Do stress hormones connect environmental effects with behavior in the forced swim test?

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Abstract. Forced swim test (FST) is a widely used test for antidepressant development. Depression is a stress related disease, as hormones of the stress-axis can modify mood. However it is not clear, how the appearance of depressive-like behavior (floating) in FST is connected with changes in stress-hormone levels. We hypothesized, that different manipulations would alter the behavior through changes in stress-hormone levels. First the effect of environmental alterations was studied. Increasing water-temperature enhanced floating time together with a decrease in adrenocorticotropin levels. During the dark phase of the day rats spent more time with floating independently from the actual lighting. Neither the phase nor the actual lighting had significant effect on adrenocorticotropin concentrations with higher corticosterone levels during the dark phase. At greater water depth rats float less but the size of animals had no effect. Water depth did not influence adrenocorticotropin and corticosterone responses, but the size of the rats significantly affected both factors. Secondly, administration of imipramine reduced floating and adrenocorticotropin level without affecting corticosterone. Despite the known connection between depression and stress we did not find a correlation between floating behavior and hormone levels. As an alternative mechanism imipramine-induced heart rate and core body temperature decrease was found by telemetric approach. This study is the first summary in rats examining the effect of wide range of environmental alterations during FST. It seems likely that both brain monoamines and stress-axis take part in the development of depression, but these pathways are regulated independently.

Key words: Forced swim test, Depression, Environment, ACTH, Corticosterone

STRESS is one of the most important etiological factors in psychiatric diseases, especially in anxiety and depression, which are highly prevalent in modern society. It is known that the hypothalamic-pituitary-adrenal (HPA)/stress-axis is designed to allow organisms to adapt to physical and psychosocial changes in their environments. The perceived stress activates the central nervous system (CNS), which leads to enhanced secretion of corticotrophin-releasing hormone (CRH) from the hypothalamus into the portal vessels and through increased release of adrenocorticotropin (ACTH) from the pituitary elevates synthesis and release of glucocorticoids (in rodents mainly corticosterone) into the general circulation from the adrenal gland [1, 2]. Exposure to repeated stress is thought to play an important role in the etiology of depression [3, 4]. Overactivation of the HPA axis is the most consistently described biological abnormality in melancholically depressed subjects [3]. Dexamethasone suppression test is one of the best diagnostic tool for this disease suggesting a strong correlation between HPA axis regulation and depression [4].

Depression is a serious medical illness that is manifested by chronic depressed mood, the inability to experience pleasure, withdrawal of interest, feelings of worthlessness and suicidal tendencies [5-7]. To date psychobiological research on depression developed various pharmacological products such as tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors etc. [8]. However, these antidepressants frequently produce side-effects and significant proportion of patients are resistant to current drug therapies [11, 12]. Hence it is a need for the development of more effective antidepressant drugs. Although depression is a human diseases but pharmacological research require animal studies.

One of the best known and widely used test for
antidepressant treatment is the forced swim test (FST) developed by Porsolt et al. [13, 14] both for rats and mice. Although chronic stress seems to be a better model of depression, but preclinical screening require a more simple method. Therefore FST is prevalent and beloved. It measures stress coping behavior with a particular sensitivity to subchronic antidepressant treatment. Since the first description a lot of different alteration is in use. Changes in environmental parameters such as the water temperature, lighting conditions etc. vary from laboratory to laboratory and may strongly influence the outcome [9]. The classical procedure uses a two-day protocol, where on the first day animals are forced to swim for 15 min to induce depressive state. On the second day a 5 min test is performed under the same conditions and subchronic treatment (2-3-times) are applied between the two sections [10].

The commonly used animal model of depression such as the above mentioned FST elevate circulating levels of corticosterone [11], however the link between behavior in the FST and the exaggerated HPA axis is not fully clarified. Our theory was that environmental alterations could have an effect on behavior through changing the level of the stress hormones. According to this assumption, the purpose of this study was to find an obvious correlation between depressive-like behavior (floating) and serum stress hormone (ACTH, corticosterone) levels during the FST. First we have examined the effect of different environmental conditions. The chosen variables may differ between laboratories as well, so our results might give an explanation for the interlaboratory differences, too. Secondly, we have chosen imipramine as a reference drug, because it is one of the oldest and most used antidepressant both during clinical and experimental conditions [12]. Based upon the original procedure we considered only the 5 min test during the second day for the evaluation. As the changes in the stress hormone levels was unable to give a clear explanation for the found behavioral difference, we tested an alternative mechanism. Data from the literature suggested that not only environmental changes but also the imipramine may induce alteration of the body temperature. The question arise if imipramine may influence the FST-behavior via influencing the thermoregulatory pathway.

Materials and Methods

Animals
Tests were done on male Wistar rats (Charles River, Hungary), weighing 300-400 g (Exp. 1, 2, 4, 5) and 200-400 g (Exp. 3). These animals were housed singly in cages under standard laboratory conditions (12h light / 12h dark cycle, light on at 7h except Exp. 2, 23 ± 1 °C, 50-70% humidity, food and water ad libitum) at least 2 days before beginning of the experiment. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine.

Forced swim test
This test is based on the method of Porsolt et al. [13, 14]. All the rats were exposed to FST on two consecutive days with one session per day. On the first day the rats were placed in a glass water tank (diameter: 14cm, high: 40cm or 60cm for Exp. 3). They were let to swim for 15 min before being removed. After that they were dried and returned to their cages. 24h later the procedure was repeated for 5 min, and this time the escape-oriented behavior of the rats was recorded by video camera. Water was changed between subjects. Behavior during the FST was scored by a trained observer - who was blind to group membership – using an event recorder software (H77, developed in our lab). The observed behavioral parameters – aligned in priority – were the time spent with floating (immobility), struggling (climbing), swimming and diving. In case of diving the frequency of the behavior was also recorded. According to Porsolt et al. [13] the floating is described as posture of the rat with those movements necessary to keep its head above the water. In this case animals can make certain, slight swimming movements in order to remain afloat. Struggling is defined as vigorous movements of the four limbs, with the front paws breaking through the surface of the water, against the wall of the tank [15]. During swimming rats made coordinated and sustained movements with all four limbs, usually traveling around the interior of the cylinder, but without breaking the surface of the water with forelimbs [16]. During diving, the rats submerge entire head and body beneath the water [17]. There was no significant alteration in swimming behavior in all of the experiments, and diving is a marginal parameter therefore we concentrated on floating and struggling behavior.

Hormone measurements
At the end of the second FST rats were decapitated
and trunk blood was collected in pre-chilled tubes. Blood samples were centrifuged for 20 min at 2500×g at 4 °C. Serum samples were stored in aliquots at −20 °C until assay. ACTH and corticosterone levels were measured in unextracted samples (50 µL and 10 µL respectively) by radioimmunoassay using antisera developed in our Institute as described earlier [18]. All samples from one experiment were measured in one assay. The intraassay coefficient of variation was 7.01% for ACTH and 7.22% for corticosterone.

**Experimental design**

**Experiment 1**

Rats were randomly assigned to one of four groups that were tested at water temperatures of 15 ± 1 °C, 20 ± 1 °C, 25 ± 1 °C or 30 ± 1 °C (n= 10, each). Animals were tested on two consecutive days during the light phase of the circadian cycle in water tank containing 30 cm of water.

**Experiment 2**

The rats used in this experiment were divided and housed in opposite diurnal cycle (for half of the rats the light was switch on at 22h), under 186 lux during the light and 26.2 lux during the dark phase. After two weeks habituation they were tested among both lighting conditions at 24 ± 1 °C and 30 cm water depth. L-L: housed in light, tested in light; L-D: housed in light, tested in dark; D-L: housed in dark, tested in light; D-D: housed in dark, tested in dark (n= 9, each).

**Experiment 3**

To assess the effect of body size and water depth on behavior in FST we used small (200-250 g) and big (350-400 g) rats (both are already adults, approx. 7 and 9 weeks old) and tested them in cylinders filled with water to a depth of 15, 30 or 45 cm (n= 9, each). The animals were tested at 24 ± 1 °C during the light phase.

**Experiment 4**

As in the above mentioned experiments we could not prove the clear connection between the HPA axis alteration and the depression-like behavior, we tested the effect of an antidepressant treatment, too. In this experiment rats were administered intraperitoneally with imipramine (TCA, Sigma, 30 mg/kg in saline) or saline (NaCl 0.9%; control group). Drugs were injected 3h and 1h before the second session (5 min) of the FST (n= 12, each). Rats were placed in a water tank containing 30 cm of 24 ± 1 °C water. They were tested during the light phase of the day.

**Experiment 5**

To test an alternative mechanism we implanted Vital View biotelemetry emitters (Minimitter Co., Bend, OR, USA) one week before first FST [19]. The emitter was placed into the abdominal cavity of rats through a midline abdominal incision under ketamine–xilazine–pseudophen anaesthesia (50–10–5mg/kg i.p.; 25–5–2.5mg/mL concentration in a volume of 2mL/kg). The negative and positive heart rate leads were attached to the anterior right side of the chest (near the clavicle) and to the posterior chest wall (left to the sternum and anterior to the last rib), respectively. Biotelemetric recordings were made by means of a 12-channel Vital View system (Minimitter Co.). Data were sampled once every minute around the clock, but on the second day the mean (heart rate, temperature) or sum (locomotion) of 10 min was presented. During the FST the biotelemetry sensors were placed near the water tank in vertical position, so horizontal+vertical locomotor behavior was monitored together during swimming sections, while otherwise only horizontal activity was recorded and was expressed as conventional units.

The experimental protocol was the same as in Exp. 4 with continuous recording of heart rate, core temperature and locomotory activity, but without hormone measurement (n= 13-12).

**Statistical analysis**

All data were analyzed using the STATISTICA 6.0 software package (Tulsa, OK, USA). Statistical differences in water temperature and the effect of imipramine were determined by one-way ANOVA (Exp. 1, 4). Two-way ANOVA was used to analyze the effects of lighting condition during housing vs. during the test and body weight vs. water depth (Exp. 2, 3). For Exp. 5 repeated measure ANOVA was used to analyse time-dependent alterations. When it was appropriate a Newman Keuls post hoc test was used. All results were expressed as the mean ± SEM and the significance level was set at p<0.05 for all statistical comparisons.

**Results**

**Experiment 1: Water temperature**

Floating behavior, as shown in Fig. 1A, showed only a tendency with minimal floating at 15 °C and maximal floating at 30 °C (F(3, 36)=2.1, p=0.12). However, comparison the floating time at 15 °C with that at 30 °C the
studied behavioral parameters and stress hormone levels.

**Experiment 2: Lighting conditions**

The lighting conditions during housing significantly affected the behavior of the animals. Rats being kept in the dark phase of the circadian rhythm spent more time with floating than rats from the light cycle (Fig. 2A) (Housing: $F(1,30)=5.74, p<0.05$). The lighting conditions during the test had no significant effect on floating behavior. Statistical analysis demonstrated a significant decrease in struggling behavior in animals from the nocturnal period (Housing: $F(1,30)=12.6, p<0.01$), independently of the lighting conditions during the test (Fig. 2B). There was no further significant alteration in other behavioral parameters. Interestingly the number of faeces boli (Table 1) was also higher in animals came from the dark phase of the cycle independently from the actual lighting (Housing: $F(1,31)=11.1, p<0.01$).

**Fig. 1** The effect of water temperature on floating (A), struggling (B) percentage and on concentration of blood ACTH (C), corticosterone (D) in the rat forced swim test. n= 10 for each of the group.

The effect of temperature became significant ($F(1,18)=5.67, p<0.05$). On the contrary there was a tendency for decreased struggling behavior (Fig. 1B) with increasing temperature, where minimal struggling could be seen at 30 °C ($F(3, 36)=2.24, p=0.099$). The animals struggled significantly less at 30 °C than at 15 °C ($F(1,18)=5.57, p<0.05$). As main effect the water temperature significantly affected only the diving behavior during the FST (Table 1). Both the time spent with diving ($F(3,36)=4.01, p=0.01$) and the number of diving ($F(3,36)=3.43, p<0.05$) was decreased with increasing water temperature.

Similarly, there was only a tendency in serum ACTH levels (Fig. 1C) being continously decreased from group 15 °C to group 30 °C ($F(3,36)=1.29, p=0.29$), but the comparison of the two extreme lead to significant alteration ($F(1,18)=4.37, p=0.05$). The corticosterone levels (Fig. 1D) were completely unchanged.

There were no significant correlation between any...
There was a tendency for reduced ACTH levels in animals came from the dark phase of the cycle (Housing: $F_{(1,31)}=3.3$, $p=0.08$), but the actual lighting conditions during the test had no effect (Fig. 2C). On the other hand the corticosterone levels were significantly increased in animals being in dark phase of the circadian rhythm (Fig. 2D) (Housing: $F_{(1,31)}=13.5$, $p<0.01$). The lighting conditions during the test had no significant effect on corticosterone concentrations.

There were no significant correlation between any studied behavioral parameters and stress hormone levels.

**Experiment 3: Body size and water depth**

In this test we demonstrated that water depth significantly affected floating behavior. At greater water depth rats float less ($F_{(2,45)}=15.3$, $p<0.01$) (Fig. 3A). The size of the animals had no effect on floating behavior at different water depth. The rats struggle more
in deeper water ($F_{(2,45)}=33.03, p<0.01$) (Fig. 3B) with reduced struggling time in bigger animals ($F_{(1,45)}=6.72, p=0.01$), however size × water depth interaction could not be manifested. The 15 cm water allowed the rats to stand on the bottom of the cylinder with their hind legs but this position differed from the floating behavior.

Water depth did not influence ACTH response, but the bigger rats had lower levels ($F_{(1,45)}=4.8, p<0.05$) (Fig. 3C). The water depth did not affect corticosterone concentrations, too, however the bigger rats have higher levels ($F_{(1,45)}=7.46, p<0.01$), (Fig. 3D). Moreover, there was a significant interaction between water depth and body size ($F_{(2,45)}=4.56, p=0.01$).

There were no significant correlation between any studied behavioral parameters and stress hormone levels.

**Experiment 4: Imipramine**
Administration of 30 mg/kg imipramine 3h and 1h before the test section had significant effects on behavior and on physiological response. Animals receiving imipramine had significantly reduced floating time compared to the control, saline treated group ($F_{(1,22)}=8.06, p<0.01$) (Fig. 4A). Imipramine significantly enhanced active behavior, i.e. struggling time, too ($F_{(1,22)}=14.83, p<0.01$) (Fig. 4B). Furthermore, imipramine treated animals had significantly less number of faeces boli in the end of the test (Control: 2.5 ± 0.7, Imipramine: 0.25 ± 0.25) ($F_{(1,22)}=12.09, p<0.01$).

Effect of imipramine on the neuroendocrine axis resulted in reduced serum ACTH level ($F_{(1,22)}=7.81, p<0.05$) (Fig. 4C), however it has no effect on serum corticosterone level (Fig. 4D).

The floating behavior and the number of faeces boli showed positive correlation ($r= 0.46, p<0.05$) (Fig. 4E).

**Experiment 5: Telemetric recordings after imipramine**
During the first 15 min FST (pretest) at 24 ± 1 °C the heart rate of the rats increased with a peak at 5 min ($F_{(253,759)}=14.06, p<0.01$) (Fig. 5A), their core temperature gradually decreased during the whole pretest.
Stress and the forced swim test

The effect of imipramine (30 mg/2 mL/kg, i.p. 3h and 1h before second swimming section for 5 min) on floating (A), struggling (B) percentage and concentration of blood ACTH (C), corticosterone (D) in the rat forced swim test. (E) represents relationship between faces boli number and floating percentage. n= 12 for each of the group. **p<0.01 vs. Control

(F(120,2520)=480.6, p<0.01) (Fig. 5B), while their activity remained constantly high (F(120, 2760)=7.14, p<0.01) (Fig. 5C). Normalisation of the heart rate lasted 4h, but the temperature and activity returned to normal level already after 90 min.

Injection of saline into the peritoneal cavity enhanced the heart rate both after the first (Time: F(16,128)=6.62, p<0.01) and second occasion (Time: F(7,70)=11.15, p<0.01) lasting approx. 30 min (Fig. 6A). Already the first injection of imipramine significantly reduced the heart rate (Treatment: F(1,18)=7.32, p<0.01; Time: F(16,288)=9.08, p<0.01; Treatment x Time interaction: F(16,288)=4.04, p<0.01) with a further reduction after the second injection (Treatment: F(1,20)=36.06, p<0.01; Time: F(5,100)=3.52, p<0.01; Treatment x Time interaction: F(5,100)=5.12, p<0.01). The 5 min FST induced a prolonged elevation of the heart rate (Time: F(9,81)=13.5, p<0.01), while imipramine treatment was able to diminish it (Treatment: F(1,19)=18.0, p<0.01; Time: F(9,171)=27.94, p<0.01; Treatment x Time interaction: F(9,171)=12.6, p<0.01).

Control injection of saline resulted in a slight, although significant elevation of the core body temperature at the first occasion (Time: F(16,128)=10.35, p<0.01), but after the second injection the elevation did not reach the level of significance (Time: F(7,56)=1.92, p=0.08) (Fig. 6B). The first injection of imipramine reduced the core body temperature (Treatment:
Intraperitoneal saline injections induced enhanced locomotion both after the first (Time: $F(16,176)=12.1, p<0.01$; Treatment x Time interaction: $F(16,176)=17.39, p<0.01$). The second injection was able to induce further reduction (Treatment: $F(1,19)=26.71, p<0.01$; Time: $F(5,95)=4.36, p<0.01$; Treatment x Time interaction: $F(5,95)=16.53, p<0.01$). The FST test section reduced the body temperature (Time: $F(9,72)=174.71, p<0.01$), while the imipramine pretreatment was able to induce further decrease (Treatment: $F(1,19)=82.68, p<0.01$; Time: $F(9,171)=238.47, p<0.01$; Treatment x Time interaction: $F(9,171)=33.77, p<0.01$).

Intraperitoneal saline injections induced enhanced locomotion both after the first (Time: $F(16,176)=12.1, p<0.01$) and second occasion (Time: $F(7,77)=3.55, p<0.01$), which effect lasted approx. 30 min (Fig. 6C). Imipramine injection slightly, but significantly reduced the general locomotor activity already after the first (Time: $F(16,352)=13.59, p<0.01$; Treatment x
Time interaction: $F_{(16,352)}=2.1, p<0.01$, but even more pronounced after the second treatment (Treatment: $F_{(1,22)}=6.69, p<0.01$; Time: $F_{(5,110)}=2.98, p=0.01$). The 5 min FST induced a prolonged elevation of the activity lasted approx. 90 min (Time: $F_{(9,99)}=7.82, p<0.01$). Although the imipramine treated animals were more reactive (bigger elevation) but their activity returned to normal level earlier than that of saline treated counterpairs (Treatment: $F_{(1,22)}=5.33, p<0.05$; Time: $F_{(9,198)}=6.47, p<0.01$; Treatment x Time interaction: $F_{(9,198)}=4.45, p<0.01$).

**Discussion**

Environmental alterations (temperature, water depth, diurnal phase of the circadian rhythm) profoundly influenced the behavior of the rats in the FST. It is clear that forced swim is a strong stress, therefore it induces
404 Pintér et al.

a significant elevation in stress hormone levels [20, 21]. Based on our assumption, that floating or immobility reflects a state of “depressiveness” [14], and depression is accompanied by hyperactive HPA axis [22], one would expect that rats floating more have an increased ACTH/corticosterone response. In contrast to our expectation, changes in the HPA axis hormone levels were not correlated with the floating behavior. After imipramine treatment the ACTH level went parallel with the reduction of the depressive-like behavior (floating), however the corticosterone levels was not changed at the end of the test. We cannot rule out that the somatic effect of imipramine (heart rate and core body temperature reduction) may contribute to its behavioral effect, however the reduction in general activity in the homebox is opposing with the enhanced struggling behavior during the test.

The effect of environmental alteration both on the behavior and stress-hormone levels were reported mainly in mice. In C57BL/6J mice the floating time enhanced with temperature (between 20 and 30 °C) without significant changes in corticosterone levels [23]. The effect of the water temperature is strongly strain dependent as in BALB/c mice the increase in water temperature went parallel with a reduction in floating and the corticosterone levels were also smaller [23]. In CD1 mice, similarly to BALB/c, the floating time was lower at 35 °C compared both to 15 and 25 °C with reduced corticosterone levels (our unpublished data). These data suggest some correlation between reduced corticosterone levels and reduced floating time in FST which was not supported by our results in rats.

The peak of behavioral activity for nocturnal rodents occurs during the dark phase of the circadian rhythm [24], however experiments in behavioral pharmacology are carried out mostly in the resting phase of the animals (during the labour hours). In contrast to the general activity rats from the dark phase spend more time with the passive behavior, with floating [25], while lighting conditions during the test had no significant effect [26]. Similar to behavioral activity, the hormones of the HPA axis also showes circadian alterations. This normal HPA rhythmicity is disturbed in depressed patients [27, 28]. As expected, corticosterone levels were higher during the dark phase of the circadian rhythm [25, 26], although the lighting conditions during the test did not affect their levels.

Even though in the original description of the FST [13] the water depth was only 15 cm, this parameter was altered in many experiments during the last three decades. There are several reports describing that rats are more active and float less at deeper, than in the smaller water depth [34, 35]. Using greater water depth it was possible even to discriminate the effects of antidepressants, like selective serotonin reuptake inhibitors and selective norepinephrine reuptake inhibitors [29]. It was hypothesized that the greater water depth may have encouraged the rats to swim and/or climb (struggle) more [30]. Our results reported here are consistent with the previous studies. Regarding the stress-hormone levels, our observation is the first on the field. We were surprised that despite the significant differences in the behavior the ACTH and corticosterone levels were not changed with the water depth.

There are only few literary data – on mice - about the effect of body weight or age on behavior in the FST. In the work of Bourin et al. 4 (20 g), 12 (40 g), 24 (46 g) and 40 (46 g) weeks old Swiss mice were compared [31]. Floating time was similar at different ages, except for 40 weeks old mice. The latter had reduced floating time, but this could be due to their age and not to their size, because they were weighing similar to 24 weeks old animals. It seems that the size of animals has no influence on the floating, but still it has an effect on serum stress hormone levels. The ACTH levels were smaller while the corticosterone levels were higher in bigger rats possibly because of the alteration in feedback regulation [32].

Dysregulation of the HPA axis activity is thought to be an important factor in pathogenesis of depression. Chronic therapy with antidepressant drugs can moderate the HPA axis function, primarily at the CRH mRNA levels [33, 34]. We showed here, that in rats subchronic treatment with imipramine profoundly decreased not only the floating behavior, but also the ACTH serum levels without an influence on the corticosterone level. Great body of evidence suggests that corticosterone secretion could be regulated independently from ACTH [35]. Although it is likely that corticosterone levels would be different at a later time-point but it could hardly has an effect on the previous behavior of the animals. It is possible that only prolonged imipramine treatment could have a significant effect on serum corticosterone levels, which is in accordance with clinical research, showing an effect just after prolonged treatment [36]. In Sprague-Dawley rats, the animals receiving chronic imipramine treatment had significantly less resting ACTH and corticosterone levels than the saline
treated once [37] and the reduction in floating behavior went together with a diminution of the corticosterone levels [44]. The behavioral effectivity of subchroinimipramine, as well as of a selective noradrenaline reuptake inhibitor, reboxetine treatment [38], despite their ineffectiveness on glucocorticoid levels suggests that the behavior of the animal in the FST is not driven through the endormone of the HPA axis. There are several other possibilities for the interaction of the HPA axis and behavior. Although CRH and ACTH are not the endormones of the axis but their levels are increased during stress and they have many other function beyond corticosterone secretion regulation. Among others chronic ACTH treatment could serve as a model of treatment-resistant depression [39]. During stress several neurotransmitter is released from different brain areas [40, 41]. Some possible candidates are the serotonin and dopamine system while their low levels thought to be strongly correlated with the development of depression. However, recent findings not fully agree with the monoamin theory of depression [42].

It is known that forced swimming have a psychological effect on the tested animals. Moreover, swimming for extensive periods (10 min or longer) in relatively cold water (25 ºC or lower) causes profound changes in the physical homeostasis of the animal, too, like increased locomotion, cardiovascular changes, decrease in body and brain temperature and changes in responsiveness of hippocampal serotonergic neurotransmission [43, 44]. As we were unable to find significant correlation between the HPA axis and FST behavior we assumed that these homeostatic changes (at least in part) may be responsible for behavioral alterations. Indeed, 15 min FST at 24 ºC (during pretest) significantly decreased core temperature accompanied by elevated activity and heart rate. Normalisation of all parameters lasted 4h. Administration of imipramine on the next day resulted in significant decreases in heart rate, core temperature and activity independently from FST. The second injection (1h before FST) escalated these effects. Similarly, Carpenter and coworkers [45] found that single injection of high dose imipramine (50 mg/kg) decreased blood pressure, heart rate and rectal temperature. In our hand the second, 5 min FST significantly decreased body temperature in all rats, but after the imipramine injection the effect was more precipitous and more prolonged. We can assume that the temperature reducing effect of imipramine may contribute to its antidepressoin-like behavioral effect as lower water temperature lead to the same result. Furthermore, contrary to imipramine, small doses of morphine is able to reduce struggling behavior [46] together with hyperthermia [47]. Lipopolisaccharide injection is also accompanied by hyperthermia and is able to enhance the floating behavior, too [38, 48, 49]. However, it is contradictory, that a more active animal (reflected by increased struggling after imipramine) has lower heart rate and temperature and its activity prior to the test was also reduced. Imipramine may reduce the horizontal activity of the animals, however its effect is dependent on the dosage and design of the administration. For example three doses of 10 mg/kg had an effect in the FST without an influence on the openfield locomotor activity [50].

Conclusions

We demonstrated that different environmental factors can strongly influence the outcome of the FST. We could not find an obvious correlation between behavior (floating, struggling) and stress hormone (ACTH, corticosterone) levels. It is quite probable, that the FST is model just some aspect of depression and HPA axis changes can be connected to depression just in part of the clinical cases [51, 52]. Clinical studies revealed additive effect between imipramine treatment and reduced corticosterone synthesis [36], which suggest that both brain monoamines and HPA axis take part in the development of depression, but these pathways are independently regulated. Drugs influencing the core body temperature may lead to alteration in floating behavior without strong correlation with depression.

Although FST could be a useful model for preclinical screening of antidepressants, but the mechanisms how a drug can influence the behavior in the FST (e.g. reduction the body temperature) and how it can influence the depressive symptoms in humans (e.g. diminution of the corticosterone levels during prolonged administration) could be different.

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Appendix

There is no conflict of interest.
References


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