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Anhedonia but not passive floating is an indicator of depressive-like behavior in two chronic stress paradigms

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Depression is the most common form of mental disability in the world. Depressive episodes may be precipitated by severe acute stressful events or by mild chronic stressors. Studies on the mechanisms of depression require both appropriate experimental models (most of them based on the exposure of animals to chronic stressors), and appropriate tests for assessment of depressive states. In this study male Wistar rats were exposed to two different chronic stress paradigms: an eight-week chronic unpredictable mild stress or a two-week combined chronic stress. The behavioral effects of stress were evaluated using sucrose preference, forced swim and open field tests. After the exposure to chronic unpredictable mild stress, anhedonia was developed, activity in the open field increased, while no changes in the duration of passive floating could be detected. After chronic combined stress, anhedonia was also evident, whereas behavior in the open field and forced swim test did not change. The levels of corticosterone in the blood and brain structures involved in stress-response did not differ from control in both experiments. The absence of significant changes in corticosterone levels and passive floating may be indicative of the adaptation of animals to chronic stress. Anhedonia appears to be a more sensitive indicator of depressive-like behavioral effects of chronic stress as compared to behavior in the forced swim or open field tests.

Key words: chronic stress, anhedonia, immobility, forced swim, corticosterone, depression

INTRODUCTION

Chronic exposure to various stressful stimuli and life threatening situations may result in the development of mood disorders, among which depression is mostly widespread. According to the estimation of World Health Organization, depression is the most common form of mental disability, affecting more than 350 million of people of various ages over the world (WHO 2016). Long-lasting depression of moderate or severe intensity may become a serious health condition and is considered as a leading cause of disability worldwide and a major contributor to the global burden of disease. In spite of huge efforts directed to investigations of this disorder, neurobiology of depression remains to be a challenge for modern clinical and basic neuroscience.

One of major problems of preclinical studies on depression is related to the ample experimental models; in many cases same models are used to study mechanisms of depression and to test depressive-like state in animals. For example, the forced swim test (FST) was initially suggested by Porsolt and others (1977, 1978) as a tool to study clinical efficacy of potential antidepressant drugs. The FST is based on a capability of animals, i.e. rodents, to exhibit resistance to repeated action of a strong stressor, i.e. placement into a cylinder filled with water. In the classic two-day FST, a 15-min pretest is a stressor suggested to induce a state of "behavioral despair" (Porsolt et al. 1978), which becomes more expressed in the 5-min retest session on the next day. In the initial version of the FST, the time spent in an immobile posture and the latency to the first immobility episode were recorded as an index of antidepressant drug effect. The modified version of the FST included the classification of active behaviors (swimming, climbing, diving) in order to facilitate the differentiation between serotonergic and noradrenergic mechanisms of antidepressant drugs effects (Cryan et al. 2002). However, in contrast to a short-term immobility in the FST, depression represents a lasting condition, which can be hardly assessed in the FST. Indeed, in animal experiments, a discrepancy exists between depressive-like behavioral manifestations in the FST and expected underlying mechanisms. For example, increased corticotropin releasing factor (CRF) level is

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expected to increase depressive-like behavior; however, opposing data have been reported. Over-expression of CRF resulted in a decrease in the immobility in mutant mice (van Gaalen et al. 2002). Interestingly, the content of CRF is persistently elevated in the CSF of patients with major depression relapsed within 6 months despite of antidepressant treatment (Banki et al. 1992). Taking this into account, many authors consider behavior in the FST as stress coping and adaptation (de Kloet and Molendijk 2016, Grigoryan and Gulyaeva 2015, West 1990).

Another popular approach to assess depressive-like state in laboratory animals is testing of anhedonia. Anhedonia is a core feature of major depressive disorder and a key diagnostic criterion according to DSM-5 (American Psychiatric Association – APA 2013). Initially, anhedonia has been considered as a "loss of pleasure", yet neuropsychological and neurobiological studies reveal a multifaceted construct of anhedonia that emphasizes different facets of hedonic function, including desire, effort/motivation, anticipation, and consummatory pleasure (Rizvia et al. 2016). In animal studies, anhedonia was estimated in various models of depressive-like conditions, including chronic unpredictable mild stress (CUMS), social defeat, and others (Duman 2010, Grigoryan and Gulyaeva 2015). In animals, anhedonia may be assessed using "primary" reward such as presentation of palatable food or drink or strong positive reinforcing stimuli such as drug injection or presentation of pups in a specific place. The CUMS model resulting in the development of depressive-like behavior was initially validated as an anhedonia-inducing model (Willner et al. 1992). The rats exposed to CUMS did not exhibit preference of a sucrose solution versus plain water. In this model, reversal of anhedonia through reinstatement of sucrose preference has been demonstrated after chronic treatment with deep brain stimulation (Hamani et al. 2012) or antidepressant drugs (Papp et al. 2003, Willner et al. 1987). Decreased place preference conditioning (Papp et al. 1991, Valverde et al. 1997) and higher brain stimulation thresholds in the experiments with intracranial self-stimulation (Moreau 1997) also suggest decreased response to rewarding stimuli after CUMS.

The development of anhedonia during the exposure to CUMS might be related to high circulating corticosterone (CORT); however, these data are quite controversial. Thus, chronic administration of CORT to mice resulted in a decrease in sucrose preference (Sturm et al. 2015), while Bowens and colleagues (2012) did not find anhedonia in mice with high CORT after social defeat but mice with low CORT exhibited decreased sucrose preference. Direct injection of CRF into the nucleus accumbens shell induced depressive-like behavior including anhedonia in rats (Chen et al. 2012), though viral vector-mediated CRF over-expression in the central nucleus of amygdala did not modify sucrose preference during first weeks after administration of CRF vector, and later on sucrose preference even increased (Flandreau et al. 2013).

The CUMS procedure was initially developed by Katz (1982) and later substantially modified by Willner and others (1987) in order to induce depressive-like behavior with anhedonia as the end-point feature, and the data on behavioral despair in the FST as a result of CUMS are usually in accordance with the effects on sucrose preference or consumption. However, many studies using CUMS to induce a depressive-like state in animals failed to define a consistent behavioral phenotype (Cancela et al. 1995, D'Aquila et al. 1994, Grønli et al. 2005, Harris et al. 1997, Hata et al. 1999, 2001, Willner 2005). In the present study we have compared the effects of two chronic stress paradigms, such as well-known CUMS and less popular combined chronic stress (CCS), on the development of two main indices of depressive-like behavior, immobility in the FST and anhedonia in the sucrose preference test.

MATERIALS AND METHODS

Animals

Male Wistar rats (Stolbovaya Breeding Center, Moscow region, Russia) were used for the study. The rats were housed five per a cage under 12:12 h light/dark cycle and free access to water and food. The experiments carried out in accordance with the EU Directive 2010/63/EU for animal experiments. The experimental protocol was approved by the Ethical Commission of the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences. The schematic drawing of the experimental protocol is presented in Fig. 1. Researchers involved in the study were blinded to the experimental conditions.

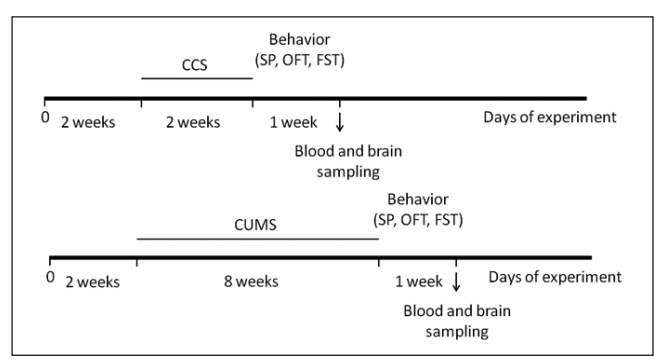

Fig. 1. Schematic drawing of the experimental protocol. CCS – chronic combined stress; CUMS – chronic unpredictable mild stress; SP – sucrose preference; OFT – open field test; FST – forced swim test.

Chronic unpredictable mild stress

For exposure to chronic unpredictable mild stress (CUMS) 4.5–5-month-old rats were used. The animals were housed individually in opaque polypropylene cages in a quiet room. In addition to social isolation, stressors presented to the animals included food or water deprivation, followed by presentation of some pellets or 1-h empty bottle, inclined cage, grouping, wet dust, stroboscope or periodical dark phase lighting. The list of stressors used is presented in Table I. All of these stressors are usually used for modeling of CUMS (Papp 2012). The stressors were changed twice a day so that each of them was presented for 12–16 h. The animals were exposed to CUMS for 8 weeks. Control animals were housed 5 per cage under the above mentioned conditions.

Combined chronic stress

Combined chronic stress was induced in a group of 5–6-month-old rats according to an "experimental neurosis" model elaborated by K. Hecht and collaborators (Baumann and Hecht 1977, Hecht and Poppei 1977) as reported elsewhere (Piskunov et al. 2016, Tishkina et al. 2012). Animals were exposed to stress stimuli daily for 2 weeks. The experimental device consisted of a rack with individual Plexiglas cells of 21×15.5×10 cm in size placed on the electro-conducting floor of steel rods of 0.4 cm in diameter and 1.5 cm between the rods. A stroboscope bulb was heightened 70 cm above the center and a speaker was placed straight above the device. The device was situated in a dark, sound protected room. At 10.00 a.m., the rats were transferred from their home cages to individual cells and stress procedure was started. In these cells, rats were exposed to asthenizing white noise (65–70 dB) for 6 h with a 1-h interval. Once per a day, during an interval between noise stimulations, they were subjected to a sequence of 12 stroboscope bursts consisting of 10 flashes (1 Hz). Time interval between bursts varied from 30 to 90 s. Each burst was combined with 0.5-mA foot shock applied with a 50% probability. Thus, the animals were exposed to six 5 sec foot shocks daily, and the scheduling of the stressors also had partial unpredictability. The control animals were transported to an experimental room but not subjected to a stress procedure.

Sucrose preference test

After the end of exposure to CUMS or CCS, the animals were tested for sucrose preference. For this purpose, two bottles were presented to the animals for two days. One bottle contained pure drinking water and the other 5% sucrose solution. Bottle positions were changed every 12 h during two last days of stress exposure in order to prevent the development of place preference. All bottles were weighted prior and after each 12-h period to control liquid consumption. The preference of sucrose solution was calculated for the last 12 h using the formula $(m_{sucrose})/(m_{sucrose}+m_{water})*100$. Food was available ad libitum during the whole period of testing.

Open field test

The open field test (OFT) was used for assessment of locomotor and exploratory activity in rats. The rats were tested in a round arena with a diameter of 100 cm surrounded with a 30-cm wall (Open Science, Russia). The floor of the arena was divided into 3 concentric zones and 18 sectors. The rat was placed into the central zone of the arena and allowed to explore it for 5 min. Animal behavior was recorded by DMK 23GV024 GigE monochrome camera (The Imaging Source Europe GmbH, Germany). The distance traveled, number of rearing, entries to the center of the arena, and defecations were measured using Ethovision XT11 software (Noldus, Netherlands).

Table I. Stressors used for treatment of animals in the chronic unpredictable stress paradigm

Stressor	Description
food and water deprivation	Periods of food or water deprivations of various lengths were applied. Complete food and water deprivation was applied during 12 h prior to sucrose preference test.
inclined cage	Housing in the cage located on an inclined shelf at an angle of 45°. Since the access of an animal to a feeder was obstructed, this stressor was applied after food deprivation.
group housing	The animals were housed three per a cage; partners were chosen randomly. The stressor was applied during the dark phase after water deprivation.
wet bedding	Housing in a cage with wet dust (250 ml of water per a cage).
stroboscope lighting	Stroboscope lighting, frequency 120 flash/min, during the dark phase.
periodical lighting	Lighting for 2 h every 2 h during the dark phase.

Forced swim test

The forced swim test (FST) was performed in clear Plexiglas cylinders (Open Science, Russia) with a diameter of 20 cm and a height of 45 cm, containing 30 cm of clean water (22°C). The cylinders were thoroughly cleaned, and water was changed from rat to rat. After the test, the animals were carefully dried and kept under a heating fan for 1 h before being returned to their home cages. On day 1, the rats were subjected to a single 15-min swimming session. On day 2, the second 5-min swimming session was conducted. Behavior was recorded by a video camera and then, the scoring was done in a blinded manner. The following parameters were measured: 1) time of struggling, that is the first period of strong movements of the limbs occurring during swimming and diving, breaking the surface of the water or scratching the walls of the tank until the first immobility episode; 2) time of swimming including the struggling period; 3) time of passive floating (immobility) i.e. an episodes when the rats remained motionless, or floating including subtle movements to keep their heads above the water.

Blood and tissue collection and preparation

After the end of behavioral testing the rats were decapitated within a 1-h interval from 4.00 to 5.00 p.m. Blood after the decapitation was collected immediately. It was centrifuged at 1500 g for 15 min and serum was sampled and kept frozen until biochemical assay.

Brains were quickly removed, thoroughly washed in 0.9% saline solution, and dissected on ice. The hippocampi and cerebral cortices were frozen and stored at –80°C. Frozen tissue was weighed and homogenized in a teflon-glass homogenizer in ice-cold Tris-HCl (pH 7.4) buffer containing 50 mM NaCl, 1 mM of EDTA, and 1 mM of EGTA at a ratio of 1:5 (w/v). The samples were centrifuged for 30 min at 10,000 g and 4°C. The cerebral supernatants and blood serum were used for corticosterone assay.

Corticosterone assay

CORT was measured in the serum and in the supernatants of brain structures using a DRG corticosterone kit (DRG Systems, Germany). This ELISA kit is based on the principle of competitive binding. Absorbance at 450 nm was measured using a microplate reader (Wallac, VICTOR 1420, PerkinElmer, Finland). CORT concentration was calculated using the standard curve method and expressed as nmol/l of the serum or per cent of the control value set as 100% for brain tissue.

Statistical analysis

Data are presented as mean ±SEM. For comparison of data on body weight repeated measures analysis of variances was applied with "group" factor for between comparisons and "duration" factor for within comparisons. Tukey HSD test was used for multiple *post hoc* comparisons. Mann-Whitney test was used for estimation of other differences between groups. The differences between the groups were considered as significant at $P<0.05$.

RESULTS

Both stress paradigms result in body weight loss in rats

The animals subjected to CUMS were weighted 7 days before the start of the exposure and the body weight was controlled weekly. Initial body weights were 385±7 (n=25) and 383±8 g (n=27) in the control rats and rats subjected to CUMS, respectively (Fig. 2A). Repeated measures ANOVA revealed that CUMS resulted in a significant weight loss in the rats with main "group" effect $F_{1,50}=5.23$, $P<0.03$; main "duration" effect $F_{8,400}=272.97$, $P<0.001$; and "group"×"duration" interaction $F_{8,400}=12.22$, $P<0.001$. The control animals gained the weight starting week 5 of the experiment ($P<0.01$ as compared to the initial body weight). CUMS induced a decrease in body weight after 1 week of exposure ($P<0.001$ as compared to the initial body weight), and this effect was evident until week 4. After that the animals started to gain the weight and the final body weight was significantly higher in this group as compared to the initial level ($P<0.001$). Significant differences between the control and CUMS groups were observed at 1, 2, and 7 weeks of treatment.

In the experiment with CCS paradigm, the initial body weights were 378±4 (n=12) and 378±5 g (n=16) in the control and CCS groups, respectively (Fig. 2B). Repeated measures ANOVA revealed main "group" effect $F_{1,25}=13.99$, $P<0.001$; a trend to significant main "duration" effect $F_{2,50}=2.59$, $P=0.085$; and significant "group"×"duration" interaction $F_{2,50}=37.55$, $P<0.001$.

In the control group body weight gain was observed during 2 weeks of the experiment ($P<0.03$ as compared to the initial level), whereas in the CCS group, the body weight gradually decreased to the end of exposure ($P<0.001$ as compared to the initial weight). Significant differences between the groups were observed at both time points studied ($P<0.001$).

Both stress paradigms induced anhedonia tested in the sucrose preference test

We applied a 12-h procedure in order to test sucrose preference in the animals. The rats exposed to CUMS

exhibited significantly lower level of sucrose preference as compared to the respective control (U=72, Z=4.85, P<0.001; Fig. 3A). Similar effect was observed in the animals subjected to CCS (U=0.00, Z=3.92, P<0.001; Fig. 3A). Thus, both animals subjected to 8-week CUMS and 2-week CCS exhibited anhedonia after the end of the stressful treatments.

Locomotion and exploratory activity are increased in the rats subjected to chronic unpredictable mild stress

The animals were tested in the OFT one day after the end of exposure to CUMS. Exposure to CUMS resulted in increased distance traveled in the OFT (Fig. 3C) and number of rearing (Fig. 3D) as compared to the control group. Furthermore, the stressed animals entered the center more frequently (2.9±0.4) as compared to the control rats (1.9±0.4; U=216.5, Z=–2.02, P<0.05).

In contrast to the animals exposed to CUMS, the rats subjected to CCS did not exhibit significant changes in locomotor activity or rearing one day after the end of exposure (Figs 3C, 3D). Both stressed and control animals exhibited similar number of entries to the center of the arena (0.25±0.13 and 0.73±0.32, respectively; U=78, Z=–0.56, P=0.57). No differences in other indices studied such as defecation boli and grooming were detected in the animals exposed to CUMS or CCS (data not shown).

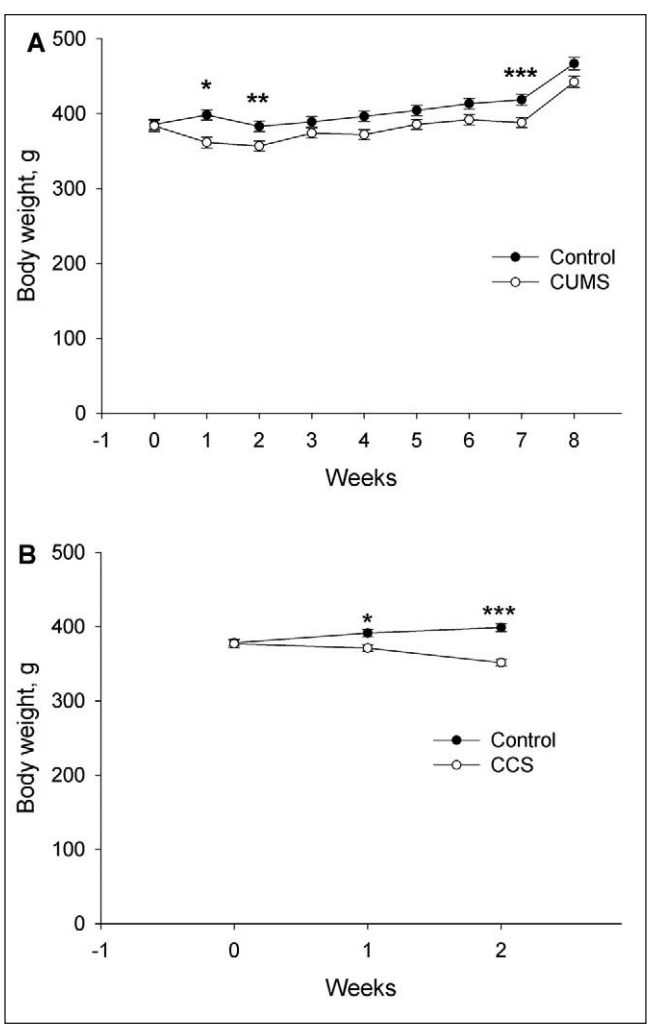

Fig. 2. Effects of CUMS or CCS on body weight in rats. * – p<0.05, ** – p<0.01, and *** – p<0.001 *vs.* respective control groups; Tukey HSD *post hoc* test. Data are presented as M±SEM.

Either stress paradigms do not affect passive floating in the forced swim test

As an additional index of depressive-like behavior in animals after CUMS or CCS, Porsolt FST was performed. The rats exposed to CUMS did not differ from the respective control group in the duration of passive floating (immobility) considered as a main feature of depressive behavior in the FST (U=259.5, Z=–0.98, P=0.32; Fig. 3B). Neither did we observe any difference in this behavioral index between the rats subjected to CCS and respective control animals (U=79.5, Z=0.00, P=1.0; Fig. 3B). Moreover, we did not find differences in the duration of active swimming (U=259.5, Z=0.98, P=0.32 and U=79.5, Z=0.00, P=1.0 for CUMS and CCS, respectively; Fig. 3 B), duration of struggling (the first episode of active swimming with strong movements of the limbs occurred during swimming and diving until the first immobility episode), number of immobility episodes, and number of diving (data not shown).

Corticosterone content in serum and in brain regions is similar in both stress paradigms and controls at the endpoint of the experiments

CORT levels in both experimental (stressed) and control groups were measured in the serum of blood collected immediately after decapitation. We did not observe any differences between the stressed animals exposed to CUMS or CCS and respective control rats at the endpoint of the experiments (Fig. 4A).

CORT contents were also measured in the frontal cortex and hippocampus of rats. Again, we did not find significant changes in the CORT levels in the rats exposed to CUMS or CCS in both structures studied (Figs 4B, 4C).

DISCUSSION

In the present study we have compared the endpoint effects of the exposure of rats to chronic stressful conditions

using two different experimental stress paradigms. We found that the experimental treatment resulted in a significant decrease in the body weight and in anhedonia development though no changes in the immobility duration in FST. Additionally, the rats exposed to CUMS exhibited increased both locomotor and exploratory activity in the OFT. No changes in CORT contents in the blood and brain structures could be detected, irrespectively of the paradigm used. Thus, both protocols used were sufficient to induce anhedonia but not immobility in the FST.

In patients, symptoms of major depression including depressed mood, loss of interest and enjoyment, and reduced energy leading to diminished activity are sometimes induced by exposure to severe acute stress or chronic low-grade stress (Kessler 1997, WHO 2016). Many people with depression also suffer from anxiety symptoms, disturbed sleep and appetite and may have feelings of guilt or low self-worth, poor concentration as well as some medically unexplained symptoms. Similarly, exposure of animals to various stressors results in behavioral alterations reminiscent of various features of depression (Duda et al. 2016, Duman 2010, Grigoryan and Gulyaeva 2015, Papp et al. 1991, Willner et al. 1987, 1992). However, one cannot be sure that long-term manipulations with animals are equal to an initiating event inducing a depressive response or leading to the development of predisposition to further depression (Willner and Mitchell 2002). Yet, Willner and Mitchell (2002) consider CUMS as a model of such initiating event, more suitable to induce depression as compared to other approaches. CUMS is well known as an effective approach to induce depression in animals (apparently similar to humans). However, it is also widely recognized

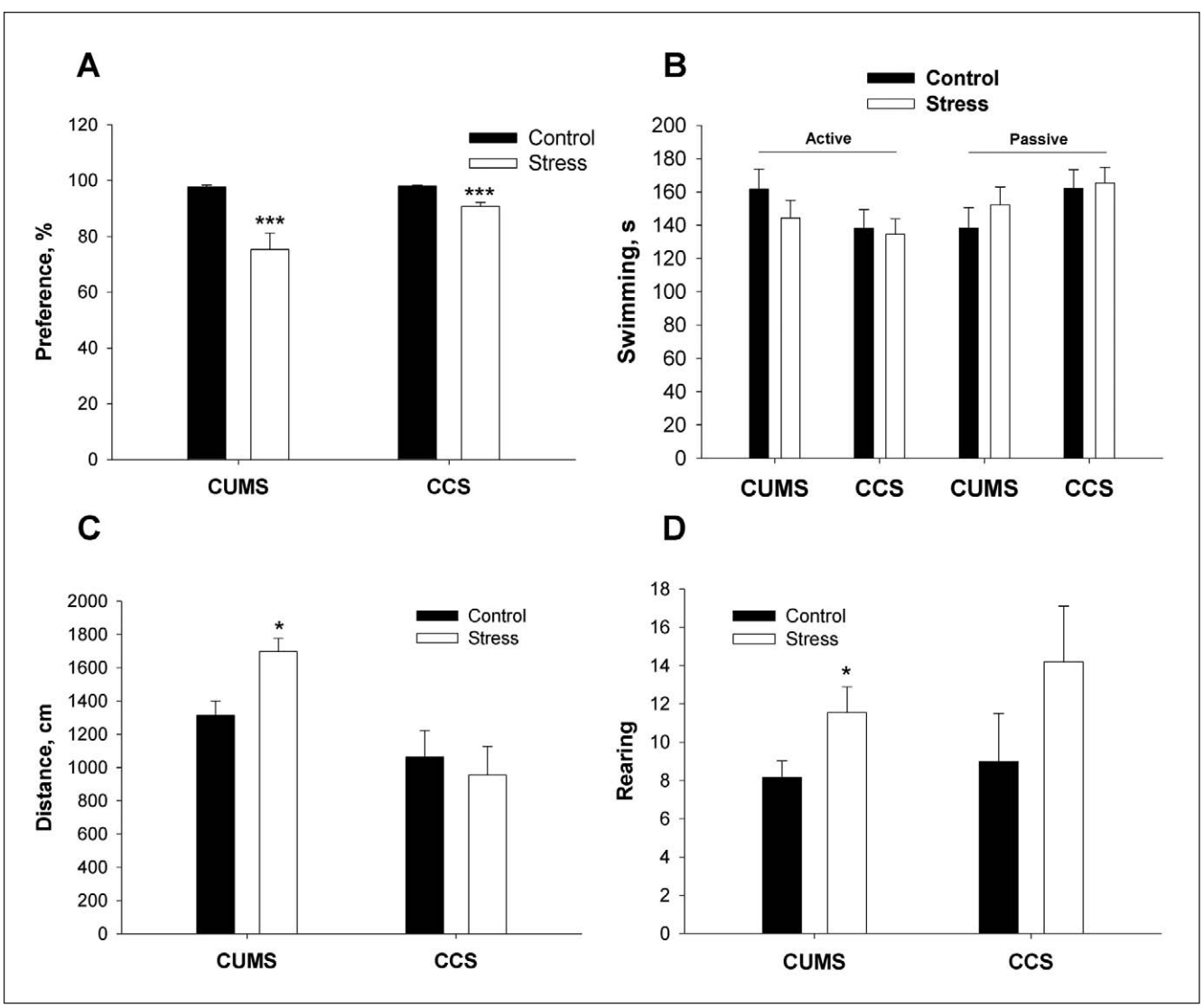

Fig. 3. Effects of CUMS or CCS on animal behavior in the sucrose preference (A), forced swim (B) and open field (C and D) tests. (B) Active and passive are duration of active or passive swimming, respectively. *** – p<0.001 *vs.* respective control groups; Mann-Whitney test. Data are presented as M±SEM.

that the effects of CUMS are dependent on various factors. For example, it depends on species, mouse/rat strain, order and duration of stressors and sex. It has also been noticed that establishing of the CUMS model varies between laboratories (see Herrera-Pérez et al. 2008, José Jaime et al. 2016, Willner 1997, 2005). Therefore, in the present study, another model of chronic stress was applied in addition to CUMS. This model, initially developed as a model of "experimental neurosis" (Baumann and Hecht 1977, Hecht and Poppei 1977) and called CCS later on (Piskunov et al. 2016, Tishkina et al. 2012), is based on the application of apparently stronger stressors, including movement restriction, white noise, stroboscope light, and short-term foot shock. Similarly to the rats exposed to CUMS, the animals subjected to CCS also developed anhedonia. Though data on a capability of various antidepressants to reverse CCS-induced anhedonia are unavailable, data on alterations in main monoamine neurotransmitter system, primarily the serotonergic system, in the prefrontal cortex and hippocampus (Chumakov et al. 2006) may indirectly support potential effect of antidepressants. In any case CUMS- or CCS-induced anhedonia was associated with significant body weight loss.

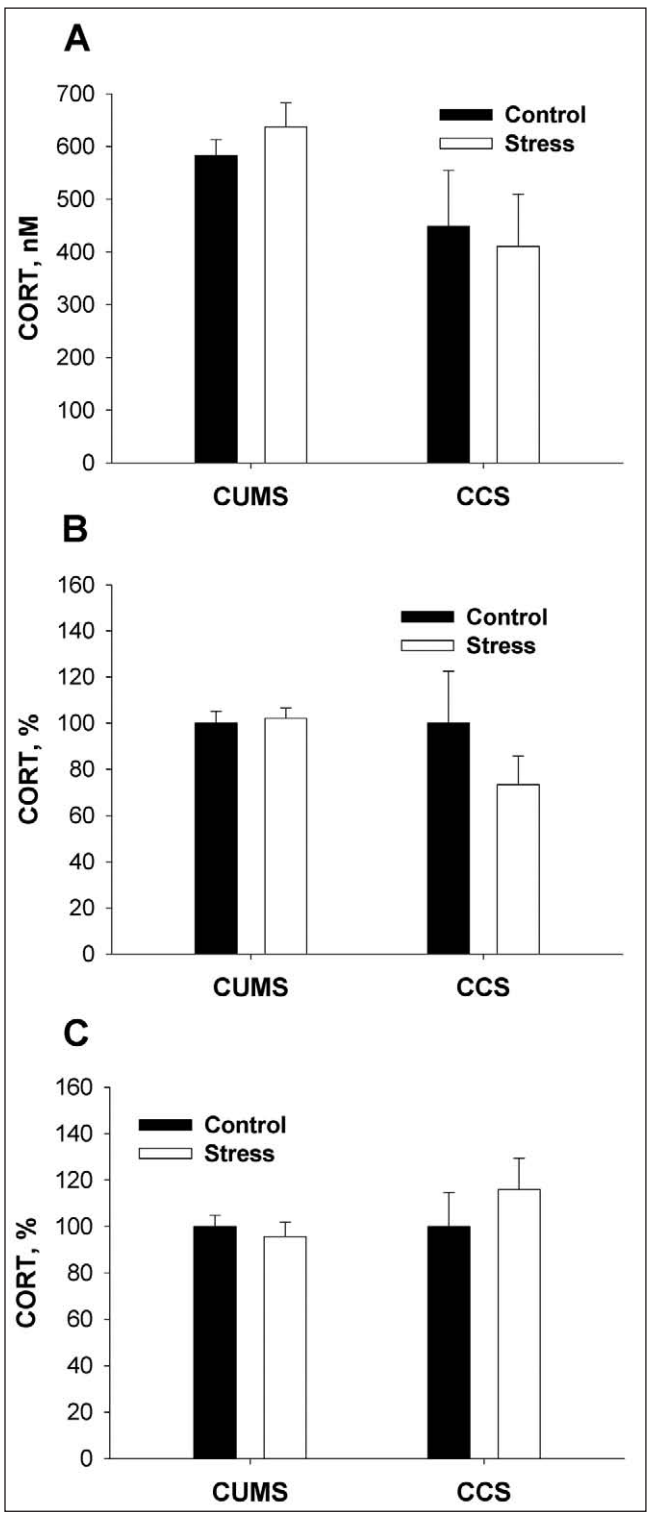

Fig. 4. Effects of CUMS or CCS on the CORT contents in the blood serum (A), frontal cortex (B) and hippocampus (C). B and C data are presented as percentage of respective averaged control levels set as 100%. Data are presented as M±SEM.

Neither CUMS, nor CCS were able to induce immobility in the FST. Although the FST was developed to test antidepressant activity of various pharmaceuticals, this test based on a stress-restress approach is often used for evaluation of a depressive-like state in animals. During the second exposure to inescapable water immersion, the animal rapidly stops active movements and exhibits immobile posture. The duration of this immobility is supposed to represent a measure of a depressive-like state. The higher immobility duration associated with CUMS-induced anhedonia has been demonstrated in several studies (Briones et al. 2012, Elizalde et al. 2008, Fu et al. 2016, Strekalova et al. 2004, Taksande et al. 2013). Interestingly, a statistically significant correlation was only found between the cognitive deficits and immobility in the FST induced by CUMS but not the anhedonia level (Elizalde et al. 2008). In the present study the effect of exposure to CUMS on the passive floating was not confirmed. However, these animals exhibited higher CUMS-induced locomotion in the OFT, including the distance traveled, exploration and number of entries into the center of the arena. Thus, in concordance with the previous proposal (Strekalova et al. 2011), this hyperactivity may be responsible for the absence of the effect of CUMS on immobility in the FST. On the other hand, increased activity in the OFT is considered as a feature of depressive-like state in an olfactory bulbectomy model of depression in rats (Song and Leonard 2005, Stepanichev et al. 2016). Increased activity in the OFT after CUMS exposure has been reported as well (Grønli et al. 2005). The specific effect of each paradigm used in the present study on the behavior in the OFT may be related to different age of animals at the end of the experiment though we were unable to find any information on the influence

of two-month age difference on behavior of adult rats. On the other hand, there exist alternative explanations of this "inconsistent" result. The above mentioned correlation of CUMS-induced immobility with cognitive impairments indicates that behavior in the FST may represent rather a learning effect than depressive state. The results of the previous studies also suggest that the behavioral process involved in the FST is rather "learning to be immobile" instead of "behavioral despair" (de Kloet and Molendijk 2016, De Pablo et al. 1989, West 1990). Moreover, the forced swim experience provides a unique paradigm to investigate the mechanistic underpinning of stress coping and adaptation (de Kloet and Molendijk 2016). The data from rats exposed to CCS also demonstrate that stress-induced anhedonia does not necessarily correlate with passive floating in the FST. Since CCS did not affect locomotor and exploratory activity in the OFT, this effect was not related to modified activity of the stressed rats.

An important role of glucocorticoids in the regulation of stress response is well recognized. Increased glucocorticoid levels are suggested to be associated with behavioral abnormalities, including anhedonia, immobility in the FST, cognitive impairments, etc. In the present study, the levels of circulating CORT and its contents in the frontal cortex and hippocampus were similar in the animals exposed to CUMS or CCS and the respective controls. The absence of a visible effect of chronic stress on the CORT level is not surprising. Measurement of faecal CORT metabolites in rats exposed to CUMS revealed the initial increase in its content and the re-establishment of CORT at the basal level after several weeks of exposure to stressors (Christiansen et al. 2012) indicating adaptation of the hypothalamo-pitutitary-adrenal axis to chronic stressful conditions. We can suppose that long-term manipulations with rats in the present study also resulted in their adaptation to stressors and this may explain the absence of clear difference in the CORT contents between the control and stressed groups. This does not mean that during the earlier periods of chronic stress CORT levels did not change; most probably, alterations in the hypothalamo-pitutitary-adrenal axis underlied the chain of events leading to depressive-like behavioral manifestations (anhedonia). On the other hand, the absence of clear difference in the CORT levels in the blood may be related to the blood sampling shortly after the end of the FST test. It has been shown that a robust CORT response peaked significantly at 30 min and had almost returned to baseline 120 min after exposure (Connor et al. 1997).

The appearance of anhedonia in depression patients as well as in animals with depressive-like conditions may be a consequence of impairment of the reward mechanisms (Rizvia et al. 2016). In the brain, activity of dopaminergic (DA) neurons of the ventral tegmental area (VTA) innervating nucleus accumbens plays an important role in these mechanisms. Phasic firing of VTA DA neurons is essential for reward behaviors (Tsai et al. 2009). It has been shown that chronic social-defeat stress, an animal model of depressive-like state (Hollis and Kabbaj 2014), increases firing rate of VTA DA neurons in susceptible animals (Cao et al. 2010). This brain region performs a stress-context detecting function mediated by the interaction between CRF and brain-derived neurotrophic factor (BDNF) on molecular level (Walsh et al. 2014). Stressors of various type and/or duration may differently modify activity of the VTA-nucleus accumbens gating system and, thus, differentially impact behavioral responses. For example, two-week exposure to cold, a comparatively mild inescapable stressor, induced a pronounced reduction in VTA DA neurons activity, whereas restraint stress increased activity in these neurons (Valenti et al. 2012). DA neurons of the VTA as well as in the substantia nigra express glucocorticoid receptors (Hensleigh and Pritchard 2013); therefore, these neurons represent an appropriate target for CORT-mediated regulation after stress exposure. However, it has been shown that significant translocation of glucocorticoid receptor signal to cell nuclei is observed after restraint in the substantia nigra, but not in the VTA (Hensleigh and Pritchard 2013). Taking this into account we can suppose that CUMS and CCS induced anhedonia may be related to impairment of neuronal interactions in the mesolimbic reward system, though they are not obviously related to stress-associated modifications in the CORT level.

CONCLUSIONS

Taken together, our data show that the exposure of rats to chronic unpredictable mild stress or combined chronic stress results in the development of anhedonia regarded as a manifestation of depressive-like behavior. Both an eight-week exposure to chronic unpredictable mild stress and a two-week exposure to combined chronic stress were sufficient to adapt the animals to stressful conditions, and this adaptation was a reason for both the absence of substantial alterations in the duration of passive floating in the forced swim test and the normal levels of corticosterone in the blood and in brain structures involved in a stress response. We suggest that anhedonia is a more sensitive measure of chronic stress consequences as compared to behavior in the forced swim or open field tests.

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