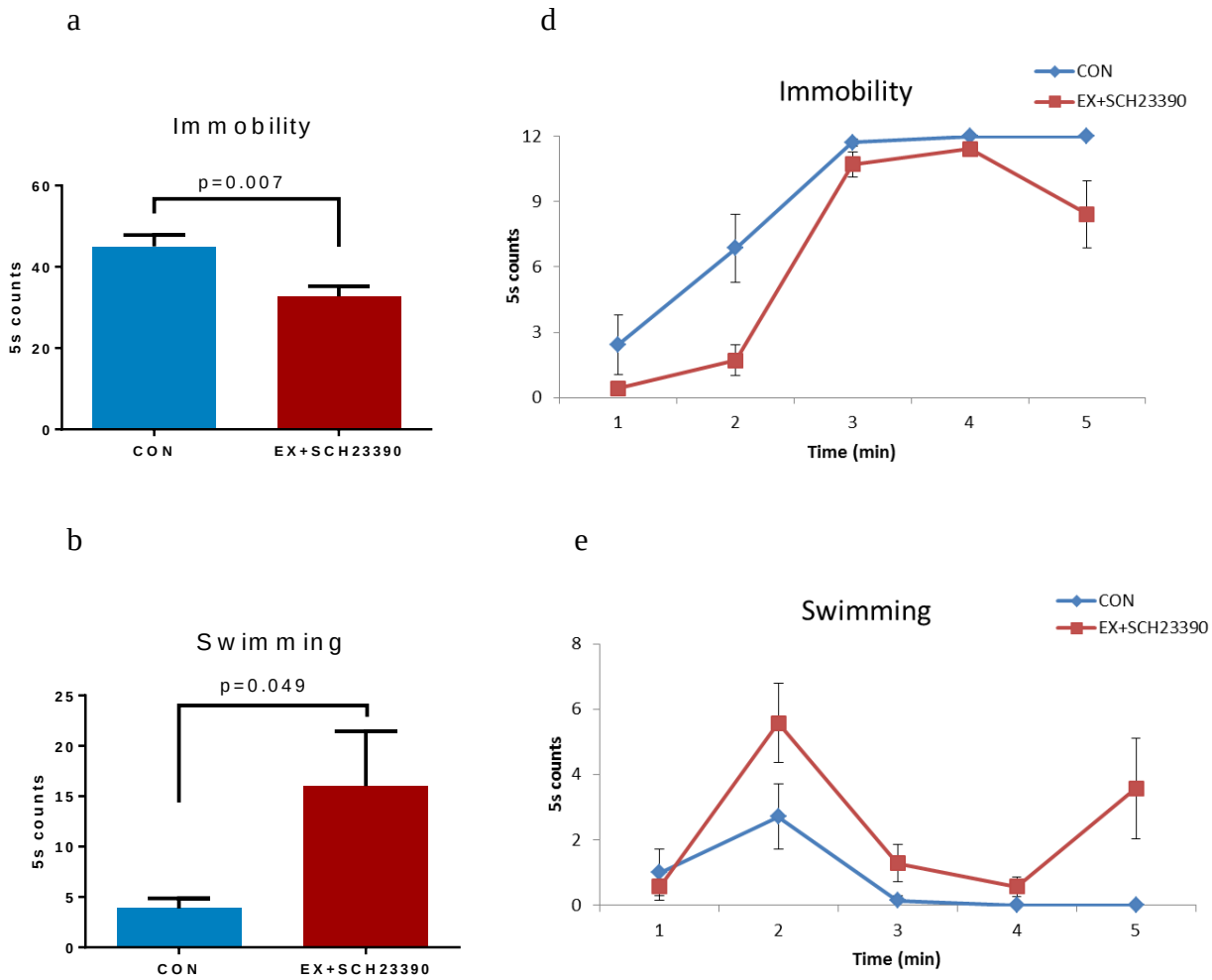


Figure 2-4 Mean running distance per day pre- and post- operation and recovery (OR) of rats for D1R (n=7) and D2R (n=6) antagonist experiment. Mean±SEM.

D1R and D2R antagonist and the antidepressant-like effect of wheel running

As shown in Figure 2-6 (a-c), bilateral intra-mPFC pre-microinjection of D1R antagonist SCH23390 into EX rats did not affect the antidepressant-like effect of exercise: immobility (t=3.247, p=0.007), swimming (t=2.190, p=0.049), climbing (t=1.597, p=0.136). EX rats still had lower immobility due to more swimming. Analysis of the FST data across the 5 min duration confirms these results (Figure 2-6 d-f, Table 2-1).



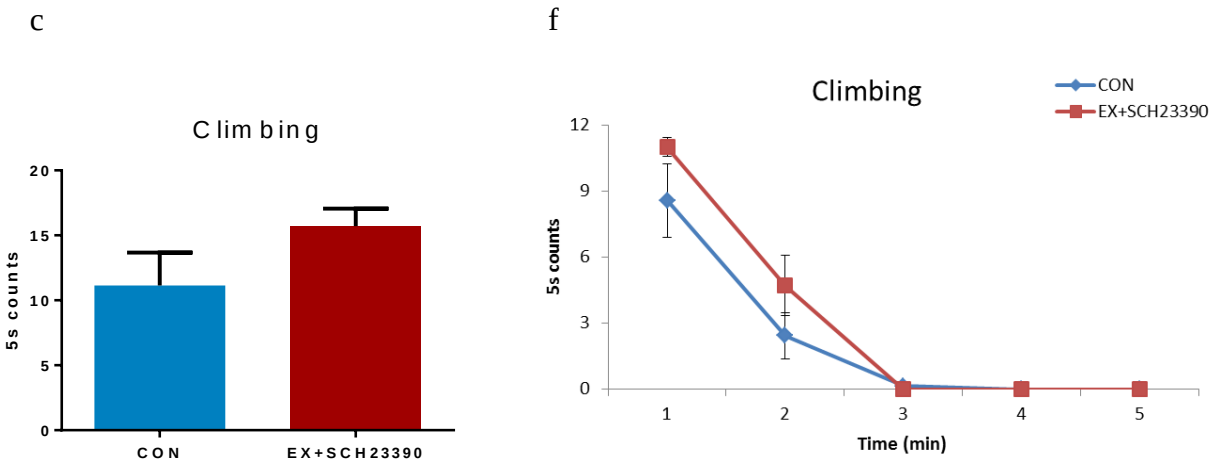


Figure 2-5 The antidepressant-like effect of exercise was not affected by pre-intra-mPFC microinjection of a D1R antagonist, SCH23390. (a-f) The FST for assessing antidepressant-like behavior. The duration of immobility, swimming, and climbing in the 5 min totally (a, b, c, in order) and across the 5 min (d, e, f, in order). (n=7/group) Mean±SEM.

	Time	Group	Time*Group
Immobility	F=70.716, p<0.001	F=10.541, p=0.007	F=2.957, p=0.064
Swimming	F=8.666, p=0.001	F=7.895, p=0.016	F=2.430, p=0.095
Climbing	F=67.304, p<0.001	F=2.549, p=0.136	F=1.617, p=0.220

Table 2-1 Statistical results of the FST data for D1R antagonist SCH23390 across the 5 min duration, by two-way ANOVA with repeated measures.

In contrast, same injection of D2R antagonist haloperidol completely abolished the antidepressant-like effect of exercise (Figure 2-6 a-c): immobility (t=0.261, p=0.800), swimming (t=0.532, p=0.606), climbing (t=0.199, p=0.847). There was no difference between EX (haloperidol injected) and CON rats regarding immobility, swimming and climbing across the 5 min duration (Figure 2-6 d-f, Table 2-2).

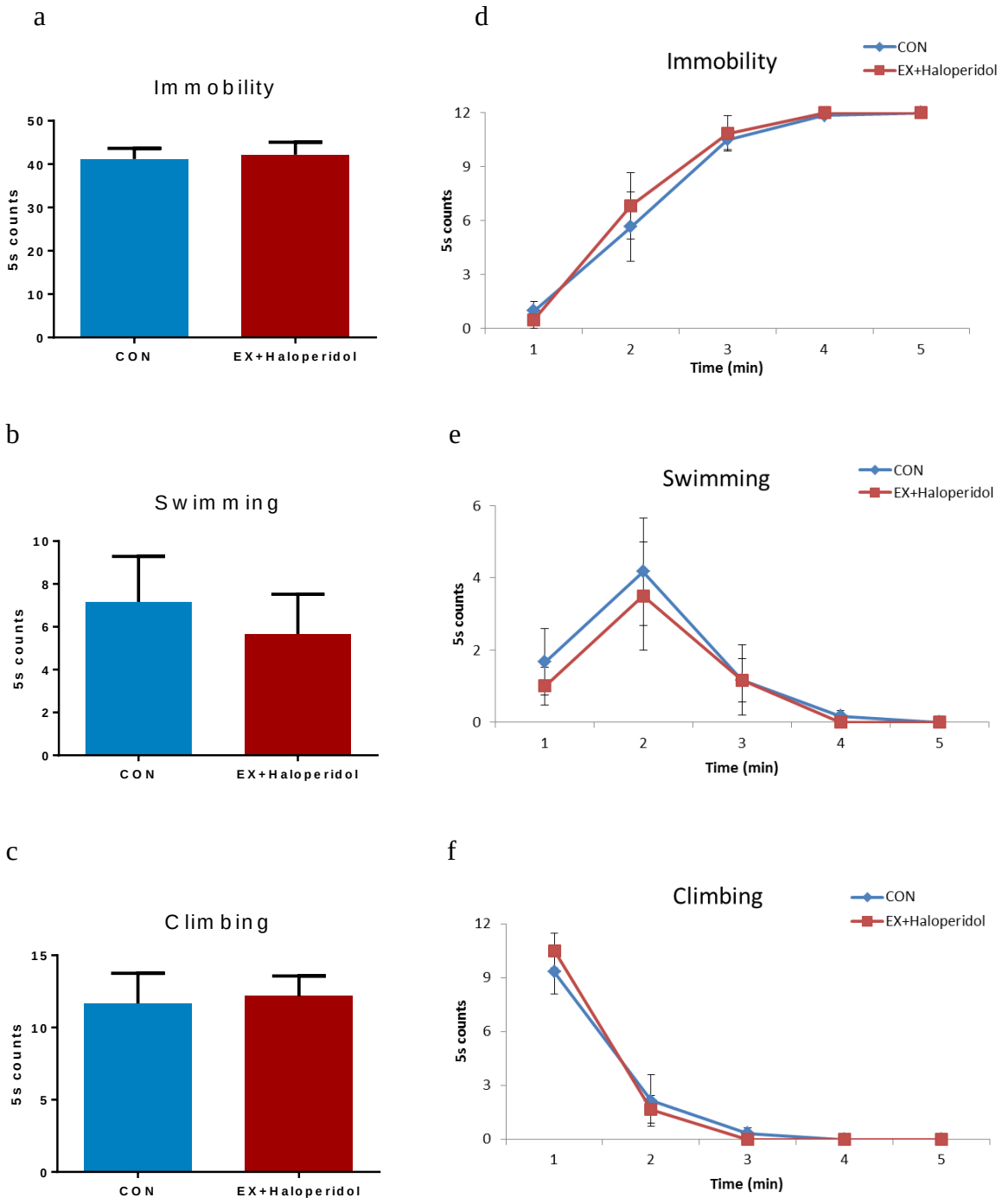


Figure 2-6 The antidepressant-like effect of exercise was completely abolished by pre-intra-mPFC microinjection of a D2R antagonist, haloperidol. (a-f) The FST for assessing antidepressant-like behavior. The duration of immobility, swimming, and climbing in the 5

min totally (a, b, c, in order) and across the 5 min (d, e, f, in order). (n=6/group) Mean±SEM.

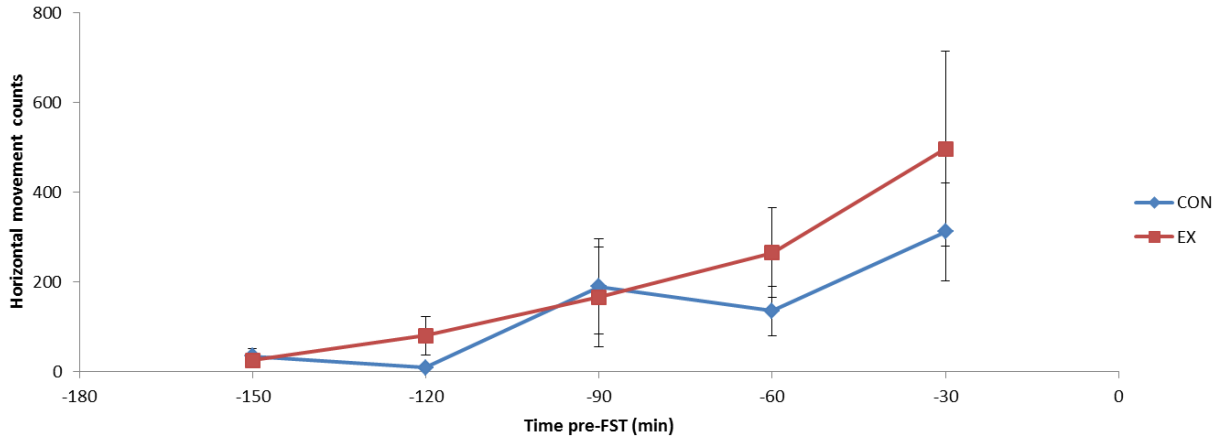
	Time	Group	Time*Group
Immobility	F=64.288, p<0.001	F=0.091, p=0.770	F=0.254, p=0.707
Swimming	F=7.180, p=0.006	F=0.283, p=0.606	F=0.087, p=0.903
Climbing	F=71.991, p<0.001	F=0.016, p=0.901	F=0.435, p=0.656

Table 2-2 Statistical results of the FST data for D2R antagonist haloperidol across the 5 min duration, by two-way ANOVA with repeated measures.

Locomotion

Two-way ANOVA with repeated measures analysis of the locomotion data from 150 min pre-FST to 30 min pre-FST (Figure 2-7a) showed a main effect of time (F=4.696, p=0.023) but not group (F=1.215, p=0.292) or time*group interaction (F=0.409, p=0.650). Nor was there any significant difference between groups regarding the total locomotion (t=-1.102, p=0.292; Figure 2-7b). These results suggest that wheel running did not affect the general motor activity of rats, even though exercise rats showed higher DA level during this time period as measured in the first experiment in Chapter one.

a



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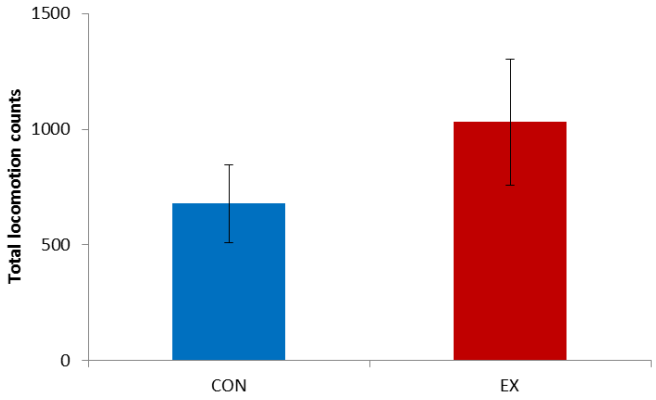


Figure 2-7 The effect of exercise on locomotion. The effect of exercise on horizontal movement counts by 30 min period (a) and in total (b) from -150 min to -30 min pre-FST. (n=7/group) Mean±SEM.

2.4 Discussion

In this experiment, employing the microinjection technique, we showed that bilateral intra-mPFC pre-microinjection of a D2R but not D1R antagonist, completely abolished the antidepressant-like effect of wheel running. Of note, D2R antagonist at this dose does not affect normal rats' performance in the FST. These results suggest that wheel running exerts the antidepressant-like effect through a D2R dependent pathway in the mPFC. Further, our pilot experiment showed that in normal rats, bilateral intra-mPFC pre-microinjection of a D2R rather than D1R antagonist increases immobility and reduces swimming, inducing depression-like behavior. It suggests that a D2R rather than D1R dependent pathway in the mPFC is essential for actively coping with stress in normal rats. These results together provide strong support to our hypothesis proposed in Chapter one that DA in the mPFC is associated with active coping and effortful behavior and that the antidepressant-like effect of exercise is accounted for by the upregulated DA in the mPFC.

Besides, in this experiment we also found that three weeks of wheel running did not change rats' general motor activity. This demonstrates that the antidepressant-like effect of

wheel running as measured by the FST is specific and validated, not reflecting a generally upregulated motor activity.

D2R, depression and cognitive flexibility

A prominent role of the D2R in the pathology and treatment of depression has already been noticed and frequently discussed [107]. For instance, several DA D2R agonists, such as pramipexole, bromocriptine, pergolide, alone or as an additive to traditional antidepressants, are effective in treating depression especially antidepressant-resistant depression in patients [173-178], and in reversing the motivational deficit induced by substantia nigra DA depletion [179] and preventing depression-like behavior [180] in rats. A recent report also showed that the D2R but not D1R antagonist abolishes the rapid antidepressant-like effect of ketamine in the FST [181]. However, the exact brain site(s) of the D2R-dependent antidepressant effect remains unclear. One commonly proposed area is the Nacc [182, 183], as various chronic antidepressant treatments selectively increase D2R-like gene expression in the Nacc but not in other areas [182], and D2R-like agonist binding potential [183]. The results of our present experiment suggest that another likely area of the D2-depedent antidepressant effect of chronic antidepressant treatments is the mPFC. This is further supported by a recent report that, pramipexole (a D2R agonist) increases the tonic activation of postsynaptic D2Rs in PFC in rats without changing their sensitivity [184].

However, it has to be noted that DA modulation in the mPFC is rather complex. D1R and D2R are present on both excitatory pyramidal neurons and inhibitory GABAergic interneurons in the mPFC, which acts on different timescales ranging from milliseconds to minutes and hours, and is also DA concentration-dependent [185-187]. It makes the precise function and role of the D1R versus D2R in the mPFC still remains unsure. Built from biophysically realistic computational models, the recent 'dual state theory of PFC DA function' [187] proposes that the PFC cognitive control network is either in a D1-dominated state favoring robust stabilization of representations and information maintenance (primarily by activating pyramidal PFC neurons), or in a D2-dominated state favoring flexible shifting of representations (primarily by inhibiting pyramidal PFC neurons).

Electrophysiological, neuroimaging, and behavioral evidences seem to support this proposal [187]. Notably, animal behavioral studies have shown that the D2 but not D1 receptor antagonist impairs cognitive flexibility as assessed by reversal learning tasks, which manifests as perseverative errors, i.e., failure to stop responding to previously rewarded stimuli and choose previously unrewarded stimuli [187].

Our result that wheel running reduced immobility through increasing swimming behavior seems to fit with the above theory. Recall that in the FST, rats show two kinds of active coping behavior, climbing and swimming. Whereas climbing is directed to escape from the swim chamber by upward movements at a certain spot(s)/place(s), swimming is horizontal movements typically shown as crossing across quadrants of the cylinder and diving downward deep in the water initiated in order to find whether there are other possibilities (spots/places) to escape [49-51]. In other words, swimming can be considered as one kind of cognitive flexibility in face of coping with the FST. Thus wheel running seems to exert its antidepressant-like effect (i.e. reducing immobility) by increasing flexible swimming behavior, which is in line with our finding that the antidepressant-like effect of wheel running is D2R-dependent.

As the primary objective of the present experiment was to test the hypothesis that the antidepressant-like effect of exercise is accounted for by the upregulated DA in the mPFC, we are not going to go on to study the dynamics of D1R versus D2R activation.

Further, our results suggest that the D1R in the mPFC may not be involved in the FST but do not exclude a role of D1R in the mPFC in depression, as studied by other paradigms rather than FST. Indeed, it has been shown that chronic restraint stress reduces D1R binding in the mPFC, which is normalized after chronic recovery [188].

What is the downstream pathway?

Although it goes beyond the objective of the present research, it is interesting and stimulating to consider the downstream pathway of the DA action in the mPFC. A brief look at the literature suggests at least two candidate brain regions as the target of the mPFC

in the antidepressant-like effect: the Nacc and the DRN [189]. The Nacc has been identified as part of the brain circuitry regulating effort-related behavior or motivation [190, 191] (or more broadly the striatum [107]), while the DRN is believed to be the key for 5-HT system in modulating learned helplessness [6, 56, 57]. Indeed, it has been shown that optogenetic activation of mPFC terminals in the Nacc elicited antidepressant-like effects following social defeat stress, as assessed by social interaction and sucrose preference [192]. Similarly, optogenetic activation of the mPFC terminals in the DRN increased active coping behavior in the FST without affecting general locomotor activity [193]. Actually the projection terminals in the Nacc and DRN primarily originate from the prelimbic part of the mPFC [194], the major region being targeted in our present experiment (see *Methodological consideration and functional dissociation within the mPFC* in Chapter three). One more possible target might be the VTA, as PFC synapses on GABAergic VTA neurons that project to the Nacc [159] and the VTA-Nacc circuit has been well implicated in depression [106, 106]. It remains for future studies to clarify whether these regions constitute the downstream pathway of the mPFC accounting for the antidepressant-like effect of exercise.

Consideration of D2 autoreceptor in the mPFC

It has been well known that the D2 autoreceptors in the striatum, when activated, inhibits DA release in this brain area [195]. Isn't it possible that D2 autoreceptors may have played a role in our experiment? Although D2 autoreceptor has also been discovered in the PFC, its density is extremely lower [195]. Unlike the DA neurons that project to the striatum, the firing rate of DA neurons that project to the PFC is not altered by intravenous administration of DA agonists or microiontophoretically applied DA [196]. Nor is DA release in the PFC facilitated by DA antagonists [197]. Further, a recent study that employed experimental neuroanatomical, electrophysiological, immunohistochemical and laser-dissection techniques demonstrated that, unlike VTA DA neurons that project to the Nacc, VTA DA neurons that project to the mPFC were unresponsive to stimulation of somatodendritic D2 autoreceptors [198]. Thus, it is unlikely that DA D2 autoreceptors have functioned in the mPFC in our experiment.

To conclude, using bilateral microinjection, we showed that the antidepressant-like effect of voluntary wheel running is completely abolished by pre-intra-mPFC injection of a D2R rather than D1R antagonist, providing strong support to our hypothesis that the antidepressant-like effect of exercise is accounted for by the upregulated DA in the mPFC.

Chapter 3 Higher basal corticosterone is responsible for the upregulated dopamine in the medial prefrontal cortex by exercise

3.1 Introduction

As discussed in Chapter one, we observed both increased basal CORT and DA in exercise rats and based on the literature we hypothesized that, higher basal CORT induces higher DA in exercise rats, the latter responsible for the antidepressant-like effect of exercise. Thus if we block the effect of higher basal CORT in exercise rats by pre-injection of a CORT antagonist, the observed higher DA in exercise rats will disappear, so does the antidepressant-like effect of exercise.

Although CORT has two types of receptors, MR and GR, only GR has been shown to mediate the upregulation effect of CORT on DA in the mPFC [158, 159]. Local injection of CORT into the mPFC increases DA in this brain area, which is blocked by a GR antagonist [157]. Indeed, it has been reported that CORT may potentiate the mPFC glutamatergic input onto DA neurons in the VTA through a GR-dependent mechanism [158]. Thus, in a third experiment we will investigate whether intra-mPFC pre-microinjection of a GR antagonist can block the antidepressant-like effect of exercise, and whether so in a DA-dependent way.

3.2 Methods

Animals and procedure for behavioral experiment

Another group of rats underwent exactly the same procedure as described in Chapter two except the solution and injection speed used. Briefly speaking, a guide cannulae for bilateral microinjection was implanted toward the mPFC after two week of wheel running (for EX rats) or no wheel running (for CON rats), and that after one more week of wheel running (for EX rats) or no wheel running (for CON rats), an injection cannulae was inserted, which was connected by polyethylene tubing to motor-driven microsyringes. The exact placement of the injector cannulae tips was verified the next day during dissection (see Figure 3-1). EX rats were injected a GR antagonist while CON rats received only the vehicle. The solution (1 µl) was infused through each injector at a rate of 0.5 µl/min for 2

min at 30 min before the day-2 FST. The injection cannulae was left in position for an additional 60 s after drug infusion. All drug solutions were freshly prepared immediately before the experiment.

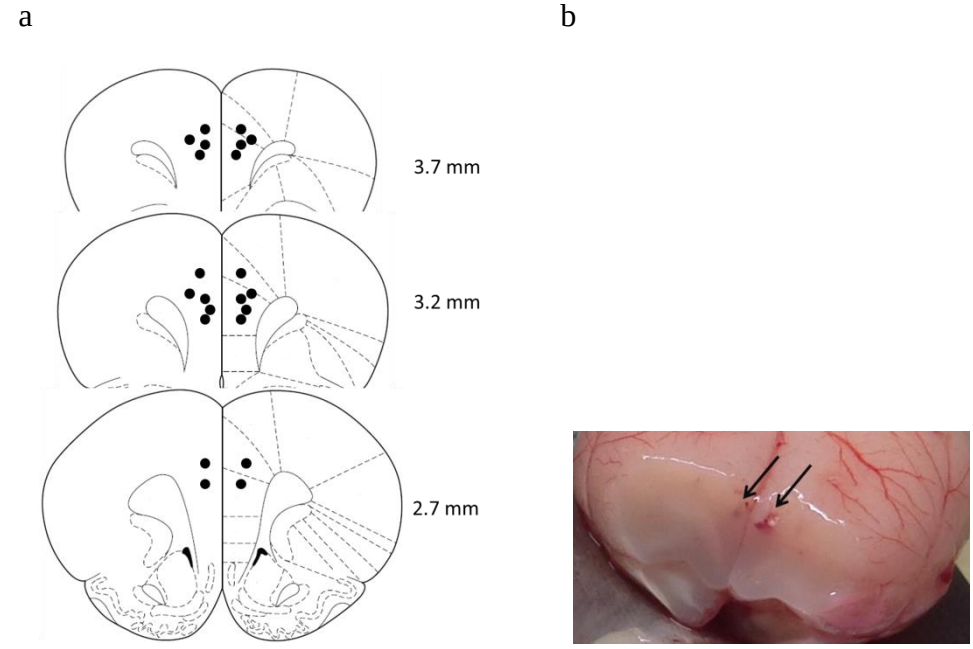


Figure 3-1 The placement of the injector cannulae tips. a, Schematic representation of bilateral placement of the injector cannulae tips; b, Actual photo taken from rats' brain during dissection showing the placement of injector cannulae tips.

Drugs

The GR antagonist RU486 (mifepristone, dissolved in ethanol, Tocris bioscience, UK) was used. Following Butts et al [157], we used a dose of 100 ng/ul, for RU486 at this dose locally delivered into the mPFC inhibits stress- or CORT- responsive DA efflux in the mPFC.

Analysis of the FST results

The procedure for the FST was completely the same as described in Chapter one for the FST. Climbing, swimming and immobility were scored using the frequency of each behavior over 5-s intervals during the day-2 5-min test session.

Animals and procedure for microdialysis experiment

Another group of rats underwent exactly the same procedure as described in Chapter one for microdialysis except the probe used and the procedure at the time of microdialysis. After day-1 test of the standard FST, under pentobarbital anesthesia (30 mg/kg i.p.), rats were implanted with a dialysis probe with a microinjection needle (MI-A-4-2) attached on it (Eicom), which allows simultaneous microdialysis and microinjection. The outer diameter of the probe was 0.22 mm, and the outer and inner diameter of the microinjection need was 0.15 mm and 0.075 mm, respectively. The probe was inserted into the guide cannulae so that 2.0 mm of the probe while 1.0 mm of the microinjection needle was exposed to the tissue of the mPFC. The next day, perfusion was started using aCSF at a flow rate of 1 μl/min at 15:00. Following the initial perfusion for 2 hours, dialysate samples were collected in sample vials every 30 min. At 18:30 namely 30 min before the onset of the dark phase, EX rats were injected a GR antagonist while CON rats only the vehicle. The solution and method for microinjection was exact the same as described in the behavioral experiment above in the present chapter. The following procedure for the day-2 FST was exactly the same to that of chapter one. Briefly speaking, at 19:00 straight rats were placed into the water tank for 5 min with the dialysis probe in their brains. Later they were dried with Kim towel and returned to the dialysis box for perfusion and sampling till 22:00. The exact placement of the probe tips was verified the next day during dissection (Figure 3-2).

a b

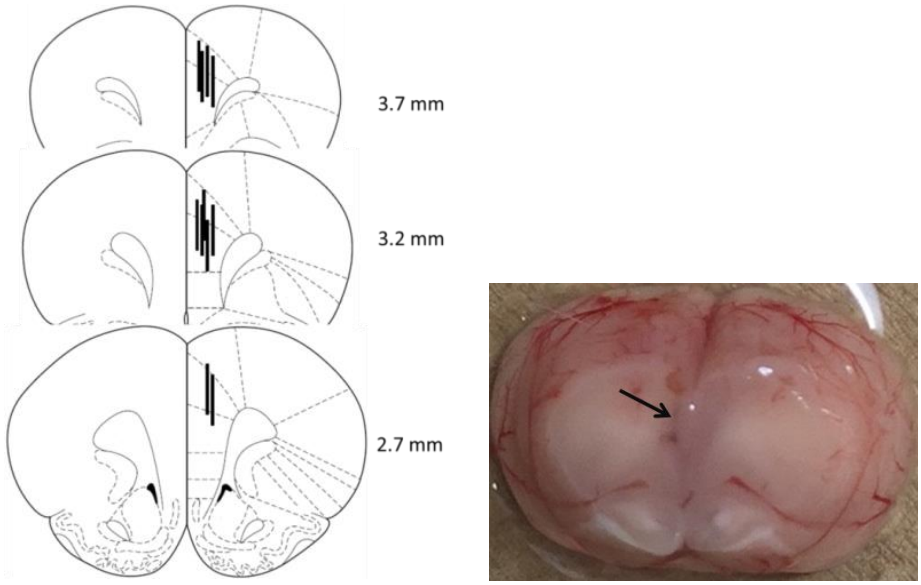


Figure 3-2 The placement of the microdialysis probe. Schematic representation of bilateral placement of the probe (a); Actual photo taken from rats' brain during dissection showing the placement of probe (b). Note that although not shown, the microinjection needles were closely coincided with the upper half of the microdialysis probe.

HPLC

For HPLC determination of DA and 5-HT levels, the procedure was completely the same as that in Chapter one.

3.3 Results

Running distance

The running distance of rats for the microinjection and microdialysis experiment is shown in Figure 3-3.

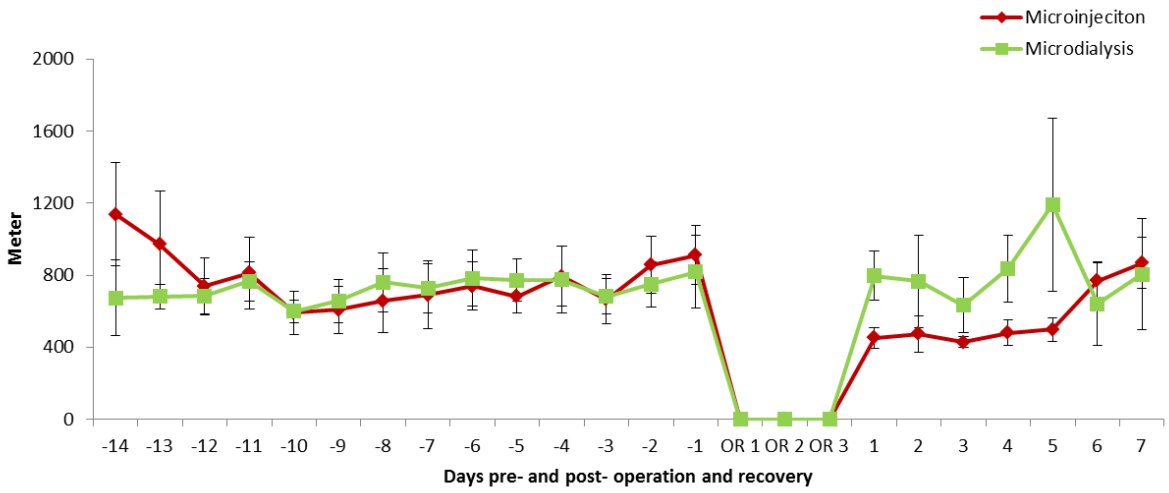
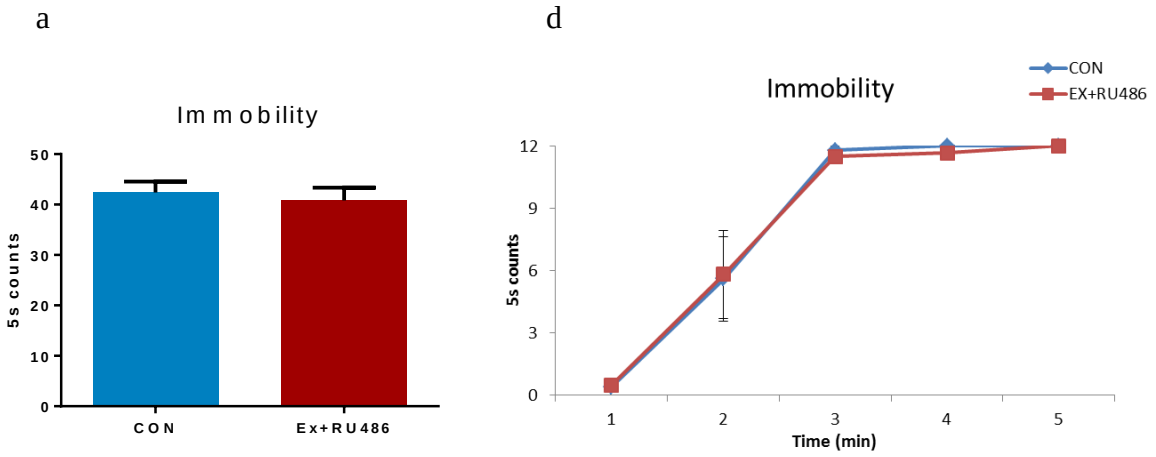


Figure 3-3 Mean running distance per day pre- and post- operation and recovery (OR) of EX rats for microinjection (n=6) and microdialysis (n=6) experiment. Mean±SEM.

GR antagonist and the antidepressant-like effect of wheel running

Bilateral intra-mPFC pre-microinjection of GR antagonist RU486 into EX rats completely abolished the antidepressant-like effect of exercise (Figure 3-4 a-c): immobility (t=0.461, p=0.656), swimming (t=0.168, p=0.871), climbing (t=1.07, p=0.314). Similar to that of D2R antagonist haloperidol, there was no difference between EX (RU486 injected) and CON rats regarding immobility, swimming and climbing across the 5 min duration (Figure 3-4 d-f, Table 3-1).



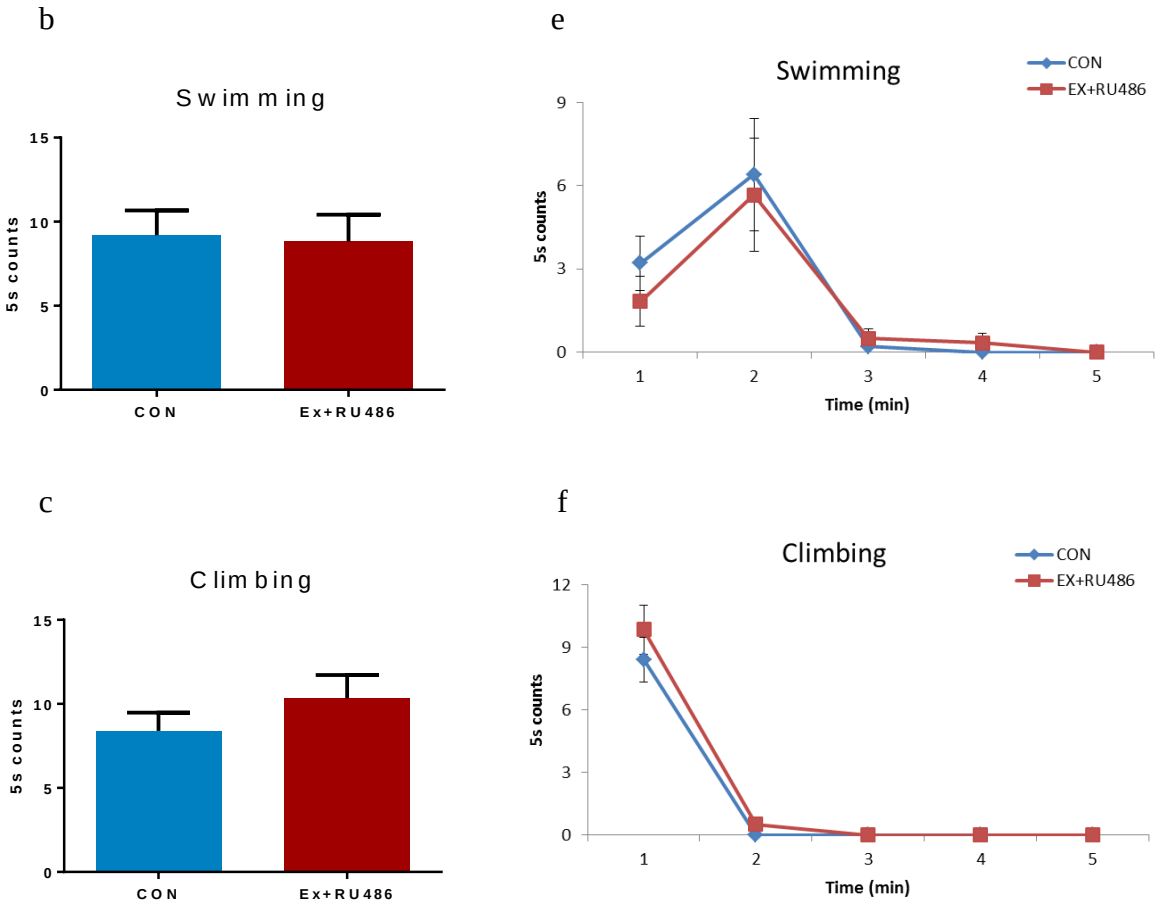


Figure 3-4 The antidepressant-like effect of exercise was completely abolished by pre-intra-mPFC microinjection of a GR antagonist, RU486. (a-f) The FST for assessing antidepressant-like behavior. The duration of immobility, swimming, and climbing in the 5 min totally (a, b, c, in order) and across the 5 min (d, e, f, in order). (EX, n=6; CON, n=5) Mean±SEM.

	Time	Group	Time*Group
Immobility	F=61.653, p<0.001	F=0.007, p=0.934	F=0.036, p=0.865
Swimming	F=11.125, p=0.007	F=0.471, p=0.510	F=0.229, p=0.627
Climbing	F=125.297, p<0.001	F=1.139, p=0.314	F=0.743, p=0.421

Table 3-1 Two-way ANOVA with repeated measures of the FST data for GR antagonist RU486 across the 5 min duration. Mean±SEM.

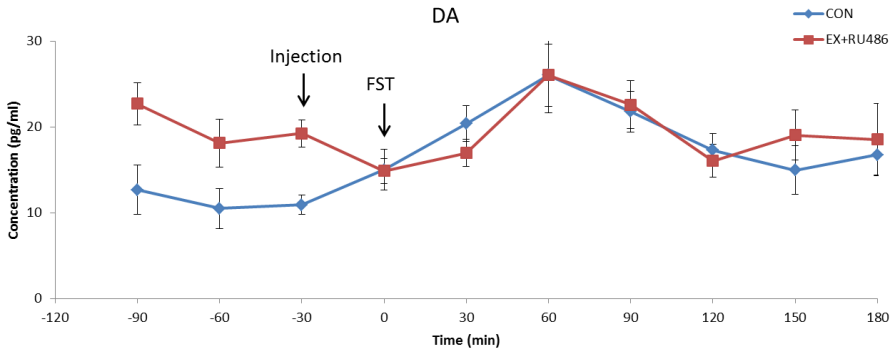
GR antagonist and the neurobiological effect of wheel running

Two-way ANOVA with repeated measures analysis of basal DA (from -90 to -30 min, Figure 3-5a) showed a significant effect of group ($F=11.082$, $p=0.010$), but not time ($F=2.342$, $p=0.128$) or time*group ($F=0.271$, $p=0.766$); in the meantime, following injection of RU486 (from -30 min to 0 min, Figure 3-5a), there is a significant effect of time*group ($F=10.314$, $p=0.012$), but not time ($F=0.010$, $p=0.924$) or group ($F=4.006$, $p=0.080$). Post hoc Student's t-test suggests that there is a significant difference between groups at -30 min ($t=-4.227$, $p=0.003$) but not 0 min ($t=0.050$, $p=0.962$). This suggests that bilateral intra-mPFC microinjection of a GR antagonist RU486 into EX rats reduced the originally upregulated DA levels in the mPFC. The percentage representation is shown in Figure 3-5b.

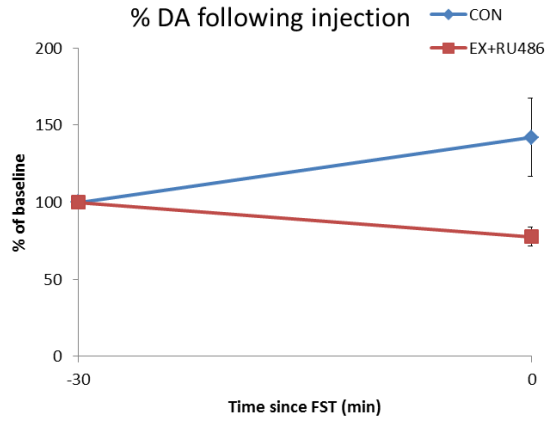
Following FST (from 0 to 180 min), there was a significant effect of time ($F=5.420$, $p<0.001$) but not group ($F=0.011$, $p=0.919$) or time*group ($F=0.513$, $p=0.795$). That is, there is no difference in DA levels between EX and CON rats in the FST. The percentage representation is shown in Figure 3-4c. %DA following the FST showed a trend toward significance with time ($F=3.127$, $p=0.072$), no effect of group ($F=0.624$, $p=0.452$) or time*group ($F=1.008$, $p=0.386$).

Comparison of AUC again suggests that EX group had higher level of DA at baseline ($t=3.079$, $p=0.015$; Figure 3-5d) but not after FST ($t=0.065$, $p=0.950$; Figure 3-5e) than CON group.

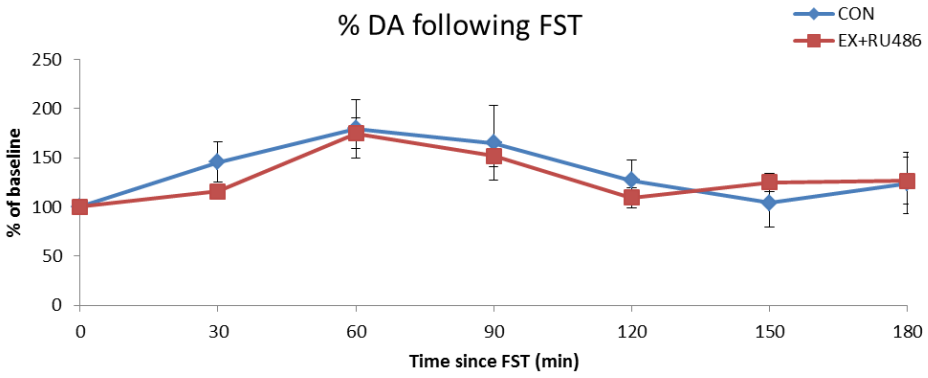
a


DA
CON
EX+RU486
Injection
FST
Concentration (pg/ml)
Time (min)

b


% DA following injection
CON
EX+RU486
% of baseline
Time since FST (min)

c


% DA following FST
CON
EX+RU486
% of baseline
Time since FST (min)

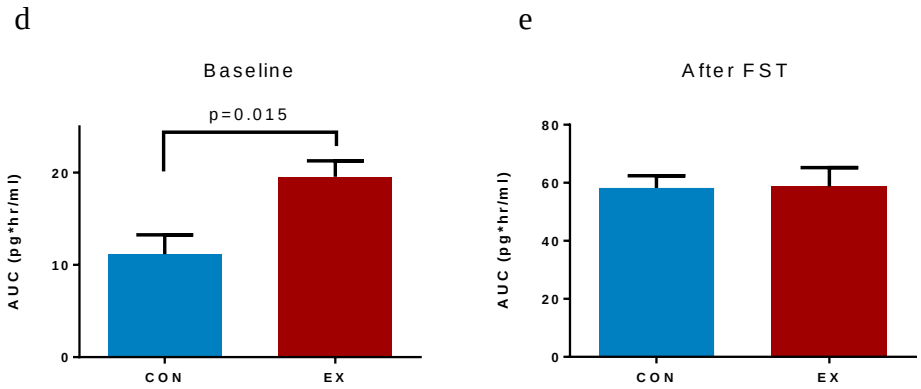


Figure 3-5 GR antagonist (RU486) reduces basal and FST-responsive DA in the mPFC of EX rats. The effect of exercise on DA (pg/ml) in the mPFC at baseline, following microinjection of RU486 and following the FST (a). % representation of DA in the mPFC following microinjection of RU486 (EX) or vehicle (CON) (b). % representation of DA in the mPFC following the FST (c). The effect of exercise on the AUC of DA (pg*hr/ml) in the mPFC at baseline (d) and following the FST (e). (n=5/group) Mean±SEM.

In contrast, regarding 5-HT (Figure 3-6), there was no significant effect of group ($F=0.015$, $p=0.907$) or time*group ($F=0.487$, $p=0.879$) but time ($F=5.113$, $p<0.001$), suggesting that GR antagonist do not affect 5-HT.

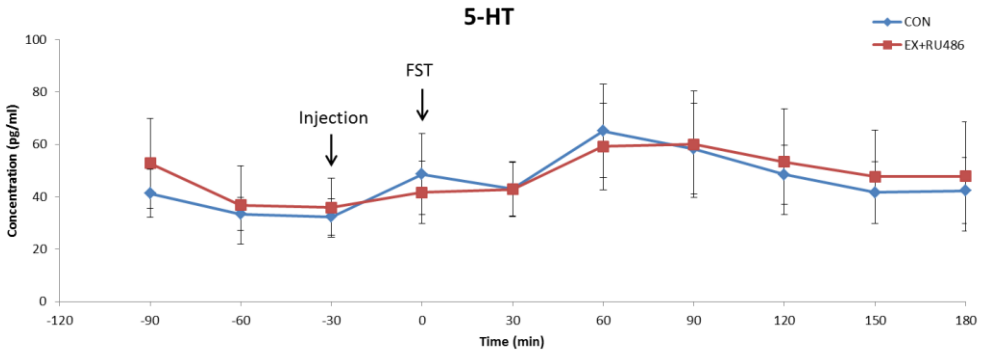


Figure 3-6 GR antagonist (RU486) did not influence basal or FST-responsive 5-HT in the mPFC of EX rats. The effect of exercise on 5-HT (pg/ml) in the mPFC at baseline, following microinjection of RU486 (EX) or vehicle (CON) and following the FST. (n=5/group) Mean±SEM.

3.4 Discussion

In this experiment, we found that the antidepressant-like effect of three weeks of wheel running in the FST was abolished by intra-mPFC pre-microinjection of a GR antagonist, RU486. In the meantime, intra-mPFC pre-microinjection of RU486 also downregulated the basal and FST-responsive DA in the mPFC of exercise rats, replicating previous findings by Mizoguchi et al [156] that suppression of CORT decreases DA in the mPFC and by Butts et al [157, 158] that pre-intra-mPFC microinjection of RU486 reduces acute tail-pinch stress-evoked DA in this brain area. These results confirm our hypothesis proposed in Chapter one and thus suggest a causal pathway linking CORT, GR, DA, and D2R, to the antidepressant-like effect of exercise. In other words, exercise increases basal CORT, which subsequently upregulates DA, the latter responsible for the antidepressant-like effect in the FST.

Methodological consideration and functional dissociation within the mPFC

It has been suggested that there is a structural and functional dissociation between the ventral (ventral prelimbic cortex and infralimbic cortex) and dorsal (cingulate gyrus and dorsal prelimbic cortex) part of mPFC [194, 199-201]. Particularly with relevance to the present research, basal DA levels are much higher in the ventral than dorsal part and the highest density of DA innervation is found in the prelimbic cortex (for a review, see [199]). A close look at the position of our microdialysis probes, in the first microdialysis experiment, we targeted both dorsal and ventral mPFC, from the cingulate gyrus, to prelimbic cortex, and to part of the infralimbic cortex (see Figure 1-2b). In contrast, in the last experiment with concurrent microinjection and microdialysis, we targeted more dorsal part of the mPFC, the cingulate gyrus and the prelimbic cortex (see Figure 3-1a). Maybe this can explain why the DA concentration in the last experiment was lower than that of the first experiment (compare Figure 1-8 and 3-4). Note that there were also methodological differences between these experiments. The length of probe membranes in the first and last experiment were 3 mm and 2 mm respectively, and the flow rate is 2 ul/min and 1 ul/min respectively. This choice was based on the fact that DA levels in the mPFC is low and challenging to

detect using HPLC and that probe longer membrane allows more fluid exchange which results in higher concentrations. Further, the choice for the last experiment was also dependent on the second experiment, microinjection. We found a causal association between DA in the mPFC and the antidepressant-like effect of wheel running and aimed to examine the relationship between CORT and DA in the mPFC and the antidepressant-like effect of wheel running. It was suggested that a 0.5 µL volume (which we used in the microinjeciton) spreads to approximately a 1.0 mm diameter sphere [202, 203]. Thus we chose the probe in the last experiment with a membrane length of 2 mm (rather than 3 mm in the first experiment), which spreads 1mm above and also below the needle for microinjection, therefore balancedly covering the whole microinjection area. Since shorter length leads to fewer fluid exchange and thus lower DA concentration, we changed the fluid rate from 2 ul/min (in the first experiment) to 1 ul/min (in the last experiment), because slower fluid rate allows more complete fluid exchange and thus lead to higher DA concentration. Indeed, 5-HT concentration measured in the last experiment was somewhat higher than that of the first experiment (compare Figure 3-5 with Figure 1-9b). On contrary, DA concentration in the last experiment was nevertheless lower than that of the first experiment. This suggests that basal DA levels are indeed much lower in the dorsal than ventral part of mPFC [199].

Returning to the functional dissociation within the mPFC, it is also assumed that under stress, the infralimbic cortex signals excitation while prelimbic cortex exerts control and inhibition [200]. For instance, lesion of infralimbic cortex decreases while lesion of prelimbic cortex prolongs HPA axis responses to acute stress (for a review, see [200]). However, it has also been reported that injection of D1R/D2R antagonist into the infralimbic cortex exaggerates the restraint stress-induced increase in ACTH and CORT [150]. Further, our own experiment (the second experiment) found that D2R antagonist injected primarily into the prelimbic cortex induces depressive states and abolishes the antidepressant-like effect of wheel running. Thus it seems DA in both infralimbic and prelimbic cortex modulates (inhibits) stress response and exerts control, although this needs future more specialized investigation.

General discussion and conclusion

In brief, by three experiments as presented in Chapter 1-3, we have shown that three weeks of voluntary wheel running exerts antidepressant-like effect in the FST, without affecting general motor activity. This is accompanied by upregulated DA in the mPFC and is blocked by intra-mPFC pre-microinjection of a D2R but not D1R antagonist. Further, wheel running upregulates basal CORT while downregulates FST-responsive CORT in the mPFC and more interestingly, blocking CORT by intra-mPFC pre-microinjection of a GR antagonist abolishes the antidepressant-like effect while reduces the originally upregulated DA in the mPFC. These results together suggest that the causal pathway of basal CORT-GR-DA-D2R accounts for the antidepressant-like effect of wheel running.

This is somewhat surprising since higher basal CORT has been believed to be detrimental. As we have discussed in section Introduction, in rodents, various chronic stress upregulates basal CORT while antidepressants normalize it (e.g. [22, 26, 32]). In humans, higher basal CORT is frequently reported in patients suffering from depression, which is normalized by antidepressant or psychological treatment (for a review, see, e.g. [41, 42]). Higher basal CORT also predicts the onset of depression in humans [44, 45]. The beneficial exercise, as we and others (e.g., [11, 14]) have demonstrated, also increases basal CORT. Thus, it has been proposed that exercise, should through some mechanism, override the detrimental effect of higher CORT [106].

The results in the present study suggest that upregulated DA may be one such mechanism. Indeed, whereas various chronic stress upregulates basal CORT (e.g. [22, 26, 32]) and downregulates DA in the mPFC (e.g. [108, 112]), exercise upregulates basal CORT but also upregulates DA in the mPFC, besides their distinct influence on GR and stress-responsive CORT (see Table 4-1). Notably, various antidepressant treatments also upregulates DA in the mPFC (e.g., [122, 123]) and higher DA in the mPFC is associated with active coping and effortful behavior (e.g., [140, 142]). In the present research, for the first time we showed that the CORT-GR-DA-D2R pathway actually accounts for the antidepressant-like effect of exercise. Thus, in contrary to the traditional view that increased basal CORT is detrimental,

it might actually be beneficial, at least in the context of exercise. Therefore these observations raise the possibility that the elevated basal CORT in humans with depression and in animals underwent various chronic stress is not dysregulated per se. Instead it may reflect a fundamental mechanism underlying CORT negative feedback (basal CORT increases DA which suppresses stress-responsive CORT), and that the dysregulation and the major problem in chronic stress and depression may locate in the DA system (for a review, see [107]): the failure of higher CORT in upregulating DA in the mPFC (the latter responsible for exerting control and coping). Although we have discussed a lot regarding the effect of chronic stress on CORT, we did not include chronic stress in our present research and this remains a limitation. Future inquiry should try to incorporate chronic stress and exercise together to further investigate the CORT paradox and provide more insights. Nevertheless, we aimed to examine the exercise-CORT paradox and have provided an answer. Future investigation is still needed to validate our finding. Future investigations may also examine how higher basal CORT upregulates DA in the mPFC in exercise and how higher DA in the mPFC induces antidepressant-like effect at the systems and cellular and molecular level.

Table 4-1 A comparison of the effect of chronic stress, antidepressants, and exercise on CORT and DA as reported previously and by the present study (see the text for references)

	Chronic stress	Antidepressants	Exercise (previous research)	Exercise (present study)
Basal CORT	↑	↬	↑	↑
MR	↓	↑ or ↬	↑ or ↓	-
GR	↓	↑ or ↬	↑ or ↭	↭ (mPFC)
Basal DA in mPFC	↓	↑ or ↬	↑ (cortetx)	↑
D1R in mPFC	-	-	-	↭
D2R in mPFC	-	-	-	↭
Stress-responsive CORT	↑ (blood)	↬	↓ or ↬ (blood)	↓ (mPFC)

↑ upregulated; ↓ downregulated; ↬ normalized; ↭ no change; - not reported

Our findings in a broader context

We have limited our discussion in the context of stress and depression. However, it has been long well-known that running is rewarding in humans ('runner's high') [204] and in rodents [205], and that reward activates DA neurons in the VTA (for a review, see [107]). Indeed, recently it has been demonstrated that tonic DA (i.e. basal DA) in the mPFC is correlated with long-term average reward [206].

Our observed increased DA in the mPFC is also consistent with the finding that exercise improves executive control and working memory [207-209], which is mPFC DA-dependent [210, 211]. Our observation therefore has great implications. Patients with Parkinson's disease suffer from motor deficits arising from depletion of striatal DA and cognitive deficits arising from depletion of PFC DA. Treatment of cognitive deficits is rather challenging because doses of DA medications that normalize striatal motor function are often too high for optimal PFC cognitive function [212]. Further, due to loss of DA in the PFC, Patients with Parkinson's disease also frequently develop depression [213, 214]. Our finding that exercise upregulates DA in the mPFC and exerts antidepressant-like effect provide powerful neurobiological support for the application of exercise therapy in treating Parkinson's disease.

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