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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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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 helplessness’ and anhedonia, which resembles human depressive states^{33, 34}. In the meantime, human studies also reported elevated basal CORT in plasma^{35, 36}, and saliva³⁷⁻³⁹ in clinically depressed patients, which is normalized by antidepressant or psychological treatment (for a review and meta-analysis, see⁴⁰⁻⁴³). 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	adrenocorticotrophic 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}.

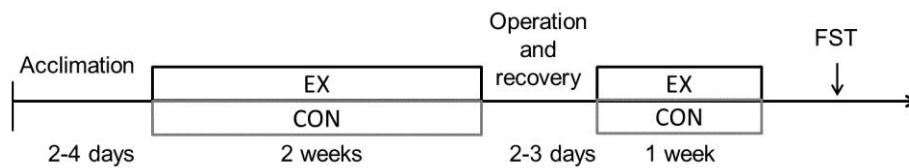
1.2 Methods

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



b

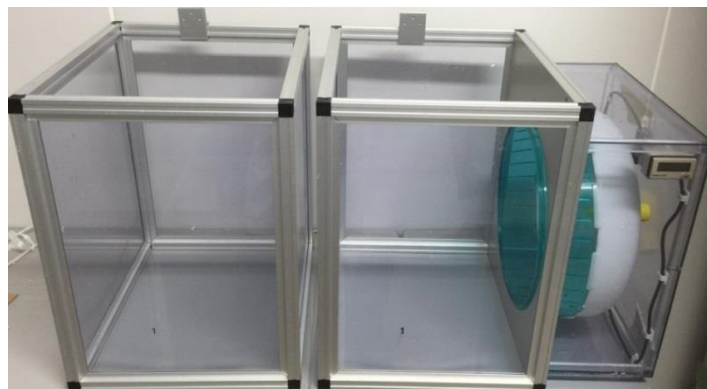


Figure 1-1 The procedure of the experiment (a) and the photo of the cage box with (b right) or without (b left) a running wheel used in the experiment

Two weeks after the allocation and treatment, all rats underwent microdialysis surgery. Stereotactically 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 times 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 paddling 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, 1.3mM CaCl_2 , 1.0mM MgCl_2) at a flow rate of 2 $\mu\text{l}/\text{min}$ 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 dialysis 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).

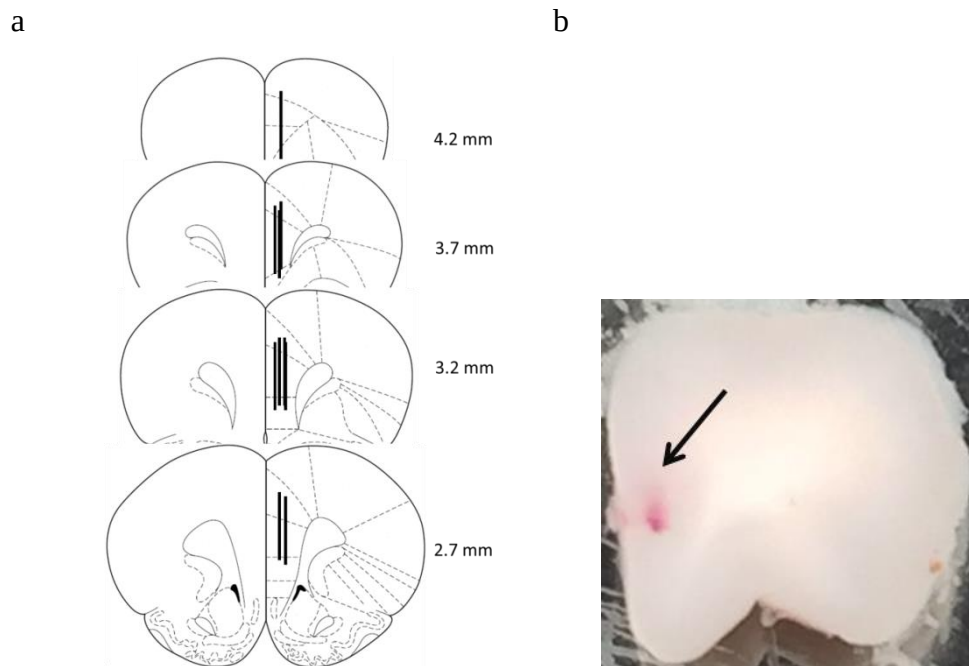


Figure 1-2 The placement of the microdialysis probe tips. Schematic representation of placement of the probe tips (a); Actual photo taken from rats' brain during dissection showing the placement of probe tips (b).

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 detected⁶¹⁻⁶⁵. 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).

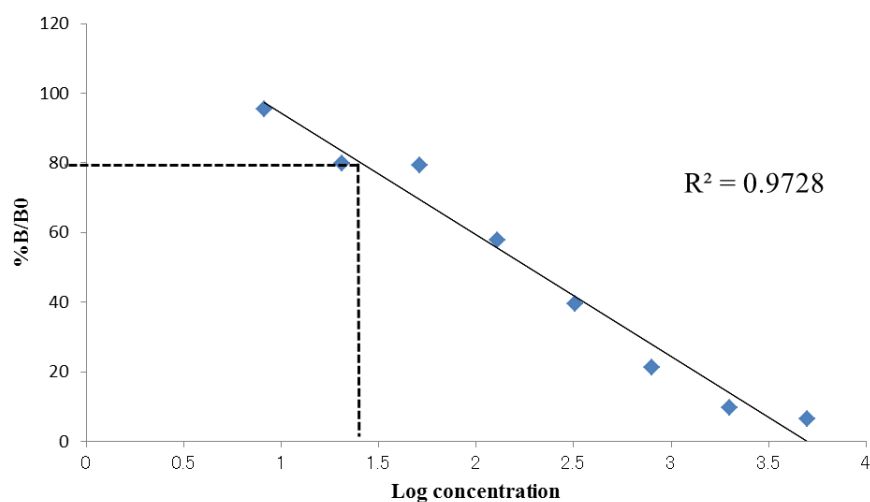


Figure 1-3 The calibration curve for quantification of CORT by Elisa. The calibration curve corresponds to the plot % bound/maximum bound for standards versus concentration of CORT from 8.2 to 5000 pg/ml using linear (y axis) and Log (x axis). The calibration curve was linear with a regression coefficient (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 previously⁵⁵. 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 $\pm$ 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).

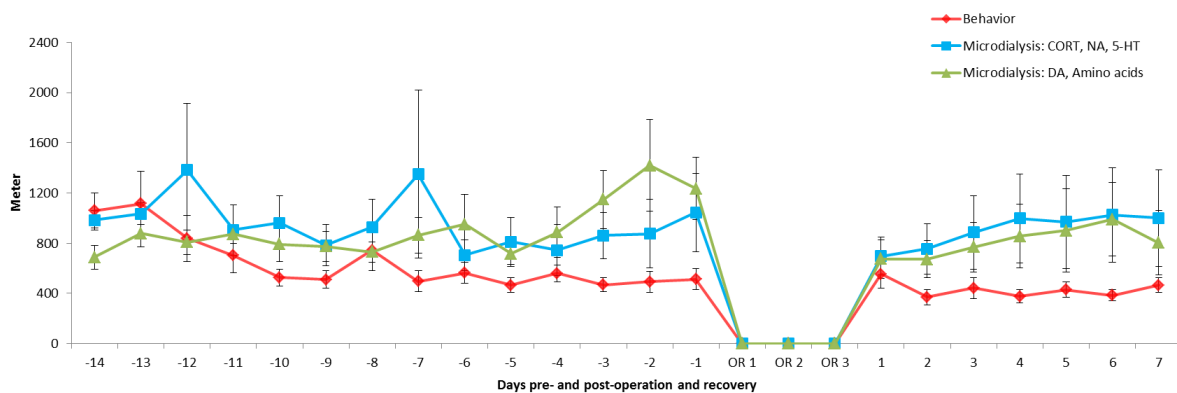


Figure 1-4 Mean running distance per day pre- and post- operation and recovery (OR) of EX rats. Red for rats that underwent the FST ($n=7$), blue for rats that underwent microdialysis for CORT, NA, and 5-HT ($n=6$), and green for rats that underwent microdialysis for DA and amino acids ($n=7$). Mean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

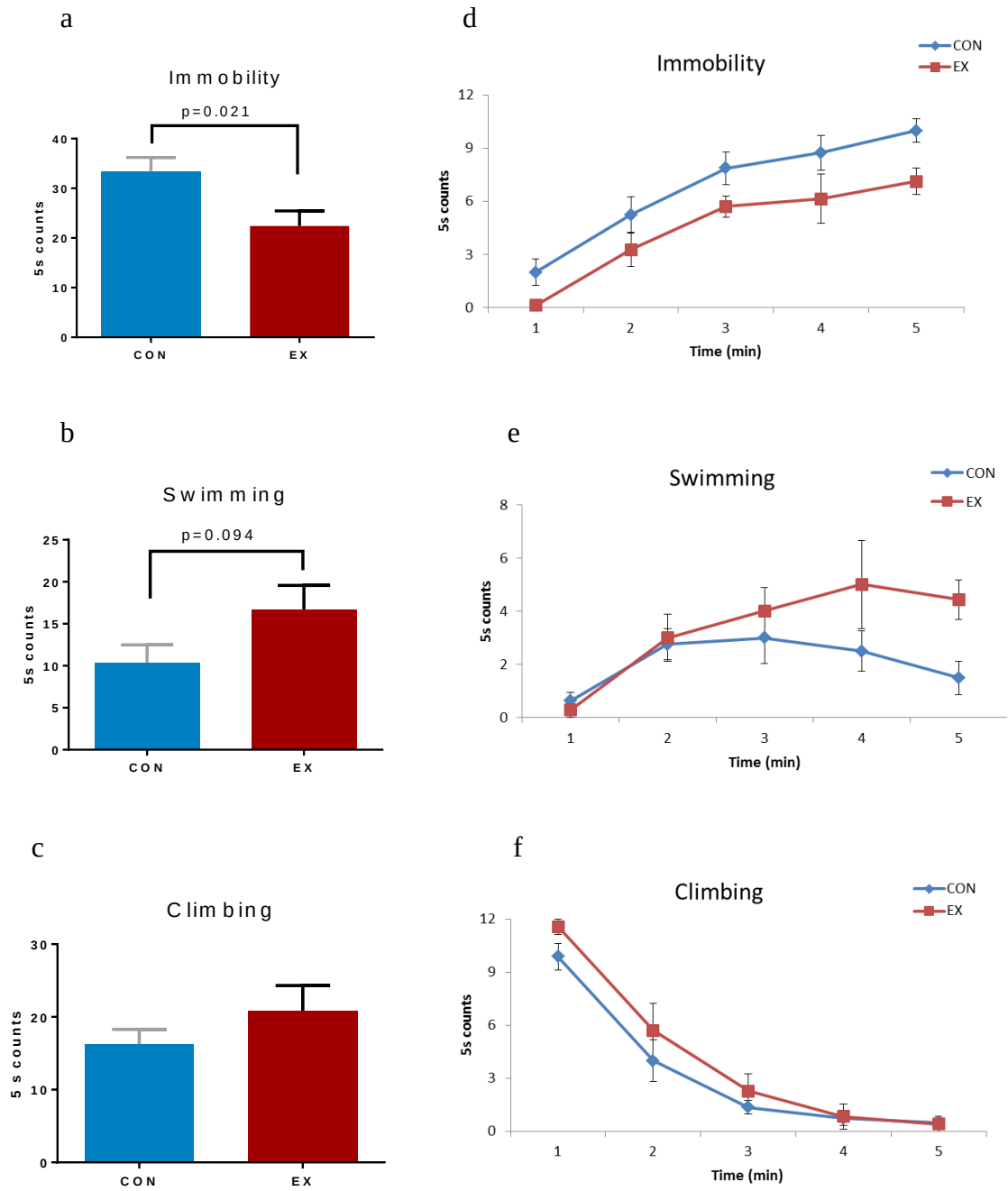


Figure 1-5 The antidepressant-like effect of exercise. (a-f) The FST for assessing antidepressant-like behavior. The duration of immobility, swimming, and climbing in the 5 min totally (a, b, c, in order) and across the 5 min (d, e, f, in order). (EX n=7; CON n=8) Mean±SEM.

	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

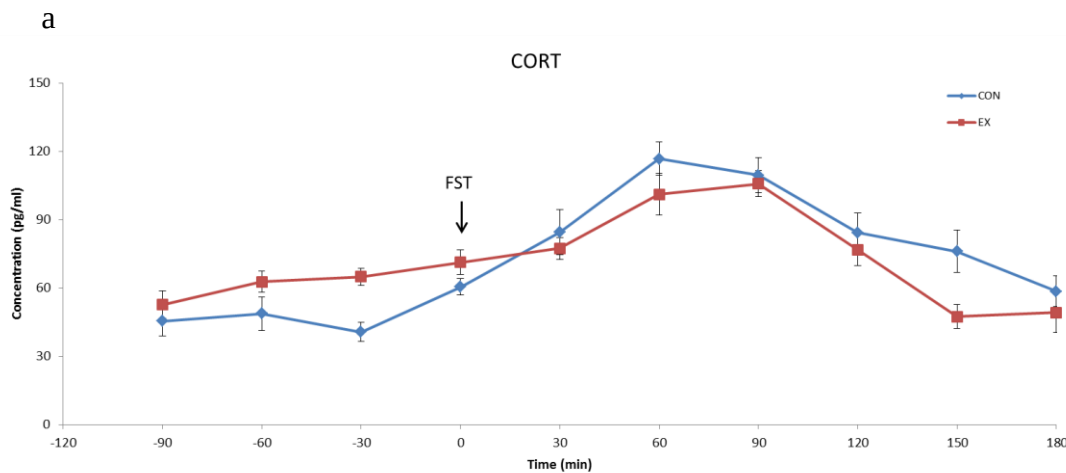
The running distance of those EX rats that underwent microdialysis is shown in Figure 1-4 (blue and green).

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.



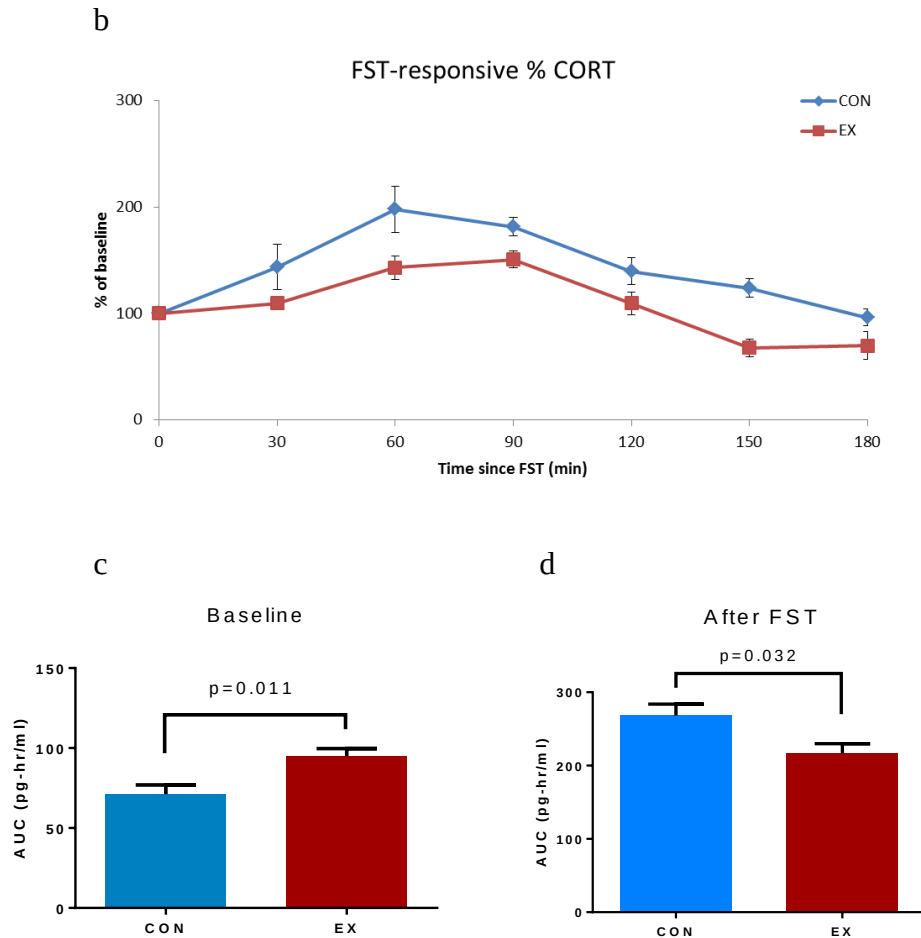


Figure 1-6 The effect of exercise on CORT in the mPFC. The effect of exercise on the concentrations of CORT (pg/ml) at baseline and following the FST (a). The effect of exercise on the FST-responsive CORT, as shown in % of baseline (b). Baseline was defined as the concentration of the last sample obtained 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.

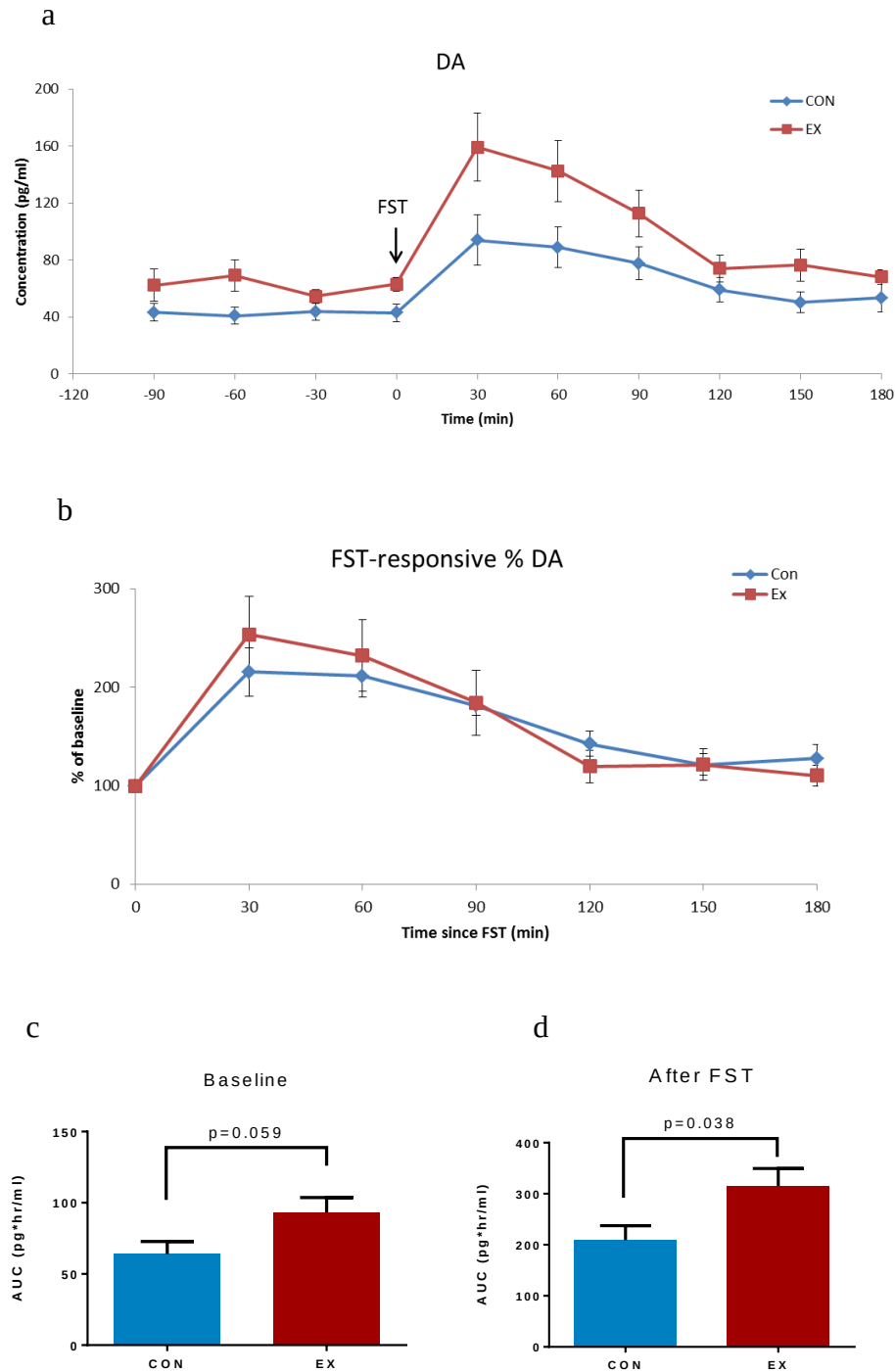
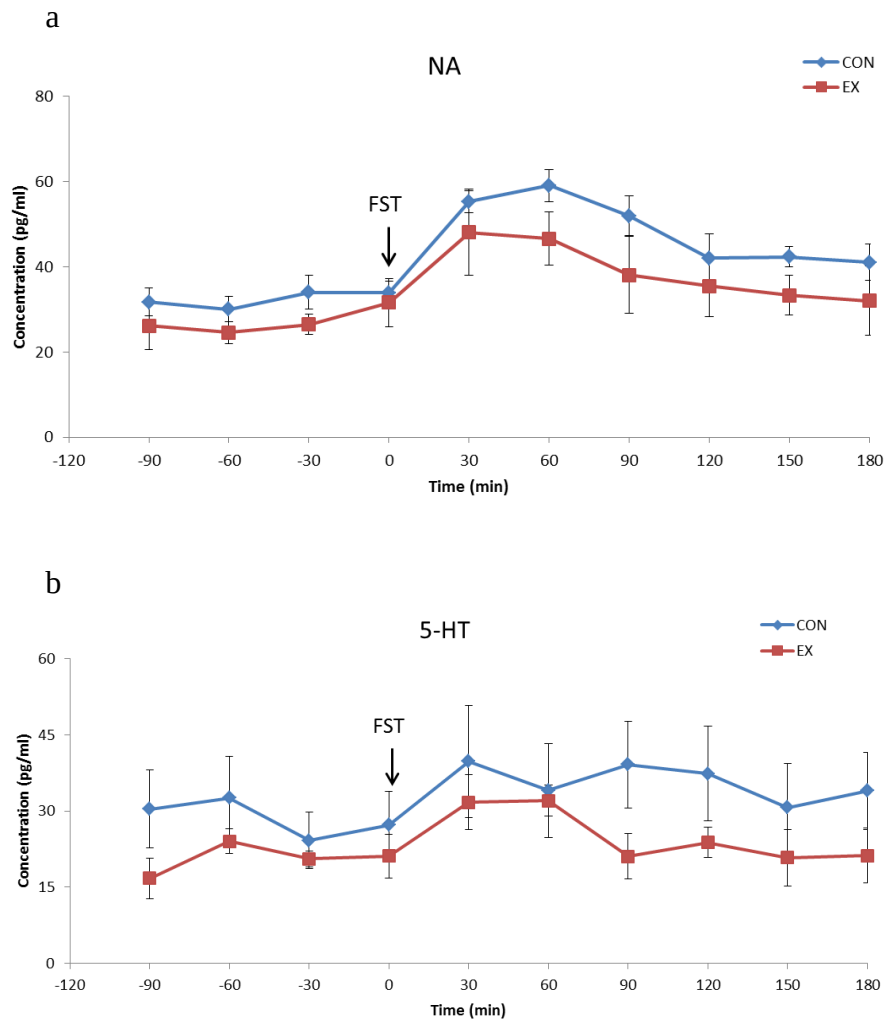
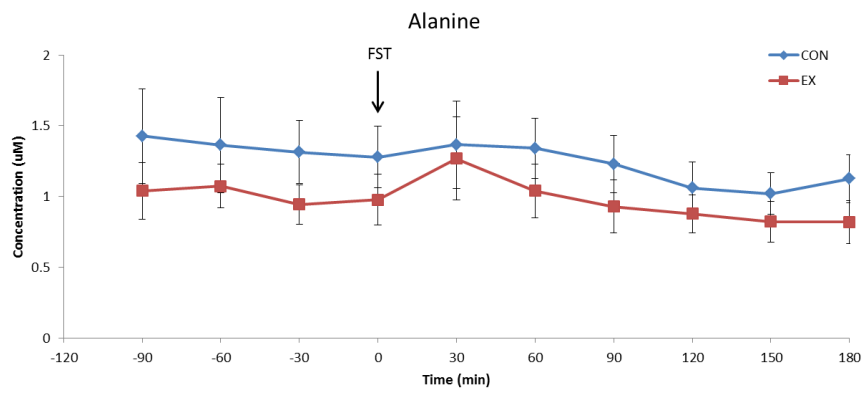


Figure 1-7 The effect of exercise on DA in the mPFC. The effect of exercise on the concentrations of DA (pg/ml) at baseline and following the FST (a). The effect of exercise on the FST-responsive DA, as shown in % of baseline (b). Baseline was defined as the concentration of the last sample obtained 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 $\pm$ SEM.

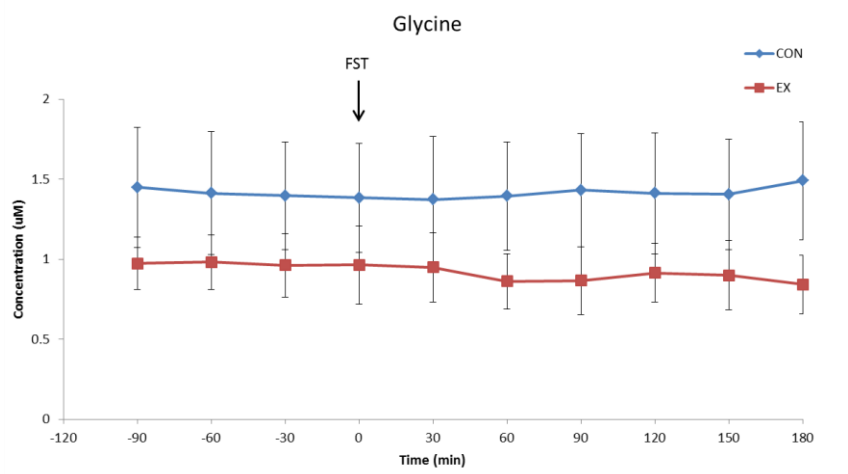
The results of other neurotransmitters are shown in Figure 1-8. Statistical analysis (as shown in Table 1-4) suggests that there was only significant effect of time with regard to NA, 5-HT, and alanine, without any other effect.



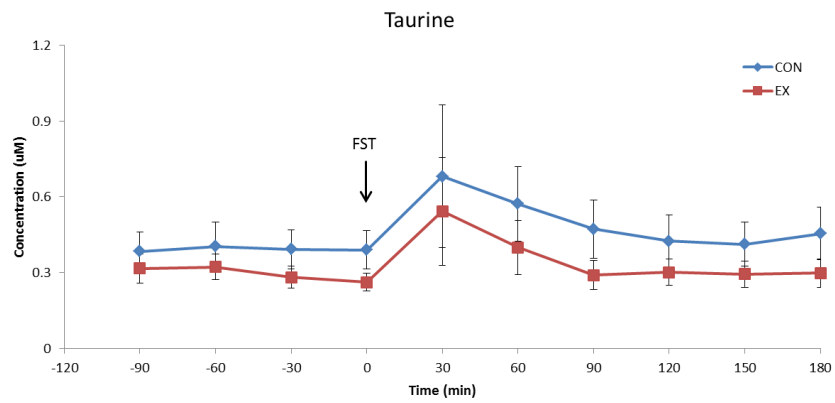
c



d



e



f

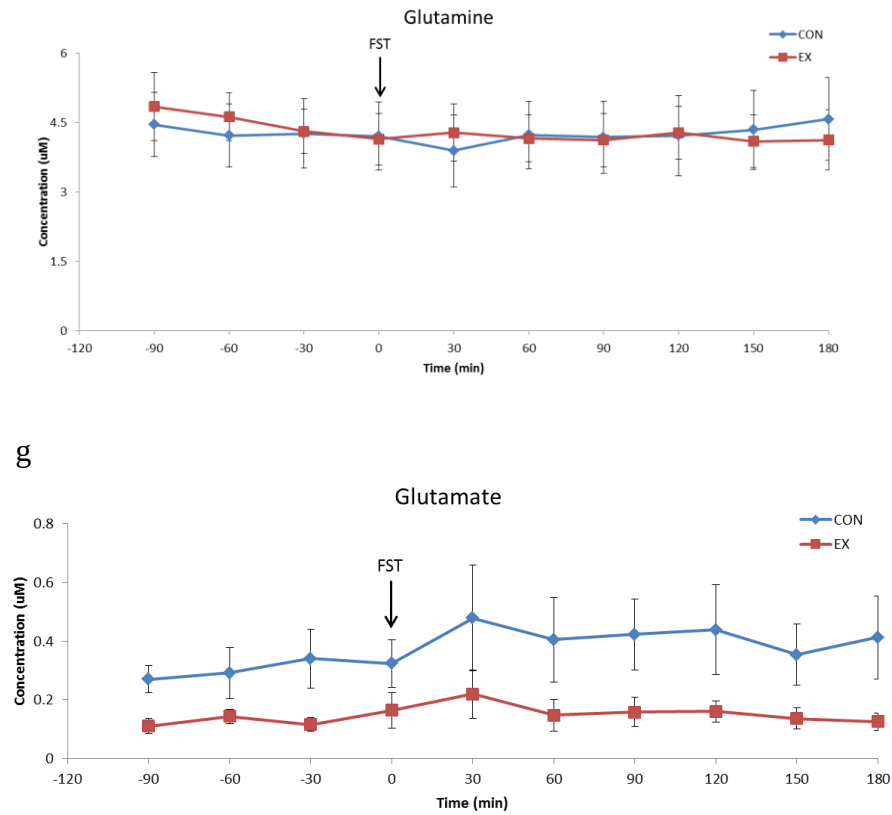


Figure 1-8 The effect of exercise on the concentrations of NA (a), 5-HT (b), alanine (c), glycine (d), taurine (e), glutamine (f), and glutamate (g) at baseline and following the FST. (n=6/group for NA and 5-HT; n=7/group for alanine and glutamine; CON n=7, EX n=6 for glycine and taurine; CON n=6, EX 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 mPFC⁶⁸, we failed to reliably detect MR in our samples.

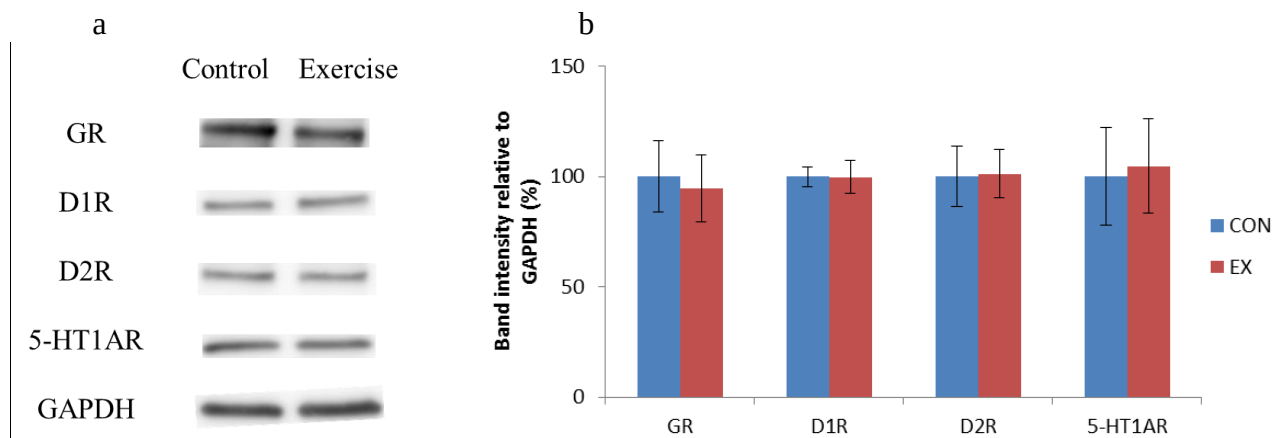


Figure 1-10 The effect of exercise on the protein expression of GR, D1R, D2R, 5-HT1AR, and GAPDH in the mPFC. Representative western blot bands for each protein (a).

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 $\pm$ 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 FST⁶⁹⁻⁷⁴, shuttle box escape deficit^{72, 75-77}, and chronic mild stress^{25, 69}. Also consistent with previous studies⁶, 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 fluids⁶². 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 plasma⁶¹, and that CORT within the amygdala fluctuates in accordance to systematic administration of CORT⁶⁴. Replicating plasma research, Droste et al⁶² 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 gyrus⁸¹. 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 phenotype⁸⁵. 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 running⁸⁶. The same study also found increased GR in dorsal but not ventral hippocampus. Several other studies reported either an increase⁸⁷ or no change^{88, 89} of GR in the hippocampus of wheel running rats or mice. With regard to MR, Droste et al¹¹ reported a 38% decrease of MR within hippocampus in exercise mice, which the authors failed to replicate in their later study⁸⁶.

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 stress¹⁰⁸⁻¹¹¹, chronic restraint^{112, 113}, chronic water bath¹¹⁴, chronic cold¹¹⁵, chronic social isolation¹¹⁶, chronic unavoidable footshock¹¹³, adolescent social defeat¹¹⁷⁻¹²⁰, and maternal separation¹²¹ all decrease DA in the mPFC. In the meantime, various antidepressant treatment, such as tricyclic antidepressants¹²²⁻¹²⁴, selective 5-HT re-uptake inhibitors (SSRIs,^{122, 124-126}), 5-HT agonist^{124, 127}, 5-HT-NA reuptake inhibitors^{128, 129}, atypical antidepressant mianserin¹³⁰, combination of atypical antipsychotic with SSRIs¹³¹⁻¹³³, or deep brain stimulation of mPFC (infralimbic)¹³⁴ increase DA in the mPFC.

Second, acute treatment with a cannabinoid receptor 1 antagonist¹³⁵ or a triple monoamine uptake inhibitor¹³⁶, or chronic antidepressant treatment with reboxetine and mirtazapine¹³⁷ or a Chinese herbal prescription¹¹⁰, 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 cortex¹³⁸, prefrontal cortex (PFC)¹³⁹, or mPFC¹⁴⁰, and reduced immobility in the FST¹³⁸, increased active escape behavior¹³⁹, or the efficacy of antidepressants in reducing immobility in the FST¹⁴⁰.

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 expend effort for larger rewards, particularly when the probability of reward receipt was low¹⁴¹. 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 depletion¹⁴⁵, leads to decreased effortful behavior and motivation. Of note, this kind of effortful behavior and motivation is dysregulated in depression¹⁰⁷.

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 adrenocorticotrophic hormone (ACTH) and CORT in response to a 20 min restraint stress¹⁴⁸, and MDD patients manifest significantly thinned mPFC and higher CORT simultaneously³⁶. 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 CORT¹⁵⁰, 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 effect¹⁵¹⁻¹⁵³. 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 restraint¹⁵⁴. 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 rats¹⁵⁵. 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 replacement¹⁵⁶. Local injection of CORT into the mPFC increases DA in this brain area, which is blocked by a GR antagonist¹⁵⁷. 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 running¹⁶¹ and 6 weeks of wheel running¹⁰³ 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 enzyme¹⁶². 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 running¹⁶⁴ 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 disease¹⁶⁷. 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 NA¹⁶⁸ 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 blood¹⁶⁹, 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 shock¹⁷⁰, and increase 5-HT1A⁷⁶ and 5-HT1B¹⁰⁵ 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 tissue¹⁰¹. It is possible that the duration of wheel running (9-12 weeks⁷⁴, 6 weeks⁷⁶; 4-5 weeks¹⁷⁰, while 3 weeks in our experiment), type of stress (electric foot shock was used by¹⁷⁰, 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 μ l) was infused through each injector at a rate of 0.25 μ l/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.

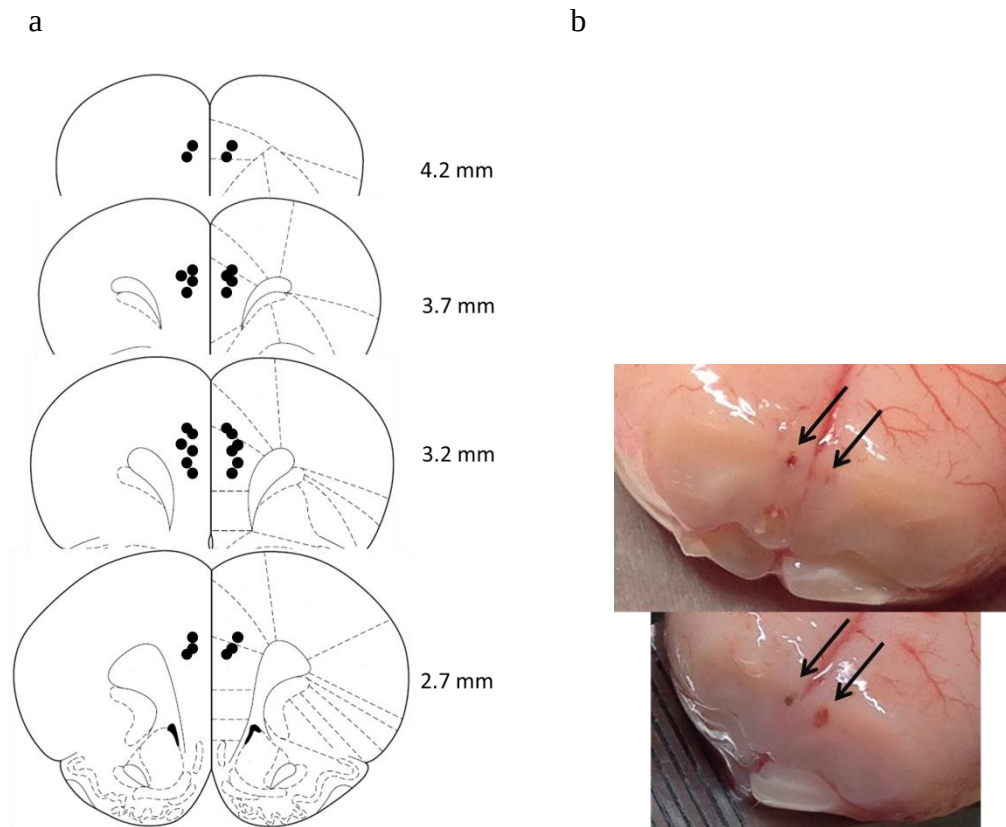


Figure 2-1 The placement of the injector cannulae tips. a, Schematic representation of bilateral placement of the injector cannulae tips; b, Actual photo taken from rats' brain during dissection showing the placement of injector cannulae tips.

Drugs

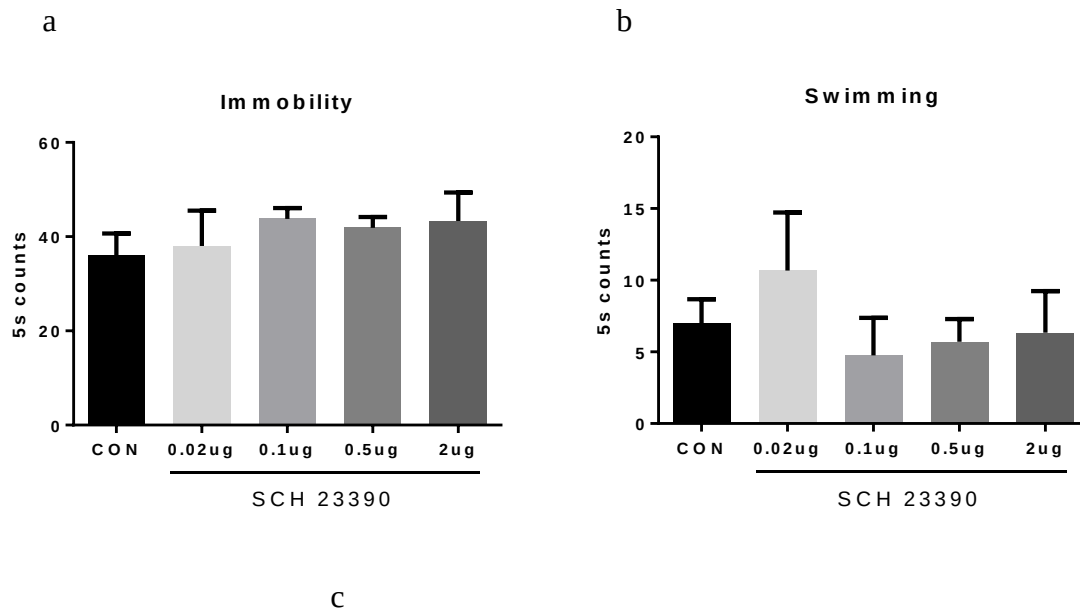
The D1R antagonist SCH 23390 (dissolved in saline, Cayman) and D2R antagonist haloperidol (dissolved in DMSO, Cayman) were used. The doses for each drug were determined by a pilot experiment below.

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.



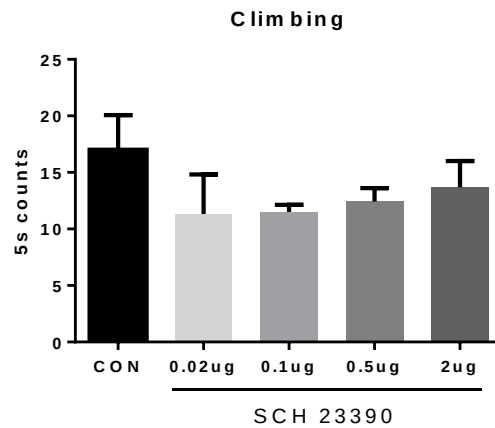


Figure 2-2 The effect of pre-intra-mPFC microinjection of SCH23390 on depression-like behavior. (a-c) The FST for assessing depression-like behavior. The duration of immobility (a), swimming (b), and climbing (c) in the 5 min FST for CON and different doses of SCH23390. (n=3-7/group) Mean±SEM.

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 μ M) which does not affect rats' performance in the FST for our experiment.

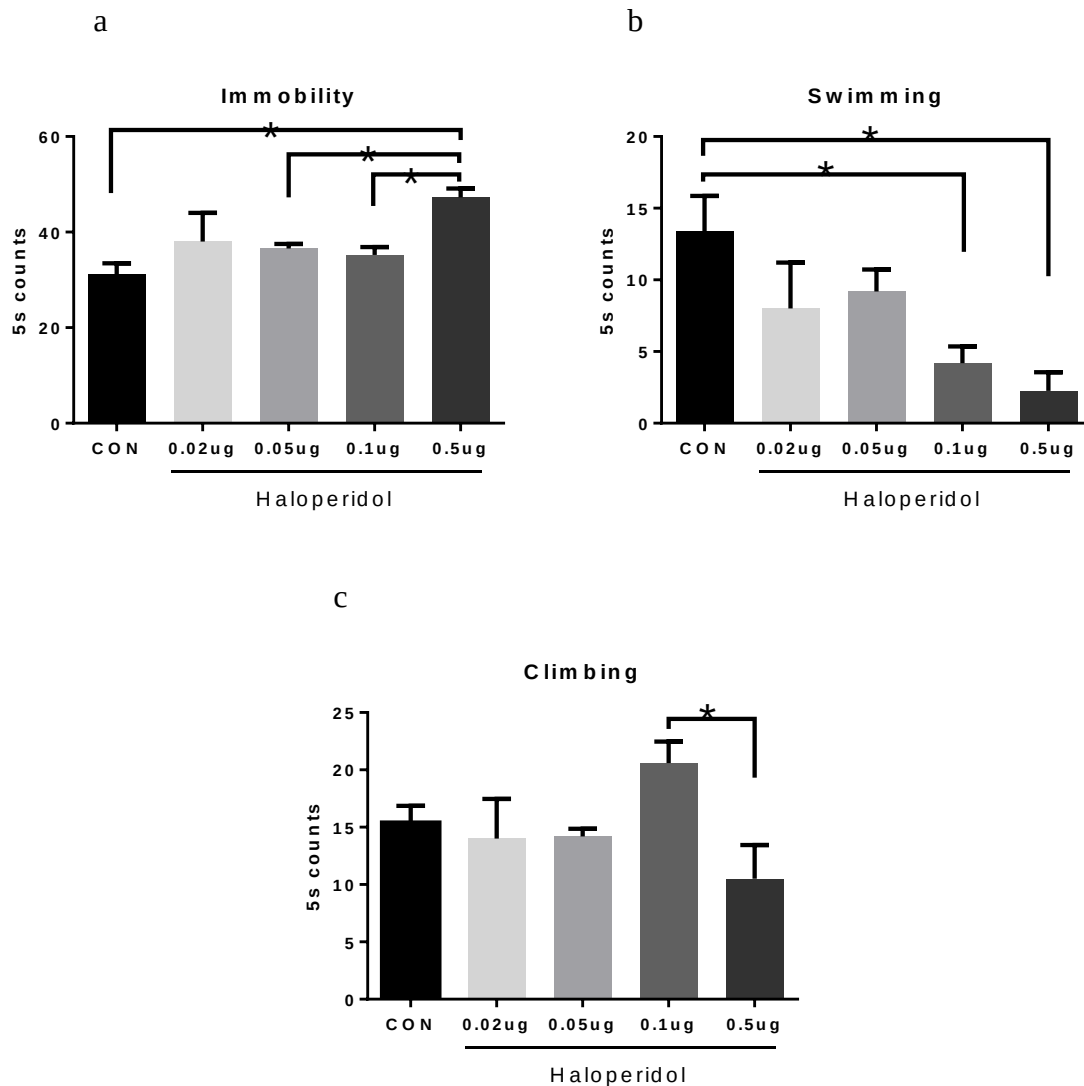


Figure 2-3 The dose-dependent effect of pre-intra-mPFC microinjection of haloperidol on depression-like behavior. (a-c) The FST for assessing depression-like behavior. The duration of immobility (a), swimming (b), and climbing (c) in the 5 min FST for CON and different doses of haloperidol. (n=3-5/group) 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 previously¹⁷². 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.

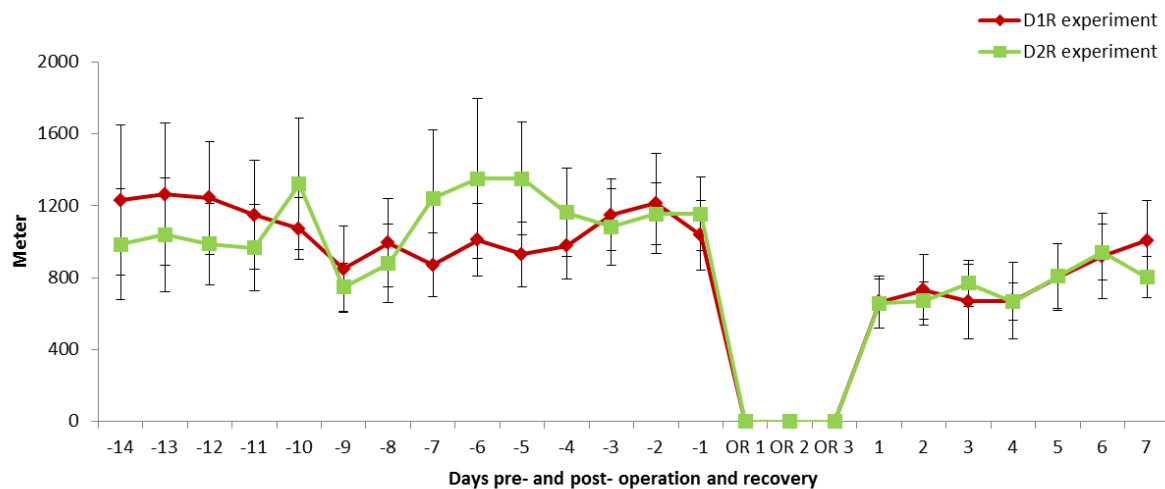
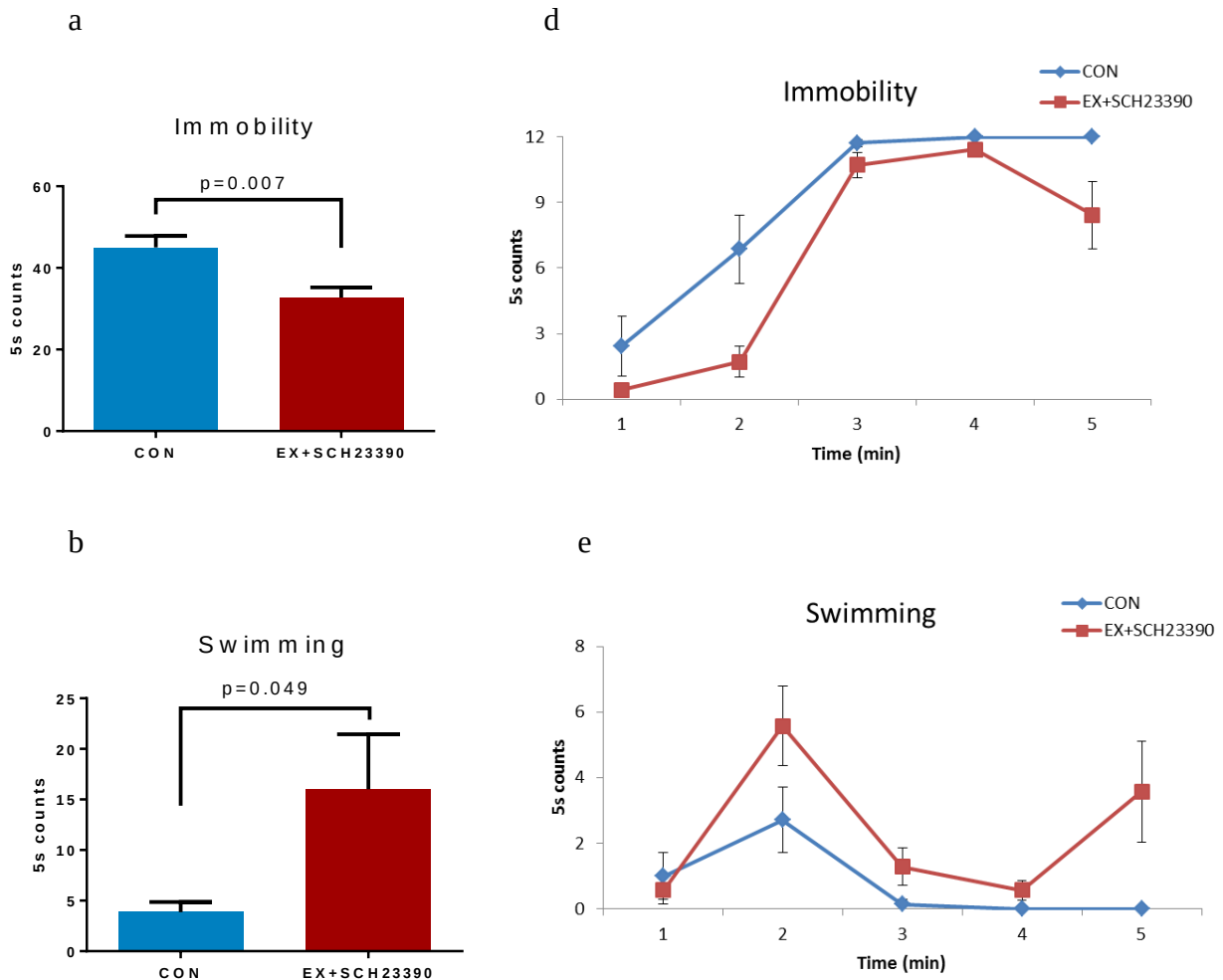


Figure 2-4 Mean running distance per day pre- and post- operation and recovery (OR) of rats for D1R (n=7) and D2R (n=6) antagonist experiment. Mean±SEM.

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).



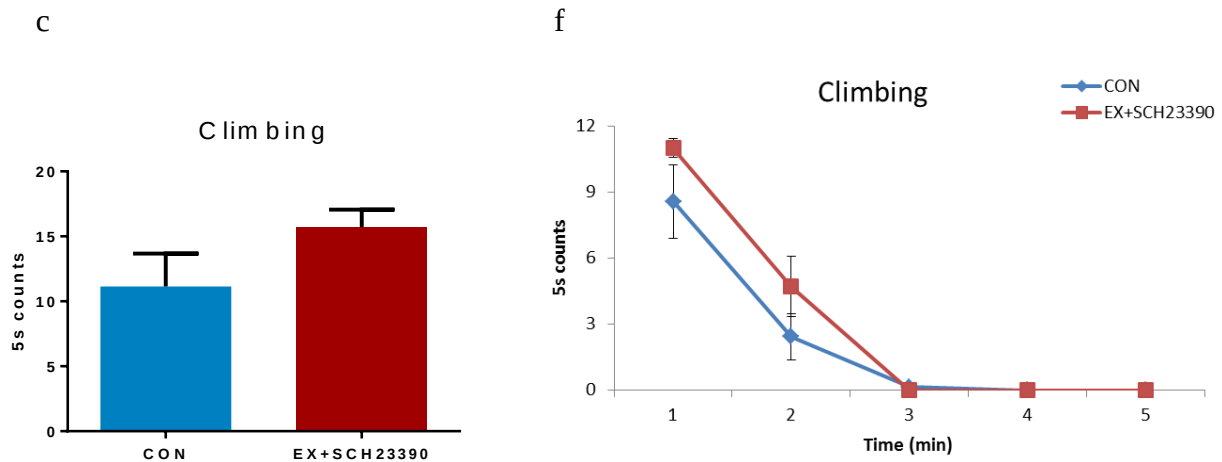


Figure 2-5 The antidepressant-like effect of exercise was not affected by pre-intra-mPFC microinjection of a D1R antagonist, SCH23390. (a-f) The FST for assessing antidepressant-like behavior. The duration of immobility, swimming, and climbing in the 5 min totally (a, b, c, in order) and across the 5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

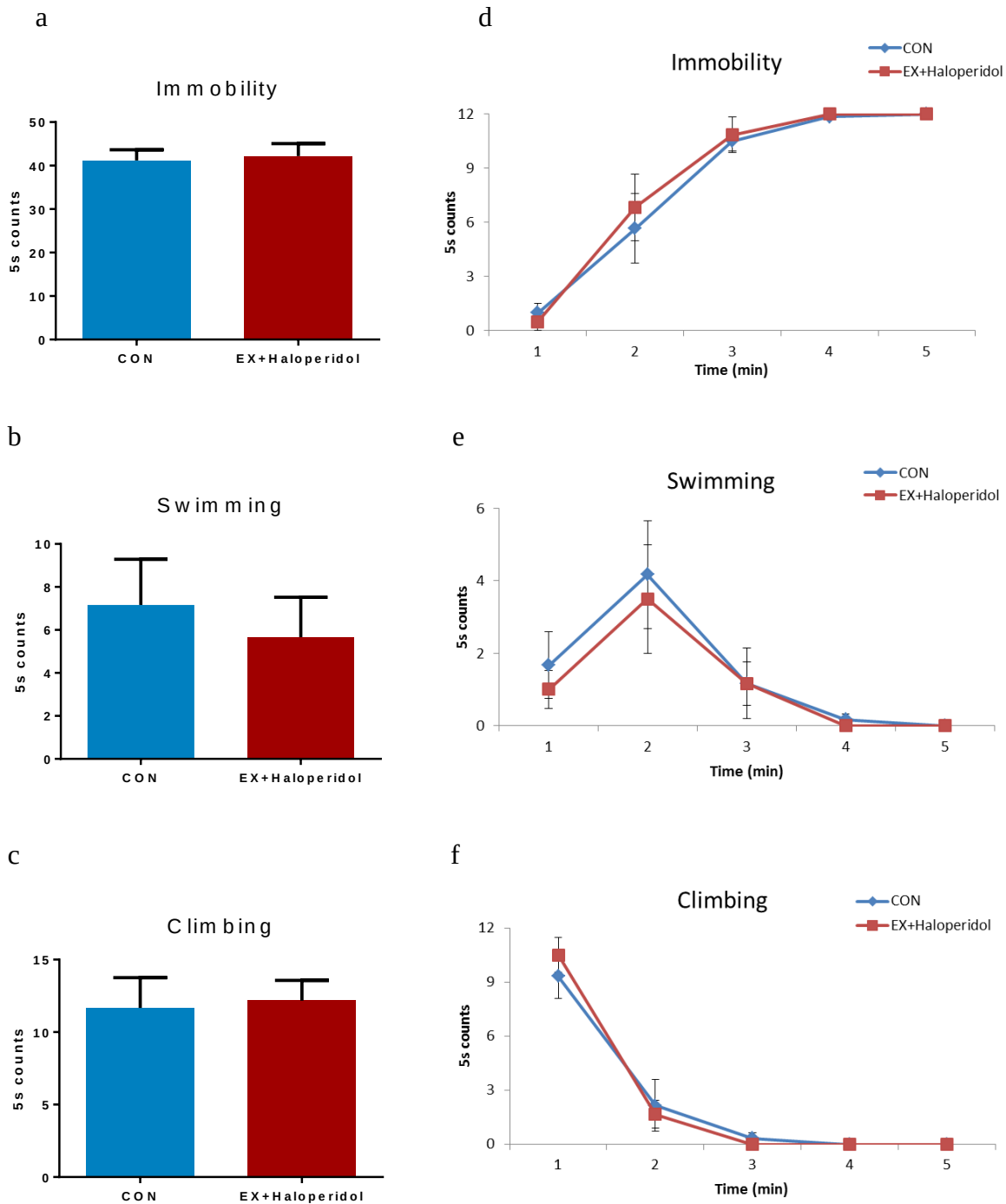


Figure 2-6 The antidepressant-like effect of exercise was completely abolished by pre-intra-mPFC microinjection of a D2R antagonist, haloperidol. (a-f) The FST for assessing antidepressant-like behavior. The duration of immobility, swimming, and climbing in the 5

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

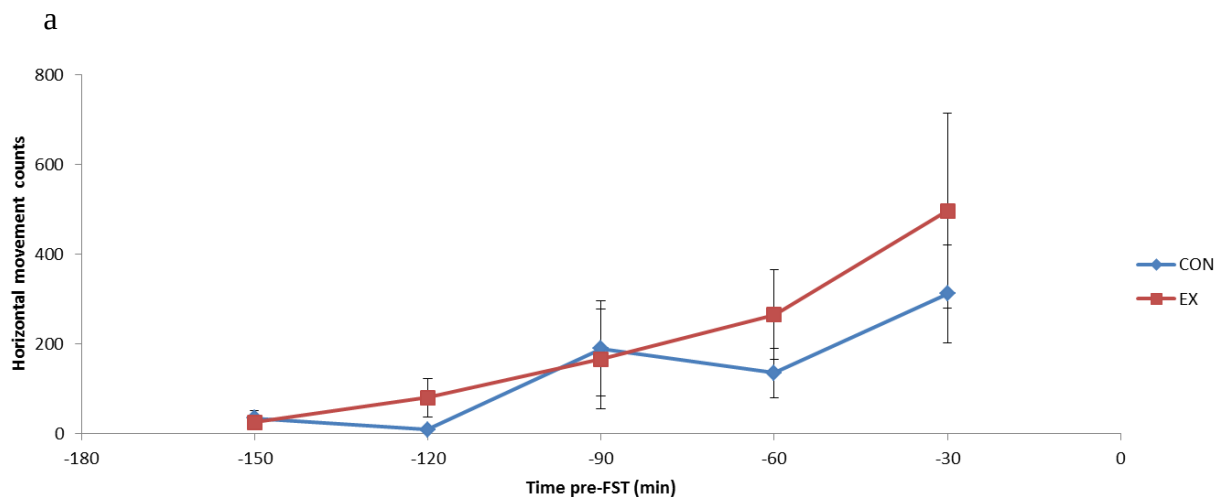
Mean±SEM.

	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.



b

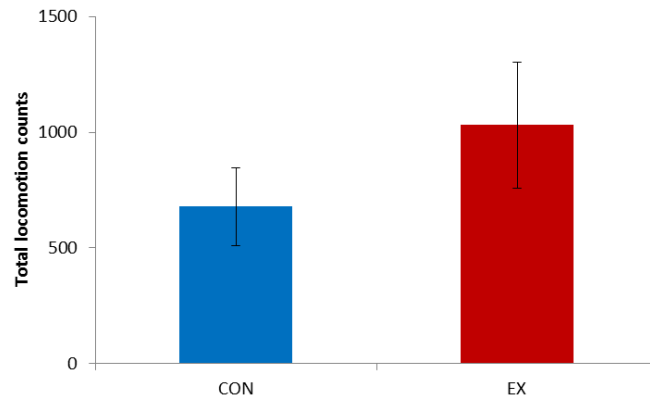


Figure 2-7 The effect of exercise on locomotion. The effect of exercise on horizontal movement counts by 30 min period (a) and in total (b) from -150 min to -30 min pre-FST. (n=7/group) Mean±SEM.

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 discussed¹⁰⁷. 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 patients¹⁷³⁻¹⁷⁸, and in reversing the motivational deficit induced by substantia nigra DA depletion¹⁷⁹ and preventing depression-like behavior¹⁸⁰ in rats. A recent report also showed that the D2R but not D1R antagonist abolishes the rapid antidepressant-like effect of ketamine in the FST¹⁸¹. 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 areas¹⁸², and D2R-like agonist binding potential¹⁸³. The results of our present experiment suggest that another likely area of the D2-dependent 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 sensitivity¹⁸⁴.

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-dependent¹⁸⁵⁻¹⁸⁷. 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 proposal¹⁸⁷. 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 stimuli¹⁸⁷.

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 escape⁴⁹⁻⁵¹. 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 recovery¹⁸⁸.

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 DRN¹⁸⁹. The Nacc has been identified as part of the brain circuitry regulating effort-related behavior or motivation^{190, 191} (or more broadly the striatum¹⁰⁷), 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 preference¹⁹². Similarly, optogenetic activation of the mPFC terminals in the DRN increased active coping behavior in the FST without affecting general locomotor activity¹⁹³. Actually the projection terminals in the Nacc and DRN primarily originate from the prelimbic part of the mPFC¹⁹⁴, 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 Nacc¹⁵⁹ 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 area¹⁹⁵. 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 lower¹⁹⁵. 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 DA¹⁹⁶. Nor is DA release in the PFC facilitated by DA antagonists¹⁹⁷. 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 autoreceptors¹⁹⁸. 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 μ l) was infused through each injector at a rate of 0.5 μ l/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.

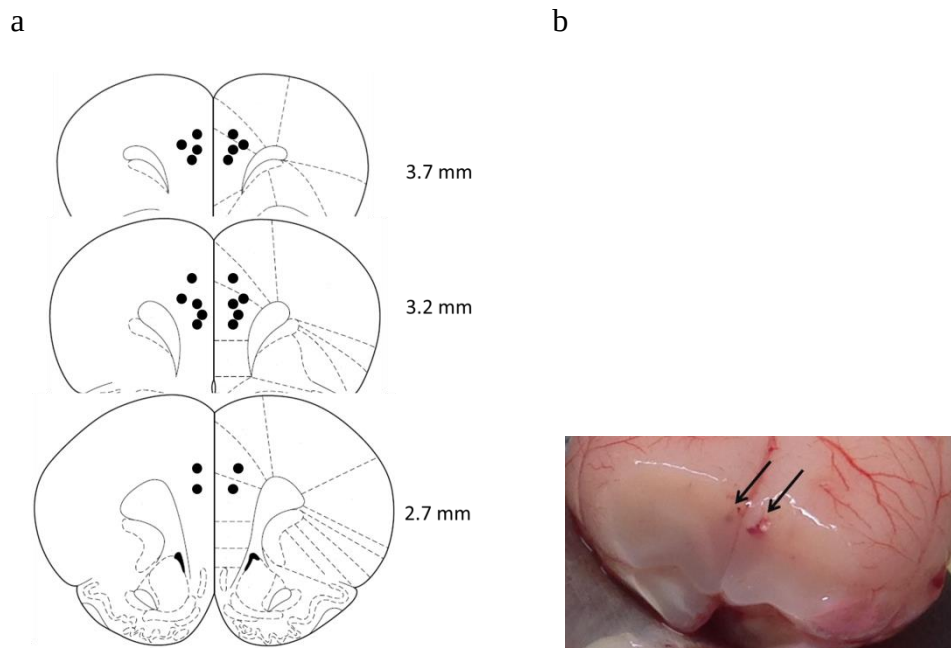


Figure 3-1 The placement of the injector cannulae tips. a, Schematic representation of bilateral placement of the injector cannulae tips; b, Actual photo taken from rats' brain during dissection showing the placement of injector cannulae tips.

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 needle 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 μ l/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).

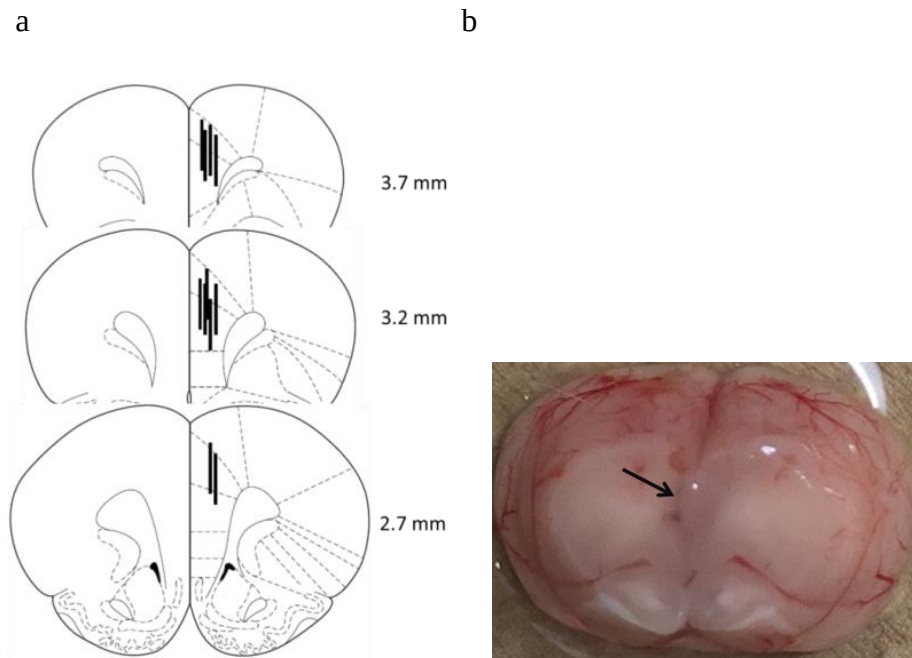


Figure 3-2 The placement of the microdialysis probe. Schematic representation of bilateral placement of the probe (a); Actual photo taken from rats' brain during dissection showing the placement of probe (b). Note that although not shown, the microinjection needles were closely coincided with the upper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

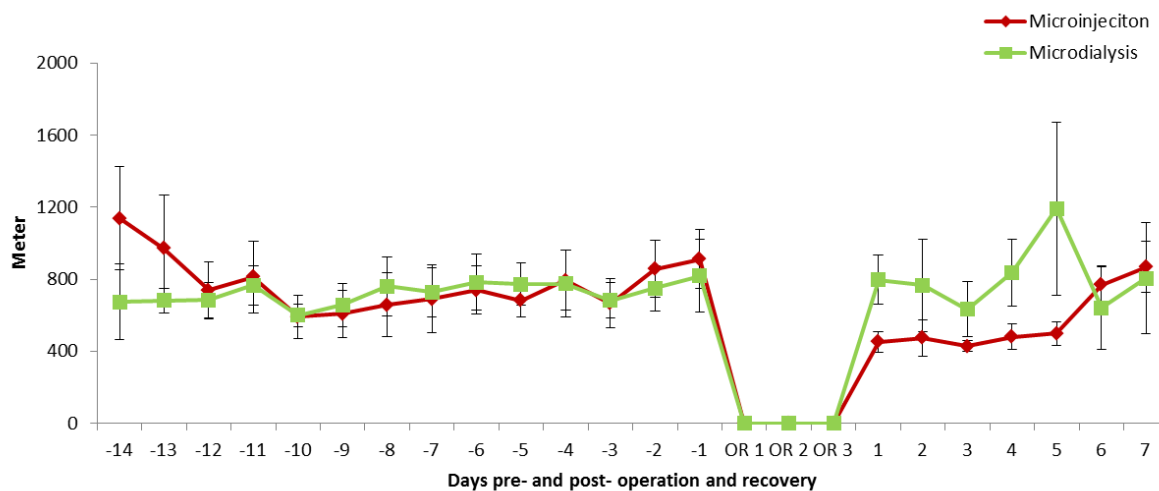
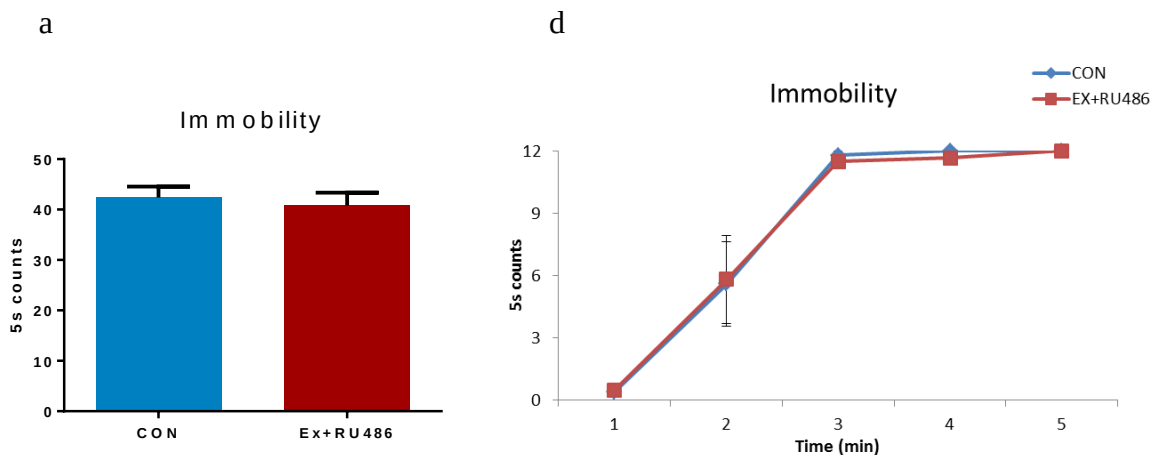


Figure 3-3 Mean running distance per day pre- and post- operation and recovery (OR) of EX rats for microinjection (n=6) and microdialysis (n=6) experiment. Mean±SEM.

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).



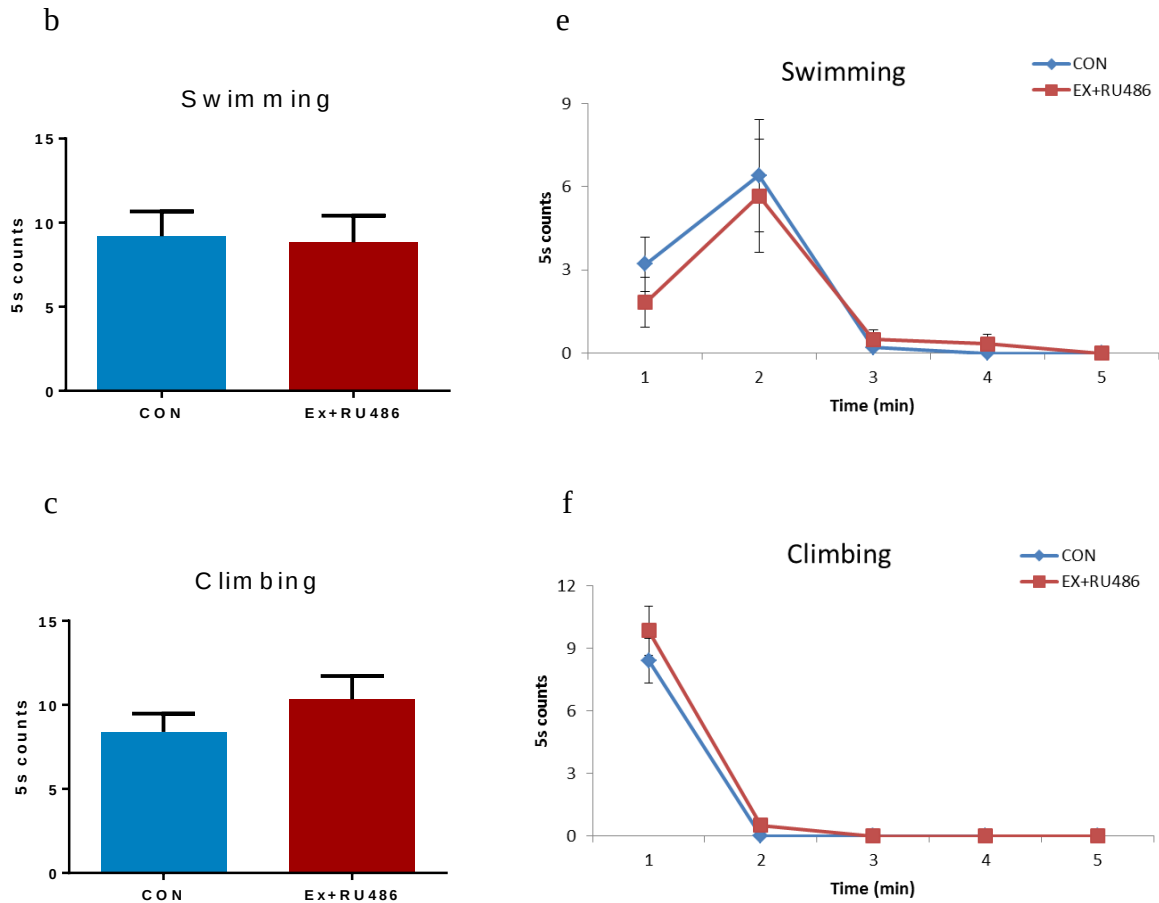


Figure 3-4 The antidepressant-like effect of exercise was completely abolished by pre-intra-mPFC microinjection of a GR antagonist, RU486. (a-f) The FST for assessing antidepressant-like behavior. The duration of immobility, swimming, and climbing in the 5 min totally (a, b, c, in order) and across the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

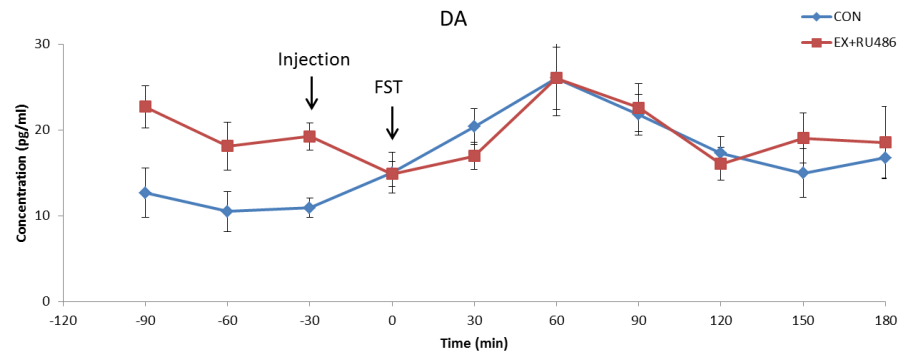
GR antagonist and the neurobiological effect of wheel running

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.

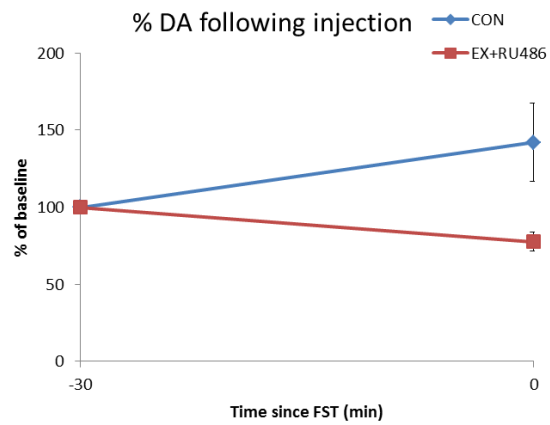
Following FST (from 0 to 180 min), there was a significant effect of time ($F=5.420$, $p<0.001$) but not group ($F=0.011$, $p=0.919$) or time*group ($F=0.513$, $p=0.795$). That is, there is no difference in DA levels between EX and CON rats in the FST. The percentage representation is shown in Figure 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.

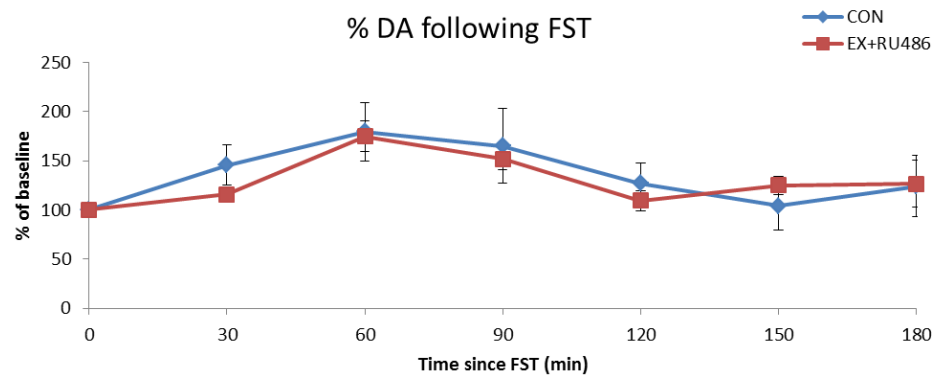
a



b



c



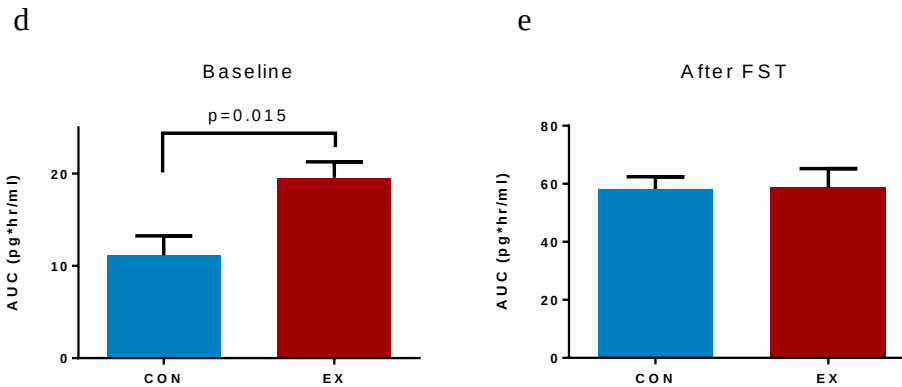


Figure 3-5 GR antagonist (RU486) reduces basal and FST-responsive DA in the mPFC of EX rats. The effect of exercise on DA (pg/ml) in the mPFC at baseline, following microinjection of RU486 and following the FST (a). % representation of DA in the mPFC following microinjection of RU486 (EX) or vehicle (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.

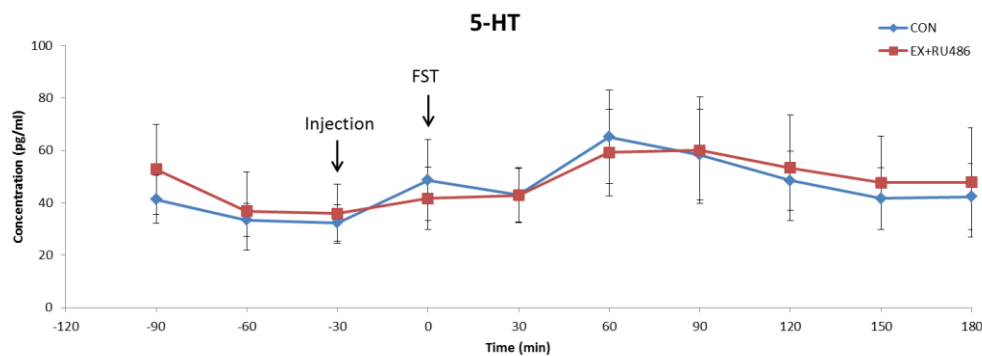


Figure 3-6 GR antagonist (RU486) did not influence basal or FST-responsive 5-HT in the mPFC of EX rats. The effect of exercise on 5-HT (pg/ml) in the mPFC at baseline, following microinjection of RU486 (EX) or vehicle (CON) and following the FST. (n=5/group) Mean±SEM.

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 μ L volume (which we used in the microinjection) 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 μ L/min (in the first experiment) to 1 μ L/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 mPFC¹⁹⁹.

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 inhibition²⁰⁰. For instance, lesion of infralimbic cortex decreases while lesion of prelimbic cortex prolongs HPA axis responses to acute stress (for a review, see²⁰⁰). 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 CORT¹⁵⁰. 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 rodents²⁰⁵, and that reward activates DA neurons in the VTA (for a review, see¹⁰⁷). Indeed, recently it has been demonstrated that tonic DA (i.e. basal DA) in the mPFC is correlated with long-term average reward²⁰⁶.

Our observed increased DA in the mPFC is also consistent with the finding that exercise improves executive control and working memory²⁰⁷⁻²⁰⁹, 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 function²¹². 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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References

- 1 Rethorst, C.D., Wipfli, B.M. & Landers, D.M. The antidepressive effects of exercise: a meta-analysis of randomized trials. *Sports Med.* **39**:491-511 (2009)
- 2 Brown, H. E. *et al.* Physical activity interventions and depression in children and adolescents. *Sports Med.* **43**, 195-206 (2013).
- 3 Cooney, G. M. *et al.* Exercise for depression. *Cochrane Database Syst Rev* **9** (2013).
- 4 Silveira, H. *et al.* Physical exercise and clinically depressed patients: a systematic review and meta-analysis. *Neuropsychobiology* **67**, 61-68 (2013).
- 5 Greenwood, B. N. & Fleshner, M. Exercise, learned helplessness, and the stress-resistant brain. *Neuromol. Med.* **10**, 81-98 (2008).
- 6 Greenwood, B. N. & Fleshner, M. Exercise, stress resistance, and central serotonergic systems. *Exerc. Sport Sci. Rev.* **39**, 140 (2011).
- 7 Novak, C. M., Burghardt, P. R. & Levine, J. A. The use of a running wheel to measure activity in rodents: relationship to energy balance, general activity, and reward. *Neurosci. Biobehav. Rev.* **36**, 1001-1014 (2012).
- 8 Prakash, R. S., Voss, M. W., Erickson, K. I. & Kramer, A. F. Physical activity and cognitive vitality. *Annu. Rev. Psychol* **66**, 769-797 (2015).
- 9 Lista, I. & Sorrentino, G. Biological mechanisms of physical activity in preventing cognitive decline. *Cell. Mol. Neurobiol.* **30**, 493-503 (2010).
- 10 Voss, M. W., Vivar, C., Kramer, A. F. & van Praag, H. Bridging animal and human models of exercise-induced brain plasticity. *TRENDS COGN. SCI.* **17**, 525-544 (2013).
- 11 Droste, S. K. *et al.* Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis. *Endocrinology* **144**, 3012-3023 (2003).
- 12 Makatsori, A. *et al.* Voluntary wheel running modulates glutamate receptor subunit gene expression and stress hormone release in Lewis rats. *Psychoneuroendocrinology* **28**, 702-714 (2003).
- 13 Stranahan, A. M., Khalil, D. & Gould, E. Social isolation delays the positive effects of running on adult neurogenesis. *Nat. Neurosci.* **9**, 526-533 (2006).

- 14 Luger, A. et al. Acute hypothalamic–pituitary–adrenal responses to the stress of treadmill exercise. *N. Engl. J. Med.* **316**, 1309-1315 (1987).
- 15 Loucks, A., Mortola, J., Girton, L. & Yen, S. Alterations in the Hypothalamic-Pituitary-Ovarian and the Hypothalamic-Pituitary-Adrenal Axes in Athletic Women*. *J. Clin. Endocrinol. Metab.* **68**, 402-411 (1989).
- 16 De Kloet, E. R., Joëls, M. & Holsboer, F. Stress and the brain: from adaptation to disease. *Nat. Rev. Neurosci.* **6**, 463-475 (2005).
- 17 Ulrich-Lai, Y. M. & Herman, J. P. Neural regulation of endocrine and autonomic stress responses. *Nat. Rev. Neurosci.* **10**, 397-409 (2009).
- 18 Sapolsky, R. M. Glucocorticoids, stress, and their adverse neurological effects: relevance to aging. *Exp. Gerontol.* **34**, 721-732 (1999).
- 19 Lupien, S. J., McEwen, B. S., Gunnar, M. R. & Heim, C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat. Rev. Neurosci.* **10**, 434-445 (2009).
- 20 Holsboer, F. & Ising, M. Stress hormone regulation: biological role and translation into therapy. *Annu. Rev. Psychol* **61**, 81-109 (2010).
- 21 Herman, J. P. Neural control of chronic stress adaptation. *Front. Behav. Neurosci.* **7** (2013).
- 22 Katz, R. J., Roth, K. A. & Carroll, B. J. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. *Neurosci. Biobehav. Rev.* **5**, 247-251 (1981).
- 23 Vogel, W. H. & Jensh, R. Chronic stress and plasma catecholamine and corticosterone levels in male rats. *Neurosci. Lett.* **87**, 183-188 (1988).
- 24 Herman, J., Adams, D. & Prewitt, C. Regulatory changes in neuroendocrine stress-integrative circuitry produced by a variable stress paradigm. *Neuroendocrinology* **61**, 180-190 (1995).
- 25 Zheng, H. et al. Beneficial effects of exercise and its molecular mechanisms on depression in rats. *Behav. Brain Res.* **168**, 47-55 (2006).

- 26 Pan, Y. *et al.* Icariin attenuates chronic mild stress-induced dysregulation of the LHPA stress circuit in rats. *Psychoneuroendocrinology* **35**, 272-283 (2010).
- 27 Yi, L.-T. *et al.* Circadian variations in behaviors, BDNF and cell proliferation in depressive mice. *Metab. Brain Dis.* 1-9 (2015).
- 28 Üresin, Y. *et al.* Losartan may prevent the elevation of plasma glucose, corticosterone and catecholamine levels induced by chronic stress. *J. Renin-Angiotensin-Aldosterone Syst.* **5**, 93-96 (2004).
- 29 Klein, F. *et al.* Prolonged increase of corticosterone secretion by chronic social stress does not necessarily impair immune functions. *Life Sci.* **50**, 723-731 (1992).
- 30 Keeney, A. J., Hogg, S. & Marsden, C. A. Alterations in core body temperature, locomotor activity, and corticosterone following acute and repeated social defeat of male NMRI mice. *Physiol. Behav.* **74**, 177-184 (2001).
- 31 Ottenweller, J. E., Natelson, B. H., Pitman, D. L. & Drastal, S. D. Adrenocortical and behavioral responses to repeated stressors: toward an animal model of chronic stress and stress-related mental illness. *Biol. Psychiatry* **26**, 829-841 (1989).
- 32 Cai, L. *et al.* Antidepressant-like effect of geniposide on chronic unpredictable mild stress-induced depressive rats by regulating the hypothalamus–pituitary–adrenal axis. *Eur. Neuropsychopharmacol.* (2015).
- 33 Sterner, E. Y. & Kalynchuk, L. E. Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: relevance to depression. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **34**, 777-790 (2010).
- 34 Fenton, E. Y. *et al.* Imipramine protects against the deleterious effects of chronic corticosterone on depression-like behavior, hippocampal reelin expression, and neuronal maturation. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **60**, 52-59 (2015).
- 35 Halbreich, U. *et al.* Cortisol secretion in endogenous depression: I. Basal plasma levels. *Arch. Gen. Psychiatry* **42**, 904-908 (1985).

- 36 Liu, X. *et al.* Relationship between the cortical thickness and serum cortisol levels in drug-naïve, first-episode patients with major depressive disorder: a surface-based morphometric study. *Depress. Anxiety* **32**, 702-708 (2015).
- 37 Lok, A. *et al.* Longitudinal hypothalamic–pituitary–adrenal axis trait and state effects in recurrent depression. *Psychoneuroendocrinology* **37**, 892-902 (2012).
- 38 Rhebergen, D. *et al.* Hypothalamic–pituitary–adrenal axis activity in older persons with and without a depressive disorder. *Psychoneuroendocrinology* **51**, 341-350 (2015).
- 39 Verduijn, J. *et al.* Pathophysiology of major depressive disorder: mechanisms involved in etiology are not associated with clinical progression. *Transl. Psychiatr.* **5**, e649 (2015).
- 40 Burke, H. M., Davis, M. C., Otte, C. & Mohr, D. C. Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology* **30**, 846-856 (2005).
- 41 Pariante, C. M. Risk factors for development of depression and psychosis. *Ann.NY Acad.Sci.* **1179**, 144-152 (2009).
- 42 Stetler, C. & Miller, G. E. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosom. Med.* **73**, 114-126 (2011).
- 43 Herbert, J. Cortisol and depression: three questions for psychiatry. *Psychol. Med.* **43**, 449-469 (2013).
- 44 Goodyer, I. M., Tamplin, A., Herbert, J. & Altham, P. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *Br. J. Psychiatry* **177**, 499-504 (2000).
- 45 Harris, T. *et al.* Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. *Br. J. Psychiatry* **177**, 505-510 (2000).
- 46 Porsolt, R. D., Le Pichon, M. & Jalfre, M. Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**, 730-732 (1977).

- 47 Detke, M. J. & Lucki, I. Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. *Behav. Brain Res.* **73**, 43-46 (1995).
- 48 Lucki, I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav. Pharmacol.* **8**, 523-532 (1997).
- 49 Cryan, J. F., Markou, A. & Lucki, I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol. Sci.* **23**, 238-245 (2002).
- 50 Cryan, J. F., Valentino, R. J. & Lucki, I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci. Biobehav. Rev.* **29**, 547-569 (2005).
- 51 Slattery, D. A. & Cryan, J. F. Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nat. Protoc.* **7**, 1009-1014 (2012).
- 52 Westerink, B. H. & Cremers, T. I. *Handbook of microdialysis: methods, applications and perspectives*. Vol. 16 (Academic Press, 2007).
- 53 Kitaichi, Y. *et al.* Effect of milnacipran on extracellular monoamine concentrations in the medial prefrontal cortex of rats pre-treated with lithium. *Eur. J. Pharmacol.* **516**, 219-226 (2005).
- 54 Kitaichi, Y. *et al.* Sertraline increases extracellular levels not only of serotonin, but also of dopamine in the nucleus accumbens and striatum of rats. *Eur. J. Pharmacol.* **647**, 90-96 (2010).
- 55 Abekawa, T., Ito, K. & Koyama, T. Role of the simultaneous enhancement of NMDA and dopamine D1 receptor-mediated neurotransmission in the effects of clozapine on phencyclidine-induced acute increases in glutamate levels in the rat medial prefrontal cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **374**, 177-193 (2006).
- 56 Maier, S. F. & Watkins, L. R. Role of the medial prefrontal cortex in coping and resilience. *Brain Res.* **1355**, 52-60 (2010).

- 57 Maier, S. F. Behavioral control blunts reactions to contemporaneous and future adverse events: medial prefrontal cortex plasticity and a corticostriatal network. *Neurobiol. Stress* **1**, 12-22 (2015).
- 58 Paxinos, G. & Watson, C. The Rat Brain in Stereotaxic Coordinates, 6th Edition. Academic Press (2006)
- 59 Castagné, V., Moser, P. & Porsolt, R. Behavioral assessment of antidepressant activity in rodents. In: Buccafusco JJ, editor. *Methods of Behavior Analysis in Neuroscience*. 2nd edition. Boca Raton (FL): CRC Press. Chapter 6. (2009).
- 60 Gomes, K. M. *et al.* Evaluation of light/dark cycle in anxiety-and depressive-like behaviors after regular treatment with methylphenidate hydrochloride in rats of different ages. *Rev. Bras. Psiquiatr.* **33**, 55-58 (2011).
- 61 Droste, S. K. *et al.* Corticosterone levels in the brain show a distinct ultradian rhythm but a delayed response to forced swim stress. *Endocrinology* **149**, 3244-3253 (2008).
- 62 Droste, S. K. *et al.* Distinct, time-dependent effects of voluntary exercise on circadian and ultradian rhythms and stress responses of free corticosterone in the rat hippocampus. *Endocrinology* **150**, 4170-4179 (2009).
- 63 Droste, S. *et al.* The ultradian and circadian rhythms of free corticosterone in the brain are not affected by gender: an in vivo microdialysis study in Wistar rats. *J. Neuroendocrinol.* **21**, 132-140 (2009).
- 64 Bouchez, G. *et al.* Quantification of extracellular levels of corticosterone in the basolateral amygdaloid complex of freely-moving rats: a dialysis study of circadian variation and stress-induced modulation. *Brain Res.* **1452**, 47-60 (2012).
- 65 Qian, X. *et al.* Circadian and ultradian rhythms of free glucocorticoid hormone are highly synchronized between the blood, the subcutaneous tissue, and the brain. *Endocrinology* **153**, 4346-4353 (2012).
- 66 Boku, S. *et al.* Glucocorticoids and lithium reciprocally regulate the proliferation of adult dentate gyrus-derived neural precursor cells through GSK-3 β and β -catenin/TCF pathway. *Neuropsychopharmacology* **34**, 805-815 (2009).

- 67 Narushima, M. *et al.* Depolarization-induced suppression of inhibition mediated by endocannabinoids at synapses from fast-spiking interneurons to medium spiny neurons in the striatum. *Eur. J. Neurosci.* **24**, 2246-2252 (2006).
- 68 Reul, J. & Kloet, E. D. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology* **117**, 2505-2511 (1985).
- 69 Solberg, L. C., Horton, T. H. & Turek, F. W. Circadian rhythms and depression: effects of exercise in an animal model. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **276**, R152-R161 (1999).
- 70 Bjornebekk, A., Mathé, A. A. & Brené, S. The antidepressant effect of running is associated with increased hippocampal cell proliferation. *Int. J. Neuropsychopharmacol.* **8**, 357-368 (2005).
- 71 Bjørnebekk, A., Mathé, A. A., Gruber, S. H. & Brené, S. Housing conditions modulate escitalopram effects on antidepressive-like behaviour and brain neurochemistry. *Int. J. Neuropsychopharmacol.* **11**, 1135-1147 (2008).
- 72 Duman, C. H., Schlesinger, L., Russell, D. S. & Duman, R. S. Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain Res.* **1199**, 148-158 (2008).
- 73 Cunha, M. P. *et al.* The antidepressant-like effect of physical activity on a voluntary running wheel. *Med Sci Sports Exerc* **45**, 851-859 (2013).
- 74 Kondo, M., Nakamura, Y., Ishida, Y. & Shimada, S. The 5-HT₃ receptor is essential for exercise-induced hippocampal neurogenesis and antidepressant effects. *Mol. Psychiatry*. **20**, 1428-1437 (2015).
- 75 Dishman, R. K. *et al.* Activity wheel running reduces escape latency and alters brain monoamine levels after footshock. *Brain Res. Bull.* **42**, 399-406 (1997).
- 76 Moraska, A. & Fleshner, M. Voluntary physical activity prevents stress-induced behavioral depression and anti-KLH antibody suppression. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281**, R484-R489 (2001).

- 77 Greenwood, B. N. *et al.* Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. *J. Neurosci.* **23**, 2889-2898 (2003).
- 78 Otawa, M., Arai, H. & Atomi, Y. Molecular aspects of adrenal regulation for circadian glucocorticoid synthesis by chronic voluntary exercise. *Life Sci.* **80**, 725-731 (2007).
- 79 Ondkova, S. *et al.* Changes in retinoic acid receptor status, 5'-deiodinase activity and neuroendocrine response to voluntary wheel running. *Gen. Comp. Endocrinol.* **165**, 304-308 (2010).
- 80 Hare, B. D. *et al.* Exercise-associated changes in the corticosterone response to acute restraint stress: evidence for increased adrenal sensitivity and reduced corticosterone response duration. *Neuropsychopharmacology* **39**, 1262-1269 (2014).
- 81 Klok, M. D. *et al.* Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. *J. Psychiatr. Res.* **45**, 871-878 (2011).
- 82 ter Heegde, F., De Rijk, R. H. & Vinkers, C. H. The brain mineralocorticoid receptor and stress resilience. *Psychoneuroendocrinology* **52**, 92-110 (2015).
- 83 Barden, N. Implication of the hypothalamic–pituitary–adrenal axis in the physiopathology of depression. *J. Psychiatry Neurosci.* **29**, 185 (2004).
- 84 Anacker, C., Zunszain, P. A., Carvalho, L. A. & Pariante, C. M. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? *Psychoneuroendocrinology* **36**, 415-425 (2011).
- 85 Boyle, M. P. *et al.* Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 473-478 (2005).
- 86 Droste, S. K. *et al.* Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. *Neuroendocrinology* **86**, 26-37 (2007).

- 87 Clemmensen, C. *et al.* Enhanced voluntary wheel running in GPRC6A receptor knockout mice. *Physiol. Behav.* **118**, 144-151 (2013).
- 88 Fediuc, S., Campbell, J. E. & Riddell, M. C. Effect of voluntary wheel running on circadian corticosterone release and on HPA axis responsiveness to restraint stress in Sprague-Dawley rats. *J. Appl. Physiol.* **100**, 1867-1875 (2006).
- 89 Griesbach, G. S. *et al.* Differential effects of voluntary and forced exercise on stress responses after traumatic brain injury. *J. Neurotrauma* **29**, 1426-1433 (2012).
- 90 Santos-Soto, I. J. *et al.* Voluntary running in young adult mice reduces anxiety-like behavior and increases the accumulation of bioactive lipids in the cerebral cortex. *PLoS One* **8**:e81459 (2013).
- 91 Droste, S., Schweizer, M., Ulbricht, S. & Reul, J. Long - Term Voluntary Exercise and the Mouse Hypothalamic - Pituitary - Adrenocortical Axis: Impact of Concurrent Treatment with the Antidepressant Drug Tianeptine. *J. Neuroendocrinol.* **18**, 915-925 (2006).
- 92 Campeau, S. *et al.* Hypothalamic Pituitary Adrenal Axis Responses to Low - Intensity Stressors are Reduced After Voluntary Wheel Running in Rats. *J. Neuroendocrinol.* **22**, 872-888 (2010).
- 93 Speaker, K. J. *et al.* Six weeks of voluntary wheel running modulates inflammatory protein (MCP-1, IL-6, and IL-10) and DAMP (Hsp72) responses to acute stress in white adipose tissue of lean rats. *Brain Behav. Immun.* **39**, 87-98 (2014).
- 94 Starzec, J. J., Berger, D. F. & Hesse, R. Effects of stress and exercise on plasma corticosterone, plasma cholesterol, and aortic cholesterol levels in rats. *Psychosom. Med.* **45**, 219-226 (1983).
- 95 Sasse, S. K. *et al.* Chronic voluntary wheel running facilitates corticosterone response habituation to repeated audiogenic stress exposure in male rats: Original Research Report. *Stress* **11**, 425-437 (2008).
- 96 Radley, J. J. & Sawchenko, P. E. Evidence for Involvement of a Limbic-Paraventricular Hypothalamic Inhibitory Network in HPA Axis Adaptations to Repeated Stress. *J. Comp. Neurol.* (2015).

- 97 Reul, J., Stec, I., Söder, M. & Holsboer, F. Chronic treatment of rats with the antidepressant amitriptyline attenuates the activity of the hypothalamic-pituitary-adrenocortical system. *Endocrinology* **133**, 312-320 (1993).
- 98 Korte, S. *et al.* Conditioned neuroendocrine and cardiovascular stress responsiveness accompanying behavioral passivity and activity in aged and in young rats. *Physiol. Behav.* **51**, 815-822 (1992).
- 99 Ising, M. *et al.* The combined dexamethasone/CRH test as a potential surrogate marker in depression. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **29**, 1085-1093 (2005).
- 100 Hasler, G., Drevets, W. C., Manji, H. K. & Charney, D. S. Discovering endophenotypes for major depression. *Neuropsychopharmacology* **29**, 1765-1781 (2004).
- 101 Renoir, T., Chevarin, C., Lanfumey, L. & Hannan, A. J. Effect of enhanced voluntary physical exercise on brain levels of monoamines in Huntington disease mice. *PLoS curr.* **3** (2011).
- 102 MacRae, P. *et al.* Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine metabolites in presenescent older rats. *Psychopharmacology* **92**, 236-240 (1987).
- 103 Greenwood, B. N. *et al.* Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. *Behav. Brain Res.* **217**, 354-362 (2011).
- 104 Bronikowski, A. *et al.* The evolution of gene expression in mouse hippocampus in response to selective breeding for increased locomotor activity. *Evolution* **58**, 2079-2086 (2004).
- 105 Foley, T. E. *et al.* Elevated central monoamine receptor mRNA in rats bred for high endurance capacity: implications for central fatigue. *Behav. Brain Res.* **174**, 132-142 (2006).
- 106 Russo, S. J. & Nestler, E. J. The brain reward circuitry in mood disorders. *Nat. Rev. Neurosci.* **14**, 609-625 (2013).

- 107 Chen, C. *et al.* Reinforcement learning in depression: A review of computational research. *Neurosci. Biobehav. Rev.* **55**, 247-267 (2015).
- 108 Ahmad, A., Rasheed, N., Banu, N. & Palit, G. Alterations in monoamine levels and oxidative systems in frontal cortex, striatum, and hippocampus of the rat brain during chronic unpredictable stress. *Stress* **13**, 356-365 (2010).
- 109 Rasheed, N. *et al.* Differential response of central dopaminergic system in acute and chronic unpredictable stress models in rats. *Neurochem. Res.* **35**, 22-32 (2010).
- 110 Sun, X.-P. *et al.* Antidepressant-like effects and memory enhancement of a herbal formula in mice exposed to chronic mild stress. *Neurosci. Bull.* **29**, 737-744 (2013).
- 111 Matuszewich, L., McFadden, L. M., Friedman, R. D. & Frye, C. A. Neurochemical and behavioral effects of chronic unpredictable stress. *Behav. Pharmacol.* **25**, 557-566 (2014).
- 112 Mizoguchi, K. *et al.* Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. *J. Neurosci.* **20**, 1568-1574 (2000).
- 113 Mangiavacchi, S. *et al.* Long-term behavioral and neurochemical effects of chronic stress exposure in rats. *J. Neurochem.* **79**, 1113-1121 (2001).
- 114 Mizoguchi, K. *et al.* Persistent depressive state after chronic stress in rats is accompanied by HPA axis dysregulation and reduced prefrontal dopaminergic neurotransmission. *Pharmacol. Biochem. Behav.* **91**, 170-175 (2008).
- 115 Gresch, P. J., Sved, A. F., Zigmond, M. J. & Finlay, J. M. Stress-induced sensitization of dopamine and norepinephrine efflux in medial prefrontal cortex of the rat. *J. Neurochem.* **63**, 575-583 (1994).
- 116 Heidbreder, C. *et al.* Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. *Neuroscience* **100**, 749-768 (2000).
- 117 Watt, M. J., Burke, A. R., Renner, K. J. & Forster, G. L. Adolescent male rats exposed to social defeat exhibit altered anxiety behavior and limbic monoamines as adults. *Behav. Neurosci.* **123**, 564 (2009).

- 118 Burke, A. R., Watt, M. J. & Forster, G. L. Adolescent social defeat increases adult amphetamine conditioned place preference and alters D2 dopamine receptor expression. *Neuroscience* **197**, 269-279 (2011).
- 119 Burke, A. R. *et al.* Effects of adolescent social defeat on adult amphetamine-induced locomotion and corticoaccumbal dopamine release in male rats. *Neuropharmacology* **67**, 359-369 (2013).
- 120 Novick, A. M. *et al.* Increased dopamine transporter function as a mechanism for dopamine hypoactivity in the adult infralimbic medial prefrontal cortex following adolescent social stress. *Neuropharmacology* **97**, 194-200 (2015).
- 121 Kwon, S. *et al.* Prefrontal-limbic change in dopamine turnover by acupuncture in maternally separated rat pups. *Neurochem. Res.* **37**, 2092-2098 (2012).
- 122 Jordan, S. *et al.* In vivo biogenic amine efflux in medial prefrontal cortex with imipramine, fluoxetine, and fluvoxamine. *Synapse* **18**, 294-297 (1994).
- 123 Pozzi, L. *et al.* Evidence that extracellular concentrations of dopamine are regulated by noradrenergic neurons in the frontal cortex of rats. *J. Neurochem.* **63**, 195-200 (1994).
- 124 Tanda, G., Carboni, E., Frau, R. & Di Chiara, G. Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacology* **115**, 285-288 (1994).
- 125 Matsumoto, M. *et al.* Characterization of endogenous serotonin-mediated regulation of dopamine release in the rat prefrontal cortex. *Eur. J. Pharmacol.* **383**, 39-48 (1999).
- 126 Bymaster, F. P. *et al.* Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology* **160**, 353-361 (2002).
- 127 Arborelius, L., Nomikos, G., Hacksell, U. & Svensson, T. (R)-8-OH-DPAT preferentially increases dopamine release in rat medial prefrontal cortex. *Acta Physiol. Scand.* **148**, 465-466 (1993).

- 128 Linnér, L. *et al.* Reboxetine modulates the firing pattern of dopamine cells in the ventral tegmental area and selectively increases dopamine availability in the prefrontal cortex. *J. Pharmacol. Exp. Ther.* **297**, 540-546 (2001).
- 129 Masana, M., Bortolozzi, A. & Artigas, F. Selective enhancement of mesocortical dopaminergic transmission by noradrenergic drugs: therapeutic opportunities in schizophrenia. *Int. J. Neuropsychopharmacol.* **14**, 53-68 (2011).
- 130 Tanda, G., Bassareo, V. & Chiara, D. Mianserin markedly and selectively increases extracellular dopamine in the prefrontal cortex as compared to the nucleus accumbens of the rat. *Psychopharmacology* **123**, 127-130 (1996).
- 131 Zhang, W. *et al.* Synergistic effects of olanzapine and other antipsychotic agents in combination with fluoxetine on norepinephrine and dopamine release in rat prefrontal cortex. *Neuropsychopharmacology* **23**, 250-262 (2000).
- 132 Koch, S., Perry, K. W. & Bymaster, F. P. Brain region and dose effects of an olanzapine/fluoxetine combination on extracellular monoamine concentrations in the rat. *Neuropharmacology* **46**, 232-242 (2004).
- 133 Ago, Y., Nakamura, S., Baba, A. & Matsuda, T. Sulpiride in combination with fluvoxamine increases in vivo dopamine release selectively in rat prefrontal cortex. *Neuropsychopharmacology* **30**, 43-51 (2005).
- 134 Jiménez-Sánchez, L. *et al.* Activation of AMPA Receptors Mediates the Antidepressant Action of Deep Brain Stimulation of the Infralimbic Prefrontal Cortex. *Cereb. Cortex*, bhv133 (2015).
- 135 Tzavara, E. T. *et al.* The CB1 receptor antagonist SR141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: implications for therapeutic actions. *Br. J. Pharmacol.* **138**, 544-553 (2003).
- 136 Popik, P. *et al.* Pharmacological profile of the “triple” monoamine neurotransmitter uptake inhibitor, DOV 102,677. *Cell. Mol. Neurobiol.* **26**, 855-871 (2006).
- 137 Masana, M. *et al.* Noradrenergic antidepressants increase cortical dopamine: potential use in augmentation strategies. *Neuropharmacology* **63**, 675-684 (2012).

- 138 Weiss, J. M. *et al.* Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. *Brain Res.* **3**, 167-205 (1981).
- 139 Carlson, J. N., Fitzgerald, L. W., Keller, R. W. & Glick, S. D. Lateralized changes in prefrontal cortical dopamine activity induced by controllable and uncontrollable stress in the rat. *Brain Res. Brain Res.Rev.* **630**, 178-187 (1993).
- 140 Kobayashi, T. *et al.* Neurochemical responses to antidepressants in the prefrontal cortex of mice and their efficacy in preclinical models of anxiety-like and depression-like behavior: a comparative and correlational study. *Psychopharmacology* **197**, 567-580 (2008).
- 141 Treadway, M. T. *et al.* Dopaminergic mechanisms of individual differences in human effort-based decision-making. *J. Neurosci.* **32**, 6170-6176 (2012).
- 142 Cetin, T., Freudenberg, F., Füchtemeier, M. & Koch, M. Dopamine in the orbitofrontal cortex regulates operant responding under a progressive ratio of reinforcement in rats. *Neurosci. Lett.* **370**, 114-117 (2004).
- 143 Winstanley, C. A. *et al.* Dopaminergic modulation of the orbitofrontal cortex affects attention, motivation and impulsive responding in rats performing the five-choice serial reaction time task. *Behav. Brain Res.* **210**, 263-272 (2010).
- 144 Schweimer, J. & Hauber, W. Dopamine D1 receptors in the anterior cingulate cortex regulate effort-based decision making. *Learn. Mem.* **13**, 777-782 (2006).
- 145 Schweimer, J., Saft, S. & Hauber, W. Involvement of catecholamine neurotransmission in the rat anterior cingulate in effort-related decision making. *Behav. Neurosci.* **119**, 1687 (2005).
- 146 Sullivan, R. M. & Gratton, A. Prefrontal cortical regulation of hypothalamic–pituitary–adrenal function in the rat and implications for psychopathology: side matters. *Psychoneuroendocrinology* **27**, 99-114 (2002).
- 147 Herman, J. P. *et al.* Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo–pituitary–adrenocortical responsiveness. *Front. Neuroendocrinol.* **24**, 151-180 (2003).

- 148 Diorio, D., Viau, V. & Meaney, M. J. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J. Neurosci.* **13**, 3839-3847 (1993).
- 149 Figueiredo, H. F. *et al.* The medial prefrontal cortex differentially regulates stress-induced c-fos expression in the forebrain depending on type of stressor. *Eur. J. Neurosci.* **18**, 2357-2364 (2003).
- 150 Sullivan, R. M. & Dufresne, M. M. Mesocortical dopamine and HPA axis regulation: role of laterality and early environment. *Brain Res.* **1076**, 49-59 (2006).
- 151 Radley, J. J., Gosselink, K. L. & Sawchenko, P. E. A discrete GABAergic relay mediates medial prefrontal cortical inhibition of the neuroendocrine stress response. *J. Neurosci.* **29**, 7330-7340 (2009).
- 152 Radley, J. J. & Sawchenko, P. E. A common substrate for prefrontal and hippocampal inhibition of the neuroendocrine stress response. *J. Neurosci.* **31**, 9683-9695 (2011).
- 153 Radley, J. J. Toward a limbic cortical inhibitory network: implications for hypothalamic-pituitary-adrenal responses following chronic stress. *Front. Behav. Neurosci.* **6** (2012).
- 154 Akana, S., Chu, A., Soriano, L. & Dallman, M. Corticosterone Exerts Site - Specific and State - Dependent Effects in Prefrontal Cortex and Amygdala on Regulation of Adrenocorticotrophic Hormone, Insulin and Fat Depots. *J. Neuroendocrinol.* **13**, 625-637 (2001).
- 155 McKlveen, J. M. *et al.* Role of prefrontal cortex glucocorticoid receptors in stress and emotion. *Biol. Psychiatry* **74**, 672-679 (2013).
- 156 Mizoguchi, K. *et al.* Endogenous glucocorticoids are essential for maintaining prefrontal cortical cognitive function. *J. Neurosci.* **24**, 5492-5499 (2004).
- 157 Butts, K. A., Weinberg, J., Young, A. H. & Phillips, A. G. Glucocorticoid receptors in the prefrontal cortex regulate stress-evoked dopamine efflux and aspects of executive function. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 18459-18464 (2011).

- 158 Butts, K. A. & Phillips, A. G. Glucocorticoid receptors in the prefrontal cortex regulate dopamine efflux to stress via descending glutamatergic feedback to the ventral tegmental area. *Int. J. Neuropsychopharmacol.* **16**, 1799-1807 (2013).
- 159 Carr, D. B. & Sesack, S. R. Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J. Neurosci.* **20**, 3864-3873 (2000).
- 160 Gilad, G. M., Rabey, J. M. & Gilad, V. H. Presynaptic effects of glucocorticoids on dopaminergic and cholinergic synaptosomes. Implications for rapid endocrine-neural interactions in stress. *Life Sci.* **40**, 2401-2408 (1987).
- 161 Tümer, N. *et al.* Gene expression of catecholamine biosynthetic enzymes following exercise: modulation by age. *Neuroscience* **103**, 703-711 (2001).
- 162 Sutoo, D. e. & Akiyama, K. Regulation of brain function by exercise. *Neurobiol. Dis.* **13**, 1-14 (2003).
- 163 Cotman, C. W., Berchtold, N. C. & Christie, L.-A. Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci.* **30**, 464-472 (2007).
- 164 Fisher, B. E. *et al.* Exercise-induced behavioral recovery and neuroplasticity in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-lesioned mouse basal ganglia. *J. Neurosci. Res.* **77**, 378-390 (2004).
- 165 Faherty, C. J., Shepherd, K. R., Herasimtschuk, A. & Smeyne, R. J. Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism. *Brain Res. Mol. Brain Res.* **134**, 170-179 (2005).
- 166 Lee, M.-Y. *et al.* Alteration of Synaptic Activity–Regulating Genes Underlying Functional Improvement by Long-term Exposure to an Enriched Environment in the Adult Brain. *Neurorehabil. Neural Repair* **27**, 561-574 (2013).
- 167 Ouchi, Y. *et al.* Changes in dopamine availability in the nigrostriatal and mesocortical dopaminergic systems by gait in Parkinson's disease. *Brain* **124**, 784-792 (2001).

- 168 Sciolino, N. R. & Holmes, P. V. Exercise offers anxiolytic potential: a role for stress and brain noradrenergic-galaninergic mechanisms. *Neurosci. Biobehav. Rev.* **36**, 1965-1984 (2012).
- 169 Soares, J., Naffah-Mazzacoratti, M. & Cavalleiro, E. Increased serotonin levels in physically trained men. *Brazilian J. Med. Biol. Res.* **27**, 1635-1638 (1994).
- 170 Soares, J. *et al.* Brain noradrenergic responses to footshock after chronic activity-wheel running. *Behav. Neurosci.* **113**, 558 (1999).
- 171 Greenwood, B. N. *et al.* Wheel running alters serotonin (5-HT) transporter, 5-HT 1A, 5-HT 1B, and alpha 1b-adrenergic receptor mRNA in the rat raphe nuclei. *Biol. Psychiatry* **57**, 559-568 (2005).
- 172 Ohmori, T., Abekawa, T., Muraki, A. & Koyama, T. Competitive and noncompetitive NMDA antagonists block sensitization to methamphetamine. *Pharmacol. Biochem. Behav.* **48**, 587-591 (1994).
- 173 Wæhrens, J. & Gerlach, J. Bromocriptine and imipramine in endogenous depression: a double-blind controlled trial in out-patients. *J. Affect. Disord.* **3**, 193-202 (1981).
- 174 Izumi, T. *et al.* Open pergolide treatment of tricyclic and heterocyclic antidepressant-resistant depression. *J. Affect. Disord.* **61**, 127-132 (2000).
- 175 Cassano, P. *et al.* Pramipexole in treatment-resistant depression: An extended follow-up. *Depress. Anxiety* **20**, 131-138 (2004).
- 176 Barone, P. *et al.* Pramipexole versus sertraline in the treatment of depression in Parkinson's disease. *J. Neurol.* **253**, 601-607 (2006).
- 177 Inoue, T. *et al.* Pramipexole for stage 2 treatment-resistant major depression: an open study. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **34**, 1446-1449 (2010).
- 178 Cusin, C. *et al.* A randomized, double-blind, placebo-controlled trial of pramipexole augmentation in treatment-resistant major depressive disorder. *The J. Clin. Psychiatry* **74**, e636-641 (2013).
- 179 Favier, M. *et al.* Pramipexole reverses Parkinson's disease-related motivational deficits in rats. *Mov. Disord.* **29**, 912-920 (2014).

- 180 Maj, J. & Rogoz, Z. Synergistic effect of pramipexole and sertraline in the forced swimming test. *Pol. J. Pharmacol.* **51**, 471-475 (1998).
- 181 Li, Y. *et al.* Dopamine D2/D3 but not dopamine D1 receptors are involved in the rapid antidepressant-like effects of ketamine in the forced swim test. *Behav. Brain Res.* **279**, 100-105 (2015).
- 1821 Lammers, C., Diaz, J., Schwartz, J. & Sokoloff, P. Selective increase of dopamine D 3 receptor gene expression as a common effect of chronic antidepressant treatments. *Mol. Psychiatr.* **5**, 378-388 (2000).
- 183 Gershon, A. A., Vishne, T. & Grunhaus, L. Dopamine D2-like receptors and the antidepressant response. *Biol. Psychiatry* **61**, 145-153 (2007).
- 184 Chernoloz, O., El Mansari, M. & Blier, P. Long-term administration of the dopamine D3/2 receptor agonist pramipexole increases dopamine and serotonin neurotransmission in the male rat forebrain. *J. Psychiatry Neurosci.* **37**, 113 (2012).
- 185 Seamans, J. K. & Yang, C. R. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog. Neurobiol.* **74**, 1-58 (2004).
- 186 Lapish, C. C. *et al.* The ability of the mesocortical dopamine system to operate in distinct temporal modes. *Psychopharmacology* **191**, 609-625 (2007).
- 187 Durstewitz, D. & Seamans, J. K. The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-o-methyltransferase genotypes and schizophrenia. *Biol. Psychiatry* **64**, 739-749 (2008).
- 188 Goldwater, D. S. *et al.* Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience* **164**, 798-808 (2009).
- 189 Barbas, H. General cortical and special prefrontal connections: principles from structure to function. *Annu. Rev. Neurosci.* **38**:269–89 (2015).
- 190 Salamone, J. D., Correa, M., Farrar, A. & Mingote, S. M. Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology* **191**, 461-482 (2007).

- 191 Nunes, E. J. *et al.* Nucleus accumbens neurotransmission and effort-related choice
behavior in food motivation: effects of drugs acting on dopamine, adenosine, and
muscarinic acetylcholine receptors. *Neurosci. Biobehav. Rev.* **37**, 2015-2025 (2013).
- 192 Vialou, V. *et al.* Prefrontal cortical circuit for depression-and anxiety-related
behaviors mediated by cholecystokinin: role of Δ FosB. *J. Neurosci.* **34**, 3878-3887
(2014).
- 193 Warden, M. R. *et al.* A prefrontal cortex-brainstem neuronal projection that controls
response to behavioural challenge. *Nature* **492**, 428-432 (2012).
- 194 Vertes, R. P. Differential projections of the infralimbic and prelimbic cortex in the
rat. *Synapse* **51**, 32-58 (2004).
- 195 Ford, C. P. The role of D2-autoreceptors in regulating dopamine neuron activity and
transmission. *Neuroscience* **282**, 13-22 (2014).
- 196 Chiodo, L. *et al.* Evidence for the absence of impulse-regulating somatodendritic
and synthesis-modulating nerve terminal autoreceptors on subpopulations of
mesocortical dopamine neurons. *Neuroscience* **12**, 1-16 (1984).
- 197 Cubeddu, L. X., Hoffmann, I. S. & Talmaciu, R. K. Is the release of dopamine from
medial prefrontal cortex modulated by presynaptic receptors? Comparison with
nigrostriatal and mesolimbic terminals. *Ann.NY Acad.Sci.* **604**, 452-461 (1990).
- 198 Lammel, S. *et al.* Unique properties of mesoprefrontal neurons within a dual
mesocorticolimbic dopamine system. *Neuron* **57**, 760-773 (2008).
- 199 Heidbreder, C. A. & Groenewegen, H. J. The medial prefrontal cortex in the rat:
evidence for a dorso-ventral distinction based upon functional and anatomical
characteristics. *Neurosci. Biobehav. Rev.* **27**, 555-579 (2003).
- 200 Herman, J. P. Neural pathways of stress integration: relevance to alcohol abuse.
Alcohol research: current reviews **34**, 441 (2012).
- 201 Cassaday, H. J., Nelson, A. J. & Pezze, M. A. From attention to memory along the
dorsal-ventral axis of the medial prefrontal cortex: some methodological
considerations. *Front. Syst. Neurosci.* **8** (2014).

- 202 Myers, R. D. Injection of solutions into cerebral tissue: relation between volume and diffusion. *Physiol. Behav.* **1**, 171-IN179 (1966).
- 203 Peterson, S. L. Drug microinjection in discrete brain regions. *Kopf Carrier* **50**, 1-6 (1998).
- 204 Sachs, M. & Pargman, D. Running addiction: A depth interview examination. *J. Sport Behav.* **2**, 143-155 (1979).
- 205 Kagan, J. & Berkun, M. The reward value of running activity. *J. Comp. Physiol. Psychol.* **47**, 108 (1954).
- 206 Onge, J. R. S., Ahn, S., Phillips, A. G. & Floresco, S. B. Dynamic fluctuations in dopamine efflux in the prefrontal cortex and nucleus accumbens during risk-based decision making. *J. Neurosci.* **32**, 16880-16891 (2012).
- 207 Smith, P. J. *et al.* Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. *Psychosom. Med.* **72**, 239 (2010).
- 208 Hindin, S. B. & Zelinski, E. M. Extended practice and aerobic exercise interventions benefit untrained cognitive outcomes in older adults: a meta-analysis. *J. Am. Geriatr. Soc.* **60**, 136-141 (2012).
- 209 Roig, M., Nordbrandt, S., Geertsen, S. S. & Nielsen, J. B. The effects of cardiovascular exercise on human memory: a review with meta-analysis. *Neurosci. Biobehav. Rev.* **37**, 1645-1666 (2013).
- 210 Cools, R. & D'Esposito, M. Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol. Psychiatry* **69**, e113-e125 (2011).
- 211 Spencer, R. C., Devilbiss, D. M. & Berridge, C. W. The Cognition-Enhancing Effects of Psychostimulants Involve Direct Action in the Prefrontal Cortex. *Biol. Psychiatry* **77**, 940-950 (2015).
- 212 Arnsten, A. F., Wang, M. & Paspalas, C. D. Dopamine's Actions in Primate Prefrontal Cortex: Challenges for Treating Cognitive Disorders. *Pharmacol. Rev.* **67**, 681-696 (2015).
- 213 Cummings, J. L. Depression and Parkinson's disease: a review. *Am. J. Psychiat.* **149**, 443 (1992).

- 214 Remy, P. *et al.* Depression in Parkinson's disease: loss of dopamine and noradrenaline innervation in the limbic system. *Brain* **128**, 1314-1322 (2005).