学位論文（要約）

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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北海道大学

陳 冲

CHONG CHEN

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. 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. 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 (BDNF), and insulin-like growth factor-1 (IGF-1), and change the neurotransmitter system (both basal neurotransmitter release and that in response to stress), 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), the final product of the hypothalamic–pituitary–adrenal axis (HPA axis) in response to stressful events. 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. For instance, rodent research has consistently found that, various models of stress and depression, such as chronic mild stress, chronic immobilization, chronic social defeat, and repeated electric shock, lead to increased basal CORT, which is reversed by antidepressant treatment. Further, repeated exogenous administration of CORT in rodents induces such depression-like behaviors as 'learned helpless' and anhedonia, which resembles human

depressive states. In the meantime, human studies also reported elevated basal CORT in plasma, and saliva in clinically depressed patients, which is normalized by antidepressant or psychological treatment. Longitudinal observations have found that higher basal plasma CORT (esp. morning) predicts the onset of depression.

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. 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% to 150% 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.

Chapter 1 The behavioral and neurobiological effects of exercise

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. Second, we chose the Forced Swim Test (FST) to measure the antidepressant-like effect, for it 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, which has been frequently used in our previous research. 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 control and coping and provides negative feedback regulation of the HPA axis response to stress.

Rats were randomly allocated to exercise (EX) or control (CON) group, both raised in the same cage box while only EX rats had free access to a running wheel attached on the side of the box. After three weeks of this allocation and manipulation, one subset of rats was subjected to the forced swim test (FST), while another subset was subjected to the FST under microdialysis. FST was analyzed in terms of immobility, swimming and climbing. CORT was measured using Elisa, while neurotransmitters including dopamine (DA), 5-HT, noradrenaline (NA), glycine, alanine, taurine, glutamine, and glutamate were determined by the high-performance liquid chromatography system. Protein expression of receptors including glucocorticoid receptor (GR), D1R, D2R, and 5-HT1AR was measured by western blot.

We found that three weeks of voluntary wheel running reduced immobility time in the FST, an antidepressant-like effect. Monitoring extracellular fluids in the mPFC we found that, compared to CON, EX rats had significantly higher bassal CORT, but lower CORT

overall-exposure after FST. Further, exercise rats had higher DA both at baseline and after FST. There was no significant effect of wheel running on other neurotransmitters measured, including NA, 5-HT, alanine, glycine, taurine, glutamine, and glutamate. Nor was there significant effect of wheel running on the protein expression density of GR, DA D1R, D2R, or 5-HT1AR.

In summary, the first experiment showed three weeks of voluntary wheel running reduced immobility in the FST, suggesting stress coping or antidepressant-like effect. This is in line with a dozen previous reports that wheel running exerts antidepressant-like effect as assessed by the FST, shuttle box escape deficit, and chronic mild stress. 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.

Recently the DA system has been attracting more and more attention in the context of stress and depression. At least three lines of research argue for a positive role of mPFC DA in the antidepressant-like effect. First, it has been consistently reported that various models of stress and depression, including chronic mild stress, chronic restraint, chronic water bath, chronic cold, chronic social isolation, chronic unavoidable footshock, adolescent social defeat, and maternal separation all decrease DA in the mPFC. In the meantime, various antidepressant treatment, such as tricyclic antidepressants, selective 5-HT re-uptake inhibitors (SSRIs), 5-HT agonist, 5-HT-NA reuptake inhibitors, atypical antidepressant mianserin, combination of atypical antipsychotic with SSRIs, or deep brain stimulation of mPFC increase DA in the mPFC. Second, acute treatment with a cannabinoid receptor 1 antagonist or a triple monoamine uptake inhibitor, or chronic antidepressant treatment with reboxetine and mirtazapine or a Chinese herbal prescription, concurrently increases DA in the mPFC and decreases animal's immobility in the FST. More importantly, several studies found a correlation between DA levels in the anterior cortex, prefrontal cortex (PFC), or mPFC, and reduced immobility in the FST, increased active escape behavior, or the efficacy of antidepressants in reducing immobility in the FST. Third, in the literature of

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

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, 4 weeks chronic unpredictable stress, or 11 days of 98-dB noise. Interestingly, it has also been found that various forms of chronic or repeated stress increase overall CORT exposure to subsequent novel stress, which is attenuated by chronic antidepressant treatment. Elevated stress-responsive CORT has been linked to passive coping behavior, such as staying immobile when confronted with inescapable electric shock. On the other hand, patients suffering from depression have hyper-responsive CORT in the dexamethasone/CRH test and demonstrate higher CORT during the recovery period after stress, which are also normalized after successful antidepressant treatment. 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.

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. Lesions of the mPFC significantly increase plasma levels of both adrenocorticotropic hormone (ACTH) and CORT in response to a 20 min restraint stress, and MDD patients manifest significantly thinned mPFC and higher CORT simultaneously. Interestingly, lesion of the mPFC does not affect basal ACTH and CORT, 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, suggesting that DA in the mPFC normally acts to suppress the HPA activity. Supporting this, research has indicated that the mPFC-GABAergic interneurons at the anterior bed nucleus of the stria terminalis-paraventricular hypothalamic nucleus may mediate this effect. 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. Further, this effect is likely to be GR-dependent, for GR knockdown in the mPFC (infralimbic and prelimbic) leads to hyper-responsive CORT to acute restraint in normal and chronically stressed rats. Our results may provide a potential explanation to these reports.

On the other hand, suppression of CORT by adrenalectomy decreases DA in the mPFC, which is prevented by CORT replacement. Local injection of CORT into the mPFC increases DA in this brain area, which is blocked by a GR antagonist. 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. These results suggest that CORT may potentiate the mPFC glutamatergic input onto DA neurons in the VTA. 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.

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

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.

25-30 min (27.23±0.33) before the FST, EX rats were injected a D1R or D2R antagonist while CON rats received only the vehicle. In the meantime, we monitored the general motor activity of a subset of EX and CON rats using an apparatus with an infrared sensor that detects thermal radiation from animals. We observed that, the antidepressant-like effect of wheel running in the FST was completely abolished by intra-mPFC pre-microinjection of a D2R but not D1R antagonist, at a dose that 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.

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

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. Local injection of CORT into the mPFC increases DA in this brain area, which is blocked by a GR antagonist. Indeed, it has been reported that CORT may potentiate the mPFC glutamatergic input onto DA neurons in the VTA through a GR-dependent mechanism. 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.

A GR antagonist was injected into the mPFC of EX rats 30 min before the FST, while CON rats only received the vehicle. One subset of rats underwent the FST only, while another subset of rats underwent the FST under microdialysis. 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. In the meantime, intra-mPFC pre-microinjection of the GR antagonist also downregulated the basal DA as well as the FST-responsive DA in the mPFC of exercise rats, without any effect on the 5-HT in the mPFC.

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.

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. 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. In humans, higher basal CORT is frequently reported in patients suffering from depression, which is normalized by antidepressant or psychological treatment. Higher basal CORT also predicts the onset of depression in humans. The beneficial exercise, as we and others have demonstrated, also increases basal CORT. Thus, it has been proposed that exercise, should through some mechanism, override the detrimental effect of higher CORT.

The results in the present study suggest that upregulated DA may be one such mechanism. Indeed, whereas various chronic stress upregulates basal CORT and downregulates DA in the mPFC, exercise upregulates basal CORT but also upregulates DA in the mPFC, besides their distinct influence on stress-responsive CORT. Notably, various antidepressant treatments also upregulates DA in the mPFC and higher DA in the mPFC is associated with active coping and effortful behavior. 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: 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.