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Original research paper

The role of medial prefrontal corticosterone and dopamine in the antidepressant-like effect of exercise

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ABSTRACT

Despite the well-documented beneficial effect of exercise on stress coping and depression treatment, its underlying neurobiological mechanism remains unclear. This is further complicated by a 'side effect' of exercise: it increases basal glucocorticoid (CORT), the stress hormone, which has been shown to be a mediator linking stress to depressive disorders. Here we show that three weeks of voluntary wheel running reduced rats' immobility in the forced swim test (FST), an antidepressant-like effect. Monitoring extracellular fluids in the medial prefrontal cortex PFC (mPFC) using microdialysis we found that, wheel running was associated with higher baseline CORT, but lower FST-responsive CORT. Further, wheel running resulted in a higher dopamine (DA) both at baseline and following FST. Interestingly, the antidepressant-like effect of wheel running was completely abolished by intra-mPFC pre-microinjection of a D2R (haloperidol) but not D1R (SCH23390) antagonist, at a dose that does not affect normal rats' performance in the FST. It suggests that exercise exerts antidepressant-like effect through upregulated DA and in a D2R dependent way in the mPFC. Importantly, the antidepressant-like effect of wheel running was also abolished by intra-mPFC pre-microinjection of a GR antagonist (RU486). Finally, intra-mPFC pre-microinjection of RU486 also downregulated the originally elevated basal and FST-responsive DA in the mPFC of exercise rats. These results suggest a causal pathway linking CORT, GR, DA, and D2R, to the antidepressant-like effect of exercise. In conclusion, exercise achieves antidepressant-like effect through the CORT-GR-DA-D2R pathway and that the increased basal CORT by exercise itself may be beneficial rather than detrimental.

KEYWORDS: corticosterone, depression, dopamine, exercise, medial prefrontal cortex, stress

1. INTRODUCTION

The beneficial effects of exercise, or physical activity, on stress coping and mental health have been well documented. For instance, meta-analysis of randomized controlled trials has shown that habitual exercise can significantly reduce depressive mood (Cooney et al, 2013). In parallel with the finding in humans, animal studies also found that exercise, such as wheel and treadmill running, improves stress coping and exerts antidepressant-like effects (Greenwood and Fleshner, 2008). However, despite these well replicated beneficial outcomes, the precise neurobiological mechanism underlying such beneficial outcomes remains to be completely elucidated.

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 and insulin-like growth factor-1, and change the neurotransmitter system which are believed to account for the above beneficial outcomes of exercise (Prakash et al, 2015). However, to our knowledge, rarely has any research examined the causality between the observed neurobiological changes and the beneficial outcomes.

On the other hand, this lack of knowledge on the neurobiological mechanism 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., Droste et al, 2003; Makatsori et al, 2003; Stranahan et al, 2006 in rodents; Luger et al, 1987; Loucks et al, 1989 in humans). CORT, the final product of the hypothalamic–pituitary–adrenal axis (HPA axis) in response to stressful events and well known as 'the stress hormone', has been shown to be a mediator of the detrimental effects of stress on coping and mental health (Holsboer and Ising, 2010; Herman, 2013). For instance, rodent research has consistently found that, various models of stress and depression, such as chronic mild stress (Zheng et al, 2006; Pan et al, 2010), chronic immobilization (Üresin et al, 2004), and repeated electric shock (Ottenweller et al, 1989), lead to increased basal CORT, which is reversed by antidepressant treatment (Pan et al, 2010). Further, repeated exogenous administration of CORT in rodents induces such depression-like behaviors as 'learned helpless' and anhedonia, which resembles human depressive states (Sterner and Kalynchuk, 2010). In the meantime, human studies also reported elevated basal CORT in clinically depressed patients, which is normalized by antidepressant or psychological treatment (for a review and meta-analysis, see Burke et al, 2005; Pariante, 2009; Holsboer and Ising, 2010; Stetler and Miller, 2011). Longitudinal observations have found that higher basal plasma CORT predicts the onset of depression (Goodyer et al, 2000; Harris et al, 2000).

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. Stranahan et al, 2006 for exercise, Pan et al, 2010 for chronic mild stress, Üresin et al, 2004 for chronic immobilization) to 150% (e.g. Makatsori et al, 2003 for exercise, Zheng et al, 2006 for chronic mild stress) of control animals. Therefore this is apparently a paradox.

In the present study we aimed to investigate this paradox, examine the role of the elevated basal CORT in the antidepressant-like effect (assessed by the FST, Cryan et al 2002) by exercise, and identify the underlying neurobiological mechanism by which exercise exerts antidepressant-like effect using in vivo microdialysis and microinjection. We employed microdialysis since it allows exploratory analysis of the neurotransmitters involved and microinjection since it establishes causality (e.g., through blocking neurotransmission by delivering an antagonist). As the medial prefrontal cortex (mPFC) is generally believed to be the final brain center for coping behavior (Maier and Watkins 2010), for regulating CORT response (Sullivan and Dufresne, 2006), and is highly involved in depression (Price and Drevets, 2012), we chose this region as the target for microdialysis and microinjection.

2. MATERIALS AND METHODS

2.1 Subjects

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/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: 0700h-1900h). 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). The torque of the wheel was 300 gf-cm (that is, if we attach a 30cm-long stick below the wheel so that it locates right below the horizontal diameter of the wheel, the load applied to one tip of the stick necessary to start the wheel turning was 20g). Running cycles were memoed every day and later transformed into running distances (m).

Two weeks after the allocation and treatment, all rats underwent a microdialysis (experiment 1 and 3) or microinjection (experiment 2 and 3) operation. Stereotaxically and under pentobarbital anesthesia (30 mg/kg i.p.), an AG-4 guide cannulae (experiment 1), an injection cannulae (bilateral, experiment 2 and 3), or a MI-AG-4 guide cannulae (experiment 3) (Eicom, Japan) was implanted into the rat brain. These cannulaes were directed toward the mPFC at the following coordinates relative to the bregma from the stereotaxic atlas of Paxinos and Watson (2006): A +3.2, ML +0.6, DV +1.8 mm. Dummy probes were inserted into the guide cannulae. Then, rats were kept in the polypropylene

cages independently for 2-3 days to recover, after which they were returned to their previous cage boxes with (EX) or without (CON) running wheels for another week. All rats were handled two or three time a week before surgery, and daily after surgery.

2.2 Drugs

The D1R antagonist SCH 23390 (2 ug or 5560 uM, dissolved in saline, Cayman, MI, USA), D2R antagonist haloperidol (0.05 ug or 266uM, dissolved in DMSO, Cayman) and GR antagonist RU486 (mifepristone, 100 ng/ul, dissolved in ethanol, Tocris bioscience, UK) were used. The doses for SCH23390 and haloperidol were determined by a pilot experiment, at which neither drugs affect control rats' performance in the FST (Figure S1). The dose for RU486 was based on Butts et al (2011), for RU486 at this dose locally delivered into the mPFC blocks stress- or CORT- responsive DA efflux in the mPFC. All drug solutions were prepared immediately before use.

2.3 FST

The procedure was based on Slattery and Cryan (2012). The test was conducted at 1900h 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, Gomes et al, 2011) on two consecutive days. On day-1, rats were placed individually in an opaque cylindrical tank (diameter 200 mm, water depth 300 mm, water temperature 25 °C) for 15 min. Then they were kept in a polypropylene cage individually. 24 hours later (day-2) subjects were placed again in the water tank for 5 min. Rats' behaviors in the water tank

were all video recorded with an infrared camera and later analyzed as climbing, swimming or immobility by one of the authors who was blind to the experimental manipulation. The scoring measures the frequency of each behavior over 5-s intervals during the day-2 5-min test session.

2.4 In vivo microdialysis and dialysate analysis

The procedure for microdialysis was similar to that described previously (Kitaichi et al, 2010). For microdialysis of experiment 1, after day-1 test, under pentobarbital anesthesia, rats were implanted with a dialysis probe (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, around 1430h, rats were transferred to a dialysis box and kept there singly and quietly. Using artificial cerebrospinal fluid at a flow rate of 2 μl/min, perfusion started at 1500h. For microdialysis of experiment 3, a different probe with an injection needle (MI-A-4-2) attached (Eicom) was used, which allows simultaneous microdialysis and microinjection (as described below). The probe was inserted into the guide cannulae so that 2.0 mm of the probe and 1.0 mm of the injection needle were exposed to the tissue of the mPFC. To improve fluid exchange, a flow rate of 1 μl/min for perfusion was used for experiment 3. Following the initial perfusion for 2 hours, dialysate samples were collected in vials every 30 min and kept at 4 °C. Four baseline samples were collected. At 1900h 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. The dislysis perfusion and sampling lasted 3 hours in dark following the start of the test till 2200h. Then

all dialysate samples were immediately transferred to a freezer and stored at -80 °C until assayed. The exact placement of the probe tips was verified the next day during dissection. Animals with cannula or probes outside of these target brain regions or with bleeding in area of the probe were excluded from the subsequent analyses.

CORT was measured using an enzymatic immunoassay (Elisa, Cayman). The calibration curve was linear with a regression coefficient (Pearson) of 0.9728, $P<0.001$. Areas under the curve (AUCs) were calculated using the standard trapezoidal method and are expressed as arbitrary units (pg*hour/ml). Noradrenaline (NA), DA and 5-HT were determined by the high-performance liquid chromatography system (HPLC) with electrochemical detection (Eicom), essentially the same as described previously (Kitaichi et al, 2010). Amino acids, including glycine, alanine, taurine, glutamine, and glutamate, were also determined by the HPLC system (Abekawa et al, 2006). In the samples from the same rats, some neurotransmitters were not reliably detected (some were not detectable while others were typically over 5 times higher than the average concentration of the same neurotransmitters from other rats, which were confirmed by Grubbs' test to be significant outliers at $p < 0.05$ level) and thus the data of the neurotransmitters in these rats were excluded from the final analysis.

2.5 Microinjection

On day-2 of the FST, a 33-gauge injection cannula, projecting 1.0 mm beyond the tips of the guide cannula (Eicom) was inserted. The injection cannula was connected by polyethylene tubing to motor-driven microsyringes. The exact placement of the injection

cannula tips was verified the next day during dissection. EX rats were injected a D1R, D2R, or GR antagonist while CON rats received only the vehicle. For D1R and D2R antagonist, the solution (0.5 µl) was infused through each injector at a rate of 0.25 µl/min for 2 min at 25-30 min (27.23±0.33) before the day-2 FST (one EX and one CON rats were always infused at the same time). For GR antagonist, the solution (1 µl) was infused through each injector at a rate of 0.5 µl/min for 2 min at 30 min before the day-2 FST. The injection cannula was left in position for an additional 60 s after drug infusion.

2.6 Western blot

The day (1000h-1600h) following day-2 test of the FST, a subset of rats (from experiment 1) were sacrificed and the brain regions containing mPFC were dissected using a Brain Slicer (Muromachi, Japan). 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). This was 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 to Boku et al (2009). The antibodies used are reported in Table S1. Protein expression was detected with the Amersham ECL Plus Western Blotting Detection System (GE Healthcare, UK) and ImageQuant LAS 4000 (GE Healthcare). The pictures were converted to digital files and the intensity of each band was analyzed with ImageQuant TL.

2.7 Locomotion

The polypropylene cage for one subset of rats was placed under a sensor before 1400h 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) began after a 2-hour habituation period. The measurement lasted from 1600h to 1830h (-180 min to -30 min of the FST), after which rats went on to subsequent experiment. Horizontal movements of the rats were digitized and stored in a computer every 5 min. For analysis we transformed all the data to a 30 min duration and a total sum.

2.8 Statistical analysis

For comparison of the data of FST, general motor activity, AUC, protein expression density of receptors, the student's t-test was employed. For the comparison of the time course data of CORT, DA, and other neurotransmitters, the two-way ANOVA with repeated measures was employed. For analysis of the correlation between the data of FST and running distances, the Pearson correlation analysis was employed. Significance was defined as $p<0.05$. Data are expressed as the means ±SEM.

2.9 Experimental design

2.9.1 Experiment 1 The behavioral and neurobiological effects of exercise

One group of rats was subjected to the FST, while another group was subjected to the FST under microdialysis.

2.9.2 Experiment 2 The role of medial prefrontal DA in the antidepressant-like effect of exercise

This experiment aimed to examine whether the upregulated DA observed in experiment 1 plays a causal role in the antidepressant-like effect of wheel running, by intra-mPFC pre-microinjection of a D1R or D2R antagonist.

2.9.3 Experiment 3 The role of corticosterone in the antidepressant-like effect of wheel running and its relation to dopamine

Experiment 3 was designed to test the hypothesis that the elevated basal CORT in the mPFC is responsible for the upregulated DA and thus plays a causal role in the antidepressant-like effect of wheel running. A GR antagonist was injected into the mPFC, and the FST and microdialysis were performed.

3. RESULTS

3.1 Experiment 1 The behavioral and neurobiological effects of exercise

Wheel running significantly reduced immobility in the FST ($t=2.638$, $df=13$, $p<0.05$), and increased swimming with a trend towards significance ($t=1.810$, $df=13$, $p=0.094$) (Figure 1c). There was no correlation between the results of FST and running distances (data not shown): whether pre-operation, post-operation, or total running distance. Further, wheel running did not significantly affect general motor activity (Figure 1d).

Two-way ANOVA with repeated measures analysis of CORT (Figure 2b) at baseline (-90 min to 0 min) indicated significantly higher basal CORT in EX than CON group ($F(1,10)=8.005$, $p<0.05$). Following the FST (0 min to 180 min), the group difference and the time*group interaction were not significant. Nevertheless, comparison of the AUC showed that EX group had higher AUC at baseline ($t=3.116$, $df=10$, $p<0.05$) but lower AUC after the FST ($t=2.484$, $df=10$, $p<0.05$) than CON group (Figure 2c).

Two-way ANOVA with repeated measures analysis of DA (Figure 2d) indicated overall higher DA in EX than CON group ($F(1,10)=6.273$, $p<0.05$). Comparison of AUC again suggested that EX group had higher level of DA both at baseline ($t=-2.131$, $df=10$, $p=0.059$) and after FST ($t=-2.382$, $df=10$, $p<0.05$) than CON group (Figure 2e).

There was no significant effect of wheel running on other neurotransmitters measured, including NA, 5-HT, alanine, glycine, taurine, glutamine, and glutamate (Figure S2). There was no significant effect of wheel running on the protein expression density of GR, D1R, D2R, or 5-HT1AR (Figure 2f). Due to its low level of expression in the mPFC, we failed to reliably detect MR.

3.2 Experiment 2 The role of medial prefrontal DA in the antidepressant-like effect of exercise

Bilateral intra-mPFC pre-microinjection of D1R antagonist SCH23390 into EX rats did not affect the antidepressant-like effect of exercise: immobility ($t=3.247$, $df=12$, $p<0.05$), swimming ($t=2.190$, $df=12$, $p<0.05$) (Figure 3b). In contrast, same injection of

D2R antagonist haloperidol completely abolished the antidepressant-like effect of exercise (Figure 3c).

3.3 Experiment 3 The role of corticosterone in the antidepressant-like effect of wheel running and its relation to dopamine

Bilateral intra-mPFC pre-microinjection of GR antagonist RU486 into EX rats also abolished the antidepressant-like effect of exercise (Figure 4b). In the meantime, two-way ANOVA with repeated measures analysis of basal DA (from -90 to -30 min, Figure 4d) indicated higher DA in EX than CON group ($F(1,8)=11.082$, $p<0.05$). However, following injection of RU486 (at -30 min), the significant group difference at -30 min ($t=-4.227$, $df=8$, $p<0.05$) disappeared at 0 min. This suggests that intra-mPFC microinjection of RU486 into EX rats reduced the originally upregulated basal DA in the mPFC. Moreover, following FST (from 0 to 180 min), there was no significant effect of group or time*group. In other words, intra-mPFC microinjection of RU486 into EX rats also reduced the originally upregulated FST-responsive DA in the mPFC. Comparison of AUC again suggested that EX group had higher level of DA at baseline ($t=3.079$, $df=8$, $p<0.05$) but not after FST than CON group (Figure 4e). In contrast, there was no significant effect of RU486 or wheel running on 5-HT in the mPFC (Figure S3).

4. DISCUSSION

Voluntary wheel running reduced immobility in the FST, an antidepressant-like effect, without affecting general motor activity. This is in line with previous reports that wheel

running exerts antidepressant-like effect as assessed by the FST, shuttle box escape deficit, and chronic mild stress (Zheng et al, 2006; Duman et al, 2008; Greenwood and Fleshner, 2008). The antidepressant-like effect of wheel running did not seem to depend on running distances.

The antidepressant-like effect of wheel running was accompanied by overall upregulated DA in the mPFC without changing DA receptors. More interestingly, intra-mPFC pre-microinjection of a D2R but not D1R receptor antagonist completely abolished the antidepressant-like effect of wheel running. It suggests that exercise exerts antidepressant-like effect through upregulating DA in the mPFC and in a D2R-dependent way. Recently the DA system has been attracting more and more attention in the context of stress and depression (Chen et al, 2015). Whereas various chronic stress, such as chronic mild stress (Sun et al, 2013) and chronic restraint (Mizoguchi et al, 2000), decrease DA in the mPFC, various antidepressant treatments, such as tricyclic antidepressants (Tanda et al, 1994), selective 5-HT re-uptake inhibitors (Tanda et al, 1994), and 5-HT-NA reuptake inhibitors (Masana et al, 2011), increase DA in the mPFC. Further, chronic antidepressant treatment with reboxetine and mirtazapine (Masana et al, 2012) or a Chinese herbal prescription (Sun et al, 2013), concurrently increases DA in the mPFC and decreases animal's immobility in the FST. Our research, for the first time, provides causal support for this association. This is consistent with the decision making literature that DA in mPFC is essential for effortful behavior (Schweimer and Hauber, 2006), which is impaired in depression (Chen et al 2015). Taken together, the evidence suggests an essential role of DA in the mPFC for active coping and antidepressant-like effect.

On the other hand, a prominent role of the D2R in the pathology and treatment of depression has already been noticed (Chen et al, 2015). Decades of research has consistently implicated a critical role of D2R rather than D1R in the treatment of depression in animals (Borsini et al 1988; Li et al, 2015) and humans (Inoue et al, 2010). However, the exact brain site(s) of the D2R-dependent antidepressant effect remains unclear. One commonly proposed area is the nucleus accumbens (Lammers et al, 2000), as various chronic antidepressant treatments selectively increase D2R-like gene expression in this area (Lammers et al, 2000). The results of our present research suggest that another likely area is the mPFC. This is further supported by a recent report that, pramipexole (a D2R agonist) increases the tonic activation of postsynaptic D2Rs in the PFC without changing their sensitivity (Chernoloz et al, 2012). However, it has to be noted that DA modulation in the mPFC is rather complex. D1R and D2R are present on both excitatory pyramidal neurons and inhibitory GABAergic interneurons in the mPFC, which acts on different timescales ranging from milliseconds to minutes and hours, and is also DA concentration-dependent (Lapish et al, 2007). It makes the precise function and role of the D1R versus D2R in the mPFC still remains unsure. More work is needed to clarify the role of the D1R and D2R interaction in the mPFC in the antidepressant-like effect of wheel running.

In the meantime, consistent with previous research, we observed that EX rats also showed higher basal CORT but overall lower FST-responsive CORT, without changing CORT receptor GR. Further, intra-mPFC pre-microinjection of a GR antagonist abolished the antidepressant-like effect of wheel running and blocked the originally upregulated basal and FST-responsive DA by wheel running. This suggests that the elevated basal CORT

might be responsible for the upregulated DA, which is essential for the antidepressant-like effect of wheel running. Indeed, suppression of CORT by adrenalectomy decreases DA in the mPFC, which is prevented by CORT replacement (Mizoguchi et al, 2004). Local injection of CORT into the mPFC increases DA in this brain area, which is blocked by a GR antagonist (Butts et al, 2011). 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 (Butts and Phillips, 2013). These results suggest that CORT may potentiate the mPFC glutamatergic input onto DA neurons in the VTA (Butts and Phillips, 2013). Prominently, an electron microscopic tract-tracing study demonstrated that VTA DA neurons that receive afferents from the mPFC preferentially project reciprocally to the PFC (Carr and Sesack, 2000). Thus, it seems that higher basal CORT plays an important role in maintaining high level of DA in the mPFC.

On the other hand, the mPFC is believed to provide negative feedback regulation of the HPA axis (Sullivan and Dufresne, 2006) such that lesions of the mPFC significantly increase plasma levels of both adrenocorticotropic hormone (ACTH) and CORT in response to a 20 min restraint stress (Diorio et al, 1993). Interestingly, lesions of the mPFC does not affect basal ACTH and CORT (Diorio et al, 1993; Figueiredo et al, 2003), suggesting that the mPFC selectively modulates stress-responsive HPA activity. More specific, DA D1/D2 antagonist injected into the mPFC enhances stress-induced increase of ACTH and CORT (Sullivan and Dufresne, 2006), suggesting that DA in the mPFC normally acts to suppress the HPA axis and inhibit CORT. In line with this, we observed

higher DA in exercise rats, together with overall reduced FST-responsive CORT. Of note, higher CORT within mPFC itself has also been reported to inhibit stress-responsive HPA activity, through an unclear mechanism. CORT locally delivered in to the mPFC rather than central amygdala of adrenalectomized rats decreases plasma ACTH response to acute restraint (Akana et al, 2001). Further, this effect is likely to be GR-dependent, for GR knockdown in the mPFC leads to hyper-responsive CORT to acute restraint in normal and chronically stressed rats (McKlveen et al, 2013). Our results provide a potential explanation to these reports, namely, higher CORT in the mPFC acts to suppress stress-responsive CORT through elevating DA in the mPFC. Interestingly, accompanying downregulated DA in the mPFC, exaggerated CORT exposure to subsequent novel stress has been reported under chronic or repeated stress (Ottenweller et al 1989; Herman 2013), which is attenuated by chronic antidepressant treatment (Reul et al 1993). Further, patients suffering from depression have hyper-responsive CORT in the dexamethasone/CRH test (Stetler and Miller 2011) and demonstrate higher CORT during the recovery period after stress (Burke et al 2005), which are also normalized after successful antidepressant treatment (Holsboer and Ising 2010). These outcomes again are in sharp contrast to that of exercise observed in our current research. Thus the effect of buffering overall CORT exposure to novel stress may be one mechanism by which exercise achieves its beneficial outcomes.

Taken together, for the first time we showed that the CORT-GR-DA-D2R pathway actually accounts for the antidepressant-like effect of exercise. Returning to the CORT paradox we discussed in the introduction, a second comparison of chronic stress and exercise reveals fundamental differences. Although both elevate basal CORT, the former

reduces while the latter (and also antidepressants, see above) increases DA in the mPFC. This might be the key why the former is detrimental while the latter is beneficial to stress coping and depression. 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 (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 (Chen et al, 2015): the failure of higher CORT in upregulating DA in the mPFC (the latter responsible for exerting control and coping). Future inquiry should try to incorporate chronic stress and exercise together to clarify the CORT paradox and provide more insights. Future investigation may also examine the downstream pathway of the DA in the mPFC in the antidepressant-like effect of exercise.

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CONFLICT OF INTEREST

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Figure legends

Figure 1 The behavioral effects of wheel running. (a) Experimental procedure. (b) Mean running distance per day pre- and post- operation and recovery (OR) of EX rats (n=7). (c) The effect of wheel running on immobility, swimming, and climbing in the FST (EX n=7; CON n=8). (d) The effect of wheel running on general motor activity, by 30 min periods (left) and in total (right) (n=7/group). * $p<0.05$ compared to CON. Data is shown as Mean±SEM.

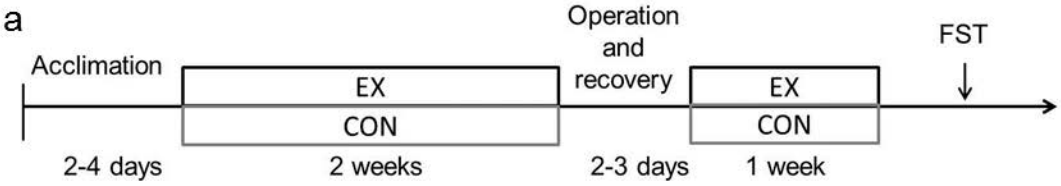
Figure 2 The neurobiological effects of wheel running. (a) Schematic representation of placement of the microdialysis probe tips. (b,c) The effect of wheel running on the concentration (b) and AUC (c) of CORT in the mPFC at baseline and following the FST (n=6/group). (d,e) The effect of wheel running on the concentration (d) and AUC (e) of DA in the mPFC at baseline and following the FST (n=6/group). (f) The effect of exercise on the protein expression of GR, D1R, D2R, 5-HT1AR, and GAPDH in the mPFC. Left, representative western blot bands for each protein. Right, relative band intensity was normalized for GAPDH and expressed as a percentage compared with the value of CON. (n=5/group for GR; n=6/group for D1R and D2R; n=4/group for 5-HT1AR). * $p<0.05$, # $p=0.059$, compared to CON in the same condition (baseline or after FST). Data is shown as Mean±SEM.

Figure 3 The antidepressant-like effect of wheel running was completely abolished by intra-mPFC pre-microinjection of a D2R but not D1R antagonist. (a) Schematic representation of placement of the injector tips. (b) The effect of intra-mPFC pre-microinjection of a D1R antagonist SCH23390 on the antidepressant-like effect of wheel running: immobility, swimming and climbing (n=7/group). (c) The effect of intra-mPFC pre-microinjection of a D2R antagonist haloperidol on the antidepressant-like effect of wheel running: immobility, swimming and climbing (n=6/group). * $p<0.05$ compared to CON. Data is shown as Mean±SEM.

Figure 4 The antidepressant-like effect of wheel running was abolished by intra-mPFC pre-microinjection of a GR antagonist. (a) Schematic representation of placement of the injector tips. (b) The effect of intra-mPFC pre-microinjection of a GR antagonist RU486 on the antidepressant-like effect of wheel running: immobility, swimming and climbing (EX n=6; CON n=5). (c) Schematic representation of placement of the microdialysis probe tips. (d,e) The effect of wheel running on the concentration (d) and AUC (e) of DA in the mPFC at baseline, following microinjection of a GR antagonist RU486, and following the FST (n=5/group). * $p<0.05$ compared to CON. Data is shown as Mean±SEM.

Color figures are for online only.

Figure 1


a
Acclimation
EX
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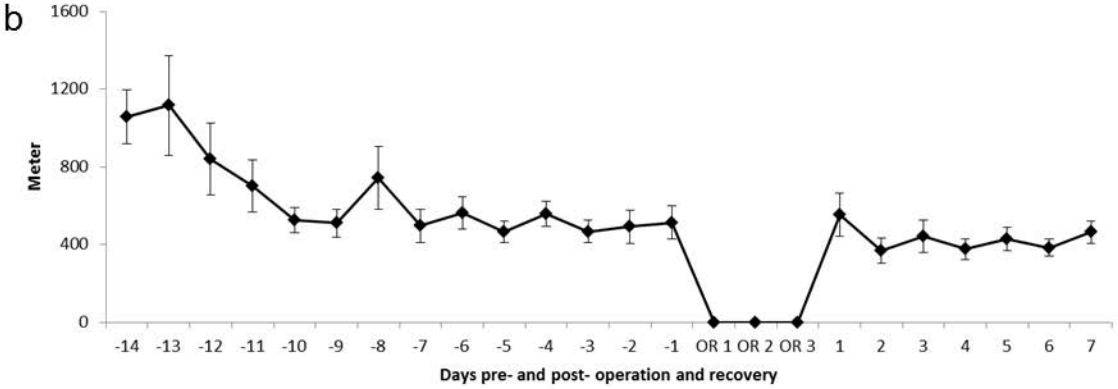
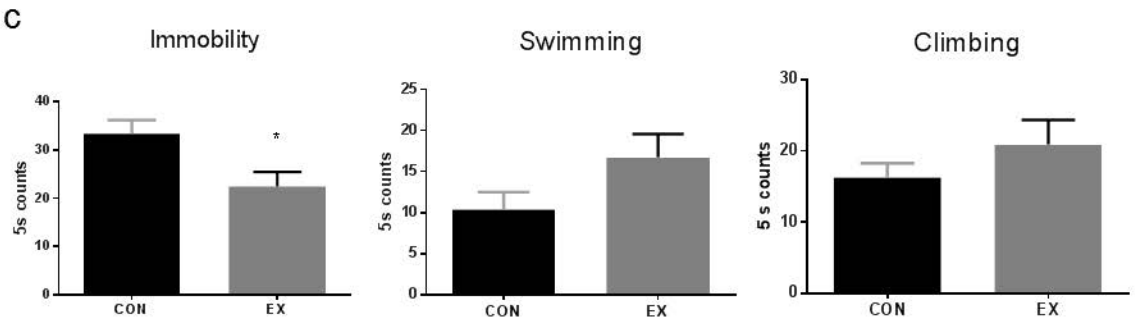
Operation
and
recovery
EX
CON
FST
2-4 days
2 weeks
2-3 days
1 week


b
Meter
1600
1200
800
400
0
-14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 OR 1 OR 2 OR 3 1 2 3 4 5 6 7
Days pre- and post- operation and recovery


c
Immobility
5s counts
40
30
20
10
0
*
CON
EX
Swimming
5s counts
25
20
15
10
5
0
CON
EX
Climbing
5 s counts
30
20
10
0
CON
EX

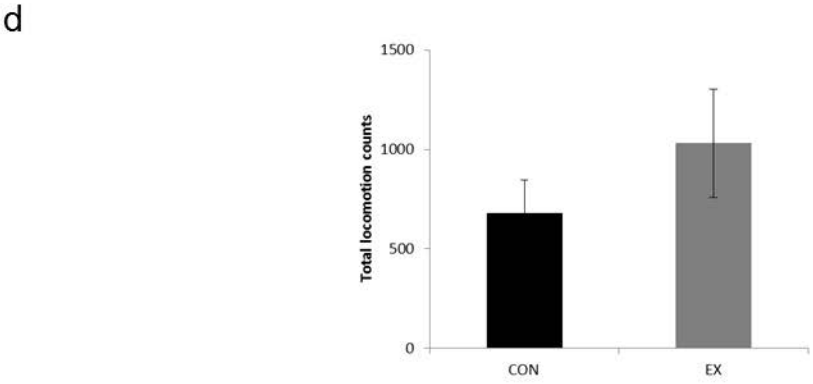

d
Total locomotion counts
1500
1000
500
0
CON
EX

Figure 2

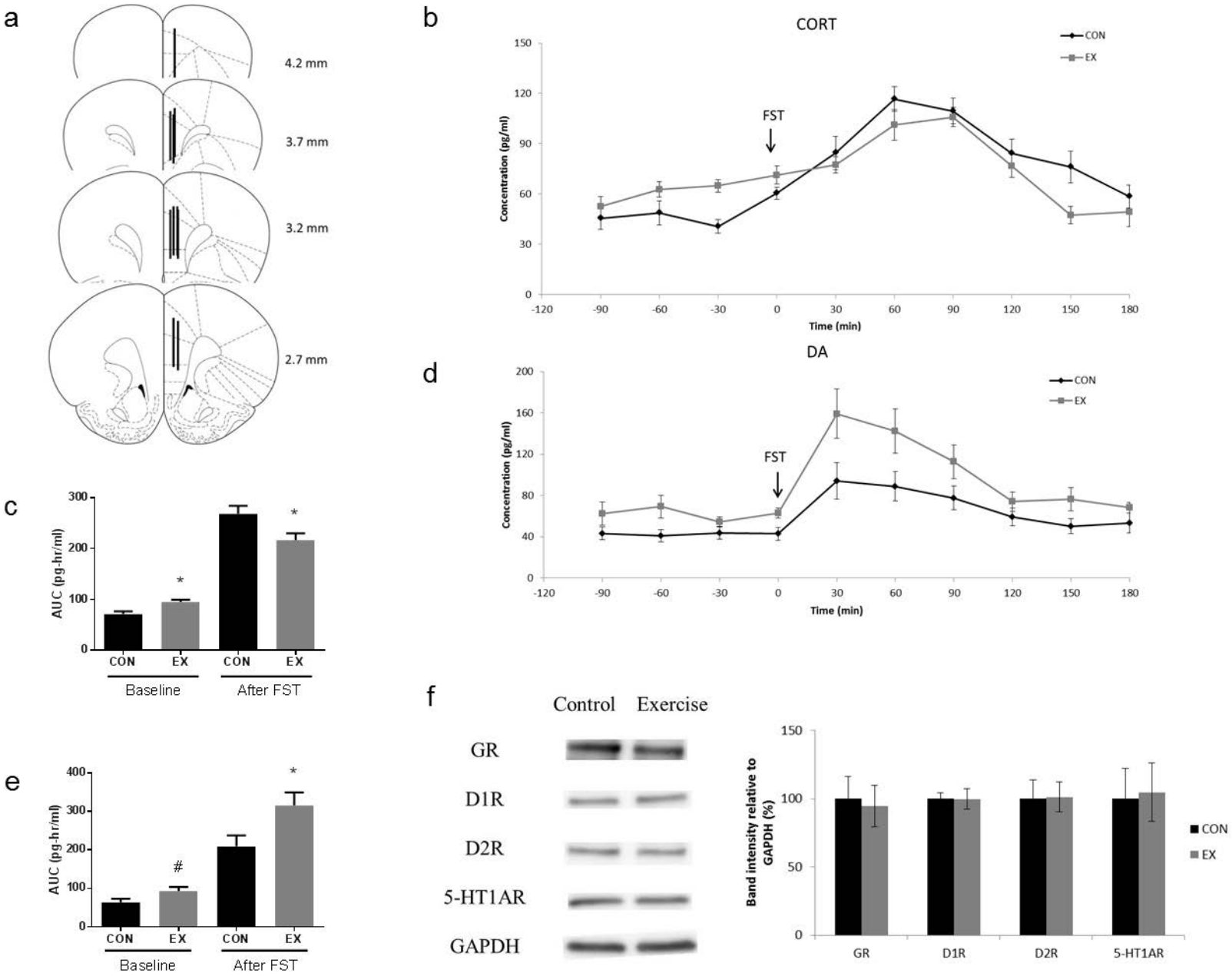
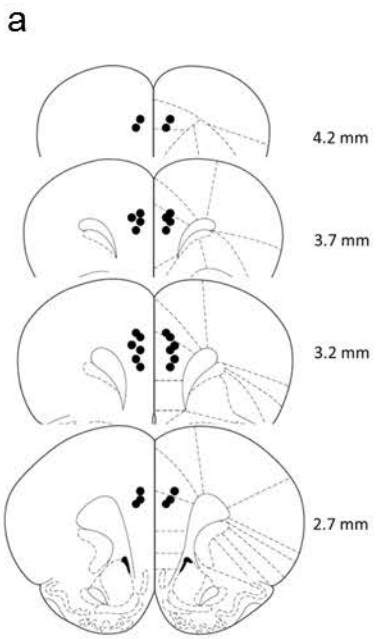

a
4.2 mm
3.7 mm
3.2 mm
2.7 mm
b
CORT
CON
EX
FST
Concentration (pg/ml)
Time (min)
c
AUC (pg-hr/ml)
CON
EX
CON
EX
Baseline
After FST
d
DA
CON
EX
FST
Concentration (pg/ml)
Time (min)
e
AUC (pg-hr/ml)
CON
EX
CON
EX
Baseline
After FST
f
Control
Exercise
GR
D1R
D2R
5-HT1AR
GAPDH
Band intensity relative to GAPDH (%)
CON
EX
GR
D1R
D2R
5-HT1AR

Figure 3


a
4.2 mm
3.7 mm
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2.7 mm

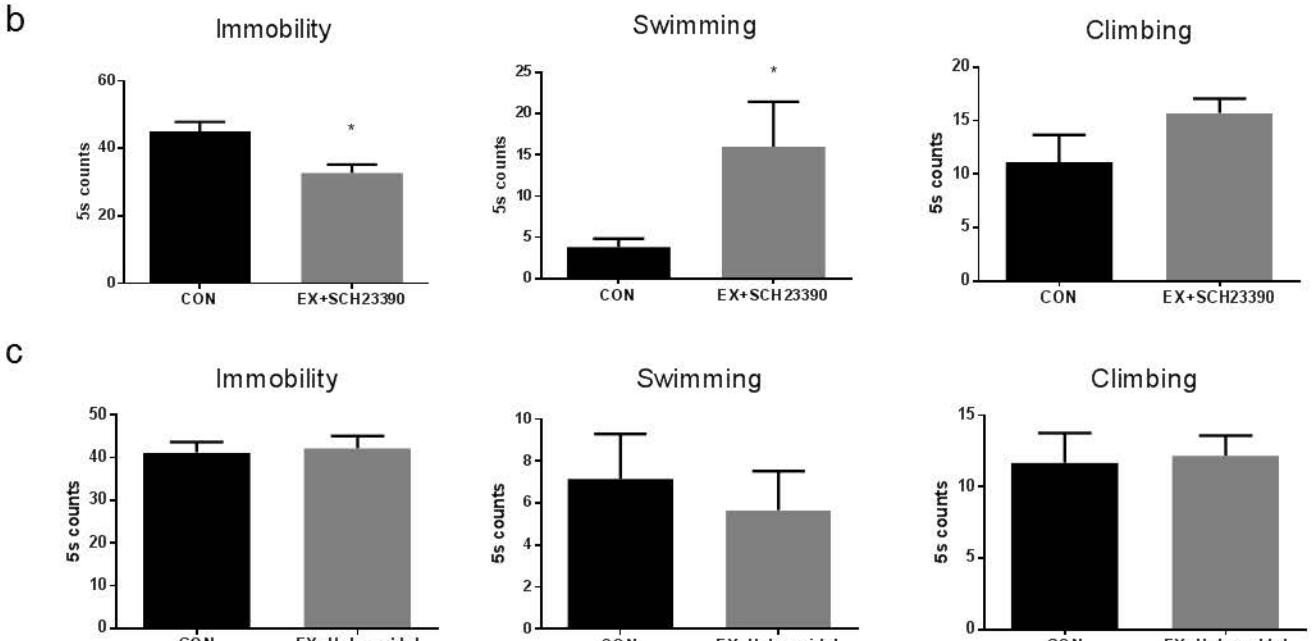
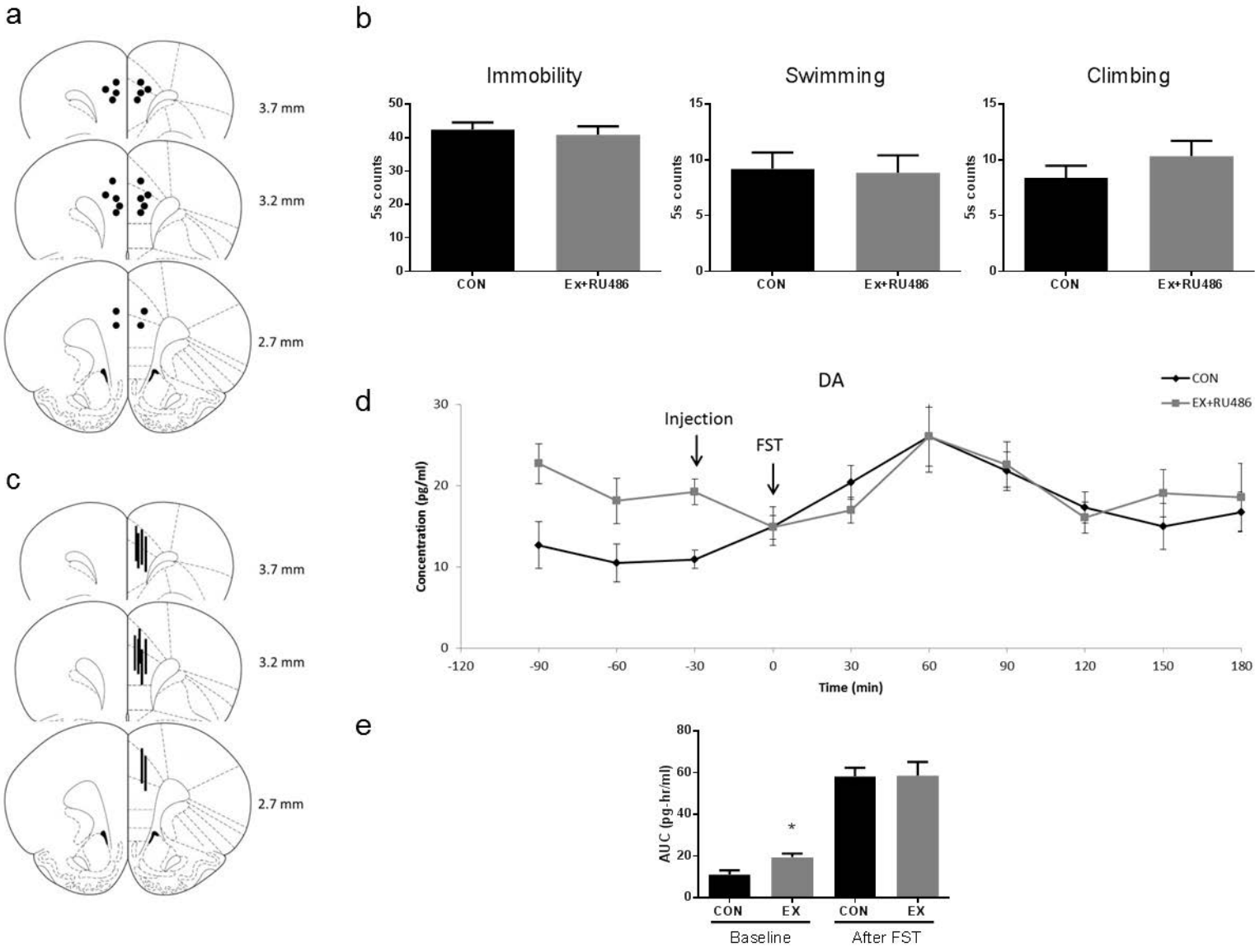

b
Immobility
5s counts
60
40
20
0
*
CON
EX+SCH23390
Swimming
5s counts
25
20
15
10
5
0
*
CON
EX+SCH23390
Climbing
5s counts
20
15
10
5
0
CON
EX+SCH23390
c
Immobility
5s counts
50
40
30
20
10
0
CON
EX+Haloperidol
Swimming
5s counts
10
8
6
4
2
0
CON
EX+Haloperidol
Climbing
5s counts
15
10
5
0
CON
EX+Haloperidol

Figure 4


a
3.7 mm
3.2 mm
2.7 mm
c
3.7 mm
3.2 mm
2.7 mm
b
Immobility
Swimming
Climbing
5s counts
CON
Ex+RU486
d
DA
CON
EX+RU486
Injection
FST
Concentration (pg/ml)
Time (min)
e
AUC (pg·hr/ml)
CON
EX
Baseline
After FST