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Middle-range exploratory activity in adult rats suggests higher resilience to chronic social defeat

Matrov D, Kõiv K, Kanarik M, Peet K, Raudkivi K, Harro J. Middle-range exploratory activity in adult rats suggests higher resilience to chronic social defeat.

Objective: Stressful life events play an important role in the aetiology of human mood disorders and are frequently modelled by chronic social defeat (SD) in rodents. Exploratory phenotype in rats is a stable trait that is likely related to inter-individual differences in reactivity to stress. The aim of the study was to confirm that low levels of exploratory activity (LE) are, in rodents, a risk factor for passive stress coping, and to clarify the role of medium (ME) and high (HE) exploratory disposition in the sensitivity to SD.
Methods: We examined the effect of SD on male Wistar rats with LE, ME, and HE activity levels as measured in the exploration box. After SD, the rats were evaluated in social preference, elevated zero maze, and open-field tests. Brain tissue levels of monoamines were measured by high-performance liquid chromatography.
Results: Rats submitted to SD exhibited lower weight gain, higher sucrose consumption, showed larger stress-induced hyperthermia, lower levels of homovanillic acid in the frontal cortex, and higher levels of noradrenaline in the amygdala and hippocampus. Open-field, elevated zero maze, and social preference tests revealed the interaction between stress and phenotype, as only LE-rats were further inhibited by SD. ME-rats exhibited the least reactivity to stress in terms of changes in body weight, stress-induced hyperthermia, and sucrose intake.
Conclusion: Both low and high novelty-related activity, especially the former, are associated with elevated sensitivity to social stress. This study shows that both tails of a behavioural dimension can produce stress-related vulnerability.

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Keywords: depression; exploratory behaviour; inter-individual differences; social defeat; vulnerability

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Significant outcomes

- Inter-individual differences exist in vulnerability to stress in rodent depression models.
- Low exploring rats consistently chose passive coping strategies after chronic social defeat (SD).
- Rats of middle-range exploratory (ME) disposition are good candidates for identifying mechanisms of stress resilience.

Limitations

- Only male rats were studied due to the low aggressiveness of female rats.
- Limited neurochemical information on only three brain regions is available.

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Introduction

Depressive disorders are widespread and by year 2010 became the second leading cause of years lived with disability, wherein depression alone accounted for 8.2% of global disability (1). It is well established that stress exposure is an independent risk factor for depressive disorders and major life events increase the likelihood of onset of the first episode of major depression several fold (2). Furthermore, stressors differ in their impact, as there exist stressor-specific central pathways that differentially regulate the sympathoneuronal and adrenomedullary outflow, as well as the activity of the hypothalamic–pituitary–adrenal (HPA) axis (3). Both clinical and translational research suggests that social relations are particularly important for mental well-being (4,5). Depression risk is estimated to be the highest when a major adverse event is coupled with social rejection. For example, a self-initiated divorce or break-up of the relationship confers smaller risk than a similar event initiated by the partner (6). Another study has found that humans who had experienced a recent major life event involving targeted rejection became depressed three times faster than their counterparts who had experienced other forms of stressful events, but without the humiliation of being rejected (7).

Improved understanding of the largely social origins of the initial episodes of mood disorders has catalysed the development and application of social stress models in translational research. Among them the SD paradigm is most widely adopted. The SD model is based on the antagonistic encounter between a resident rat, which is usually larger and selected for its aggressiveness, and an intruder rat, who is forced to enter resident's territory and in most cases suffers one or many physical attacks from the angry resident (8). Hence, the SD paradigm not only imitates the social nature of a stressful situation, but also narrows it down to the event of social rejection and humiliation, as the intruder rat is usually not able to withstand the attacks of the resident and must repeatedly engage in submissive behaviours. No habituation to the repeated SD stress occurs in glucocorticoid and sympathetic responses (9) and even a single episode of SD is sufficient to produce long-lasting behavioural and physiological changes in affected animals (10–12). Typical findings in the SD paradigm in defeated rats include potentiated release of adrenocorticotropic hormone and glucocorticoids with the concurrent inhibition of androgens, but also hyperthermia, decreased exploratory and social activity, higher immobility in the forced swim test (FST), and altered synaptic plasticity (13–15).

Still, in humans even a highly stressful social environment is depressogenic only in the subpopulation of individuals. Experimental primate studies have confirmed that individual resilience to social stress is important, as not every socially stressed monkey succumbs to the depressive-like behaviour (5). Hence, to improve the predictive validity of animal models of depression it is necessary to account for the individual sensitivity to stress, and numerous rodent models of human affective and mood disorders have been developed to capitalise on the inter-individual differences in behaviour and underlying neurobiology (16,17). Amongst the evolutionarily relevant behavioural strategies, approach-avoidance conflict as expressed in novelty-related, exploratory behaviour (EB) offers an easy distinction between animals with profoundly different adaptive responses. EB in rodents is a stable and easily quantifiable behavioural tendency that comprises both motivational and affective facets. After a series of pharmacological experiments we selected to use the exploration box test as previously described (18) to separate rats with stable expression of low versus high exploratory activity (LE- vs. HE-rats) over repeated testing for many months (19). In acute experiments, the LE-rats are more vulnerable to stress: while there was no difference in behaviour in familiar surroundings, the LE-rats displayed higher anxiety levels in the elevated plus maze (EPM), increased immobility in the FST, and retained a more enduring association between neutral and stressful stimuli in the fear conditioning test (19). Wistar LE-rats consumed less sucrose water, but also tap water during two 1 h testing sessions, which may be explained by their neophobia towards change in the drinking conditions. Nevertheless, in the social interaction test of anxiety the Wistar LE- and HE-rats behaved similarly (19). A number of neurochemical differences may underlie the behavioural variation between LE- and HE-rats: LE-rats had higher number of 5-hydroxytryptamine (5-HT) transporter-binding sites and increased citalopram-induced 5-HT release in the prefrontal cortex, whereas citalopram-induced serotonin release in the dentate gyrus was higher in HE-rats (20). In the striatum, LE-rats show lower baseline and amphetamine-stimulated dopamine (DA) levels and lower proportion of D_2 DA receptors in the high-affinity state (19,21), as well as lower glutamate levels after uptake inhibition (22).

Despite the apparent vulnerability of LE-rats in acute experiments, their adaptive capacity in a chronic mild/variable stress experiment was largely comparable with that of HE-rats, despite of a slightly larger early stress effect (23). Instead, in the course of chronic stress LE- and HE-rats became similar in a number of tests, even though the defining phenotype persisted, the LE- and HE-rats still remaining robustly different in the exploration box test. We reasoned that both active and passive coping strategy

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in novel surroundings may facilitate adaptation to challenges from physical environment and hence set out to examine whether the SD stress would reveal differential vulnerability better. We have also included rats with ME activity as an internal control phenotype, as it has been suggested to facilitate interpretation of the effects of experimental manipulations (17).

Methods

Animals and general procedures

At the age of 2–3 months male Wistar rats (Harlan Laboratories, Venray, The Netherlands) were tested in exploration box and, based on their behaviour on the 2nd day of testing, assigned into groups of LE ($n = 20$), ME ($n = 24$), and HE ($n = 20$) activity, respectively. In total, 200 animals were tested in the selection process, hence the selected HE and LE animals represent tails of the behavioural distribution (frequency distribution of selected rats can be viewed in Fig. 1). Half of each selection group was submitted to SD procedure. The rats belonging to the same experimental condition were group-housed together ($n = 4$) in standard, transparent polypropylene cages with food (Diet R70, Lactamin AB, Kimstad, Sweden) and water available *ad libitum* for the duration of the experiment. Room temperature was maintained at 21 ± 1°C and controlled 12-h light cycle (lights on 08:00–20:00 h) was implemented. After the conclusion of exploration box testing and re-housing, animals were allowed 2 weeks to become used to the new housing conditions. Two LE- and two HE-rats shared each cage, whereas ME-rats were housed with other ME-rats. A 15 days long SD procedure commenced when rats came to 4 months of age. The baseline tests for stress-induced hyperthermia (SIH) and sucrose preference were carried out just before the commencement of the SD regimen, whereas behavioural test battery that included social preference, elevated zero maze (EZM), open-field (OF) test, and FST followed after the end of SD. The rats were weighed daily during the SD. SIH was again measured at the conclusion of the SD exposure series, whereas two additional sucrose preference tests were carried out in the middle of SD and its end, respectively. Upon the conclusion of SD period, all rats were submitted to the behavioural test battery and sacrificed thereafter. Levels of biogenic amines and their metabolites were measured by high-performance liquid chromatography (HPLC) in the frontal cortex, the amygdala, and the hippocampus. Fig. 2 shows schematically the sequence of experimental procedures. All experimental procedures were carried out in accordance with EU legislation (directive 2010/63/EU) and the experimental protocol was approved by the Animal Experimentation Committee at the Estonian Ministry of Agriculture.

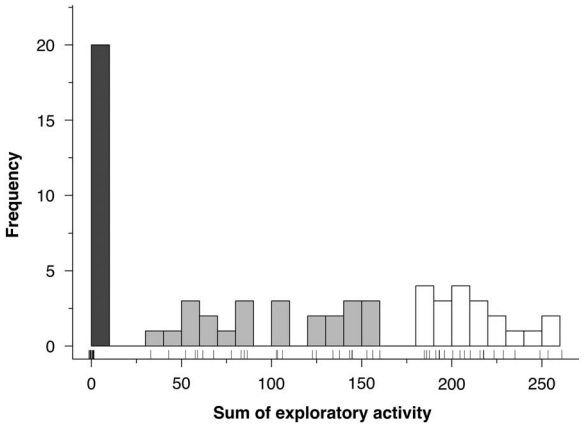

Fig. 1. Distribution of activity scores on the 2nd day of testing in the exploration box. Based on these scores three exploratory phenotypes were identified as low (dark grey bar), medium (light grey bars), and high exploratory activity rats (white bars).

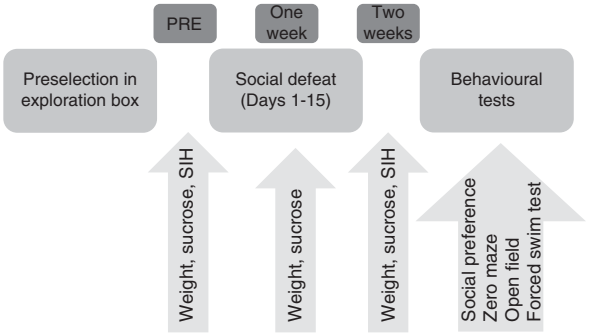

Fig. 2. The general timeline of experimental procedures. SIH, stress-induced hyperthermia.

Selection in the exploration box

The exploration box test was carried out as described (19). Briefly, the exploration box was made of metal and consisted of a 0.5×1 m open area (height of side walls 40 cm) with a $20 \times 20 \times 20$ cm small compartment opening to one of the shorter sides of the arena, which was divided into eight squares of equal size and contained four objects, three novel and one familiar. A rat was placed into the small compartment and for the next 15 min latency to enter, entries into, and time spent in the open area; as well as the exploratory events in the open area such as line crossings, rearings, and object investigations were registered. Rats were tested in the exploration box for 2 consecutive days to determine their stable exploratory activity levels and were assigned to the corresponding exploratory activity groups on the basis of the sum of exploratory activity during the second testing session (Fig. 1).

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Chronic SD regimen

Chronic SD regimen consisted of 15 daily 1 h sessions where rats assigned to stress condition (intruders) confronted a physically larger and aggressive male rat (resident) in the resident's home cage. Our SD procedure employed reliably evoked aggressive dominance on the part of the resident over intruder to produce daily repeating occurrences of social submissiveness in intruders (8). A total of 44 Wistar rats mostly in group-housing, but housed in individual cages in the beginning of the SD regimen, were used as residents. These residents were selected on the basis of their greater body weight over intruders (482–721 g at the beginning of SD regimen vs. 321–439 g weight of the intruders) to assure that all intruders were defeated. The aggressiveness in residents was further facilitated and made less variable by daily subcutaneous injections of apomorphine (1 mg/kg dissolved in 0.001% ascorbic acid) as described previously (24,25). Each day the intruders were confronted with a different resident. One of the experimenters was always observing the intruder–resident encounters ready to intervene and prevent any serious bodily harm to the intruders by dragging the rats apart. No physical damage occurred. Control animals were left alone in a novel cage in a separate room for 15 daily 1 h sessions.

Sucrose preference test

The preference for 1% sucrose solution over normal drinking water was measured three times during the experiment: right before the commencement of SD regimen, between SD days 7 and 8, and at the conclusion of the SD regimen. For the duration of the test animals were housed in single-occupancy cages and provided with a choice of two drinking bottles: one with a sucrose water and another with ordinary drinking water. The consumption of fluids was measured by weighing the filled bottle before the experiment, 1 h into the experiment, and 10 h after the beginning, which marked the conclusion of the experiment. Sucrose preference was computed by dividing the consumption of sucrose water by the total consumption of the fluids. The fluids intake was also normalised by animal's body weight. Testing took place during the dark phase of the light cycle. The position of the bottles was balanced between trials. Food was provided freely during the experiment.

SIH

SIH effect refers to the reliable short-lasting elevation of core body temperature in response to acute stress. It is modulated by anxiety phenotype and degree of habituation to a stressor (26). SIH was measured twice in an experimental room, before the commencement of the SD regimen and right after its conclusion, as described before (27).

Social preference test

A modified version of the social preference test described by Berton et al. (28) was implemented. A rat was placed for 10 min at the centre of a rectangular box (98 × 98 × 40 cm) with walls and floor painted black. Identical small wire-mesh cages were located in the two diagonally opposing corners of the box, one of them was empty and another contained an unfamiliar rat. The unfamiliar rats were male and from the same batch as experimental animals and roughly of the same age and weight distribution. For scoring purposes, the floor of the box was partitioned into several imaginary zones. One-fourth of the box that surrounded the cage with the rat was designated the 'interaction zone', whereas the opposing area surrounding the empty cage was designated as the 'novelty zone'. In addition, the 'central zone', as well as narrow corridors around the cages were designated, respectively, as 'close interaction' and 'close novelty' zones. Testing sessions were recorded on digital video and scored using behaviour tracking and analysis software EthoVision XT8 (Noldus Information Technology, Wageningen, The Netherlands). High mobility state (s) was algorithmically calculated in EthoVision as the duration for which the complete area detected as animal is changing, even if the centre point remains the same. In addition, social interaction was scored by an experimenter with a timer.

EZM test

The EZM test (29) was conducted as previously described (30). An elevated annular platform was equally divided into two opposing enclosed quadrants that were connected by open quadrants. The outer diameter of the annulus was 105 cm and its width 10 cm. The apparatus was elevated 72 cm above the floor and the height of the walls in the enclosed quadrants was 28 cm. Test animal was placed at the centre of one of the open quadrants for 10 min and recorded on digital video. Such measures as latency to enter the closed quadrant, latency to re-enter the open quadrant, time spent in open and closed quadrants, number of stretch-attend postures and head dips over the edge of the open quadrant were scored by an experimenter blind to the experimental group. The open quadrants were also divided into three equidistant parts to quantify the locomotor activity of a rat.

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OF

In this experiment, a painted black square box (78 × 78 × 34 cm) with open top was used. During automatic scoring with EthoVision XT8 software, the apparatus was divided into three imaginary parts: the centre, corners, and wall adjacent areas. The centre was defined as square with the side of 39 cm. Thigmotaxic area was 7 cm wide corridor adjacent to the walls. Each rat was placed in the centre of the box and its behaviour recorded for 10 min.

FST

FST, as first described by Porsolt et al. (31) and subsequently modified, was carried out as previously described (23). The test behaviour was manually scored into three categories of struggling, swimming, and immobility from video playback by an experimenter blind to group assignments.

HPLC

Monoamines and their metabolites were assayed by HPLC with electrochemical (amperometric) detection. Rat brain tissues were homogenised with an ultrasonic homogeniser (Bandelin Sonopuls, Berlin, Germany) in ice-cold solution of 0.1 M perchloric acid (30 µl/mg for amygdala and 50 µl/mg for hippocampus and frontal cortex) containing 5 mM of sodium bisulphite and 0.4 mM ethylenediaminetetraacetic acid (EDTA) to avoid oxidation. The homogenate was then centrifuged at 14 000 rpm for 10 min at 4°C. Aliquots (10 µl) of the obtained supernatant were chromatographed on a Luna C18(2) column (150 × 2 mm, 5 µm). The separation was done in isocratic elution mode at column temperature of 30°C using the mobile phase containing 0.05 M sodium citrate buffer at pH 3.7, 0.02 mM EDTA, 1 mM KCl, 1 mM sodium octanesulphonate, and 7.5% acetonitrile. The chromatography system consisted of an isocratic pump (Agilent, Waldbronn, Germany), a temperature-regulated autosampler, a temperature-regulated column compartment, and an HP 1049 electrochemical detector (Agilent, Waldbronn, Germany) with glassy carbon electrode. The measurements were done at an electrode potential of +0.7 V versus the Ag/AgCl reference electrode. The limits of detection at signal-to-noise ratio = 3 were as follows (expressed as pmol/mg tissue for each): 0.08 for DA, 0.10 for homovanillic acid (HVA), 0.05 for 3,4-dihydroxyphenylacetic acid, 0.08 for 5-HT, 0.04 for 5-hydroxyindoleacetic acid, 0.07 for noradrenaline (NA), and 0.03 for normetanephrine.

Data analysis

Multivariate normality of the data set was confirmed with Mardia's test of multivariate skewness and kurtosis. Kurtosis of the data did not differ from normality, whereas a slight positive skewness was present ($p < 0.05$). Overall, the data was deemed to approach the normal distribution and hence parametric statistical tests were used throughout. The only exception was the results of EB, where analysis of variance (ANOVA) score is presented for convenience, whereas a more appropriate Kruskal–Wallis test did show equally strong group differences. ANOVA was generally performed with two between-subjects factors: Stress (divided into SD and control conditions) and Exploration (divided into LE, ME, and HE conditions). Repeated measurements of the same construct were treated as within-subjects Time factor. *Post hoc* tests were performed by the least significant difference method. Correlations were computed by Pearson's product-moment method with Holm's adjustment for multiple comparisons. Statistical analysis was performed with SPSS and R software packages.

Results

Selection in the exploration box

Univariate ANOVA with subsequent *post hoc* tests confirmed the statistically significant group differences between LE-, ME-, and HE-rats in the sum of exploratory activity on the 2nd day of testing [$F(2,61) = 296.3$, $p < 0.005$; corresponding scores: LE 0 ± 0 vs. ME 101.9 ± 8.1 vs. HE 212.7 ± 23.1, $p < 0.0001$ for all comparisons; Fig. 1].

Changes in body weight

Whereas right before the commencement of the SD regimen the groups did not differ in body weight, after 1 week of the daily stress sessions its effect was statistically significant [$F(1,58) = 11.5$, $p < 0.01$] and increased further after 2 weeks [$F(1,58) = 20.2$, $p < 0.0001$; Fig. 3a]. After the first SD week the stress effect was statistically significant in HE-rats, and after full 2 weeks of stress regimen each stress group weighed less than the respective control group (*post hoc* comparisons, all $p < 0.05$). All control groups gained weight during the SD, whereas stress groups either lost or maintained their prior weight [$F(1,58) = 209.1$, $p < 0.0001$; Fig. 3b]. The mean weight gain for control animals was 22.9 ± 1.2 and −3.1 ± 1.4 g for stressed rats ($p < 0.001$). ANOVA further demonstrated the interaction between Stress and Exploration [$F(2,58) = 3.6$, $p < 0.05$]. *Post hoc* tests clarified that this effect was primarily due to the difference between Stress HE and ME groups ($p < 0.05$).

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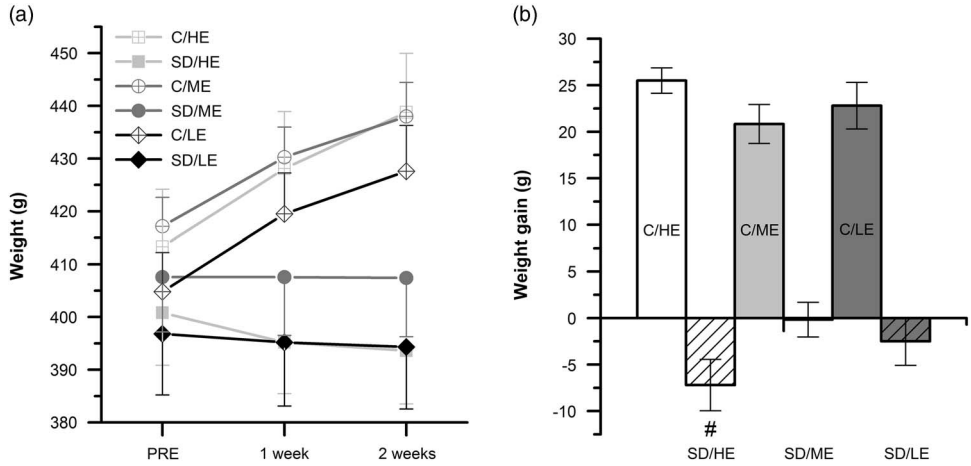

Fig. 3. (a) Body weight (g) before and over 2 weeks of the social defeat (SD) regimen. (b) Weight gain (g) after 2 weeks of SD regimen (2 weeks minus PRE): all SD groups differ from respective Controls (C) ($p < 0.001$). #$p < 0.05$ versus SD/ME group. Data expressed as means ± SEM. HE, high exploratory activity; LE, low exploratory activity; ME, medium exploratory activity.

Sucrose preference test

Rats clearly preferred sucrose water over the plain tap variety from the first baseline measurement before the commencement of the SD regimen. Henceforth, we discuss the changes of this preference after 2 weeks of stress as compared with the baseline. After 1 h of testing no significant effects of either Stress or Exploration were detected; however, after the full 10 h of testing the main effect of Stress had emerged [$F(1,58) = 6.6$, $p < 0.05$]. Whereas the sucrose water intake increased in all groups, in stress condition the gain was higher. The LE/SD group had the largest and significant gain (Fig. 4b). The repeated measures ANOVA of sucrose water intake (Fig. 4a) confirmed the increase with subsequent trials [Time $F(1,58) = 12.7$, $p < 0.001$] and the higher consumption by SD groups over time [interaction of Time and Stress $F(2,58) = 4.5$, $p < 0.05$]. When sucrose intake was normalised per kilogram of body weight, the effect of Stress in repeated measures ANOVA became stronger [$F(1,58) = 7.6$, $p < 0.001$]. Conversely, on subsequent trials rats drank less ordinary water [Time $F(1,58) = 15.5$, $p < 0.001$; data not shown]. The preference for sucrose increased during the repeated testing and started to approach ceiling on the third test (Figs 4c and d). In the repeated measures ANOVA only the Time factor was significant for both 1 and 10 h measurements [$F(1,58) = 13.3$ and 8.8, respectively; both $p < 0.001$].

SIH

Core body temperature was measured first time before the onset of the SD regimen and second time upon its conclusion. As expected, the first act of measurement (T_0) served as an acute stressor and led to the elevated temperature 15 min later (T_{15}), from 36.9 ± 0.05°C to 38.0 ± 0.04°C in the first trial [$F(1,58) = 537.0$, $p < 0.001$; Fig. 5a], while exploratory phenotype had no effect. The second trial caused the temperature rise from 36.6 ± 0.05°C to 37.8 ± 0.05°C. Herein emerged Time × Stress [$F(1,58) = 6.9$, $p < 0.05$] and Time × Stress × Exploration [$F(2,58) = 5.2$, $p < 0.01$] interactions. The difference between the initial and stress-induced body temperature was smaller for control rats (from 36.6 ± 0.06°C to 37.7 ± 0.07°C) than for SD animals (from 36.5 ± 0.07°C to 37.9 ± 0.06°C, $p < 0.05$). In stress-induced T_{15}, an interaction between Stress and Exploration factors was identified [$F(2,58) = 4.0$, $p < 0.05$]. *Post hoc* comparisons indicated a significantly higher body temperature in SD/HE and SD/LE rats as compared with respective control groups (Fig. 5b).

Social preference test

All experimental groups spent less time in the quadrant with a non-social stimulus and did not exhibit statistically significant difference in their preference for non-social novelty. Analysis of the social preference revealed the significant effects of Exploration [$F(2,58) = 3.7$, $p < 0.05$] and Stress factors [$F(1,58) = 4.1$, $p < 0.05$]. Socially defeated LE-rats displayed higher social preference than C/LE and also all other SD groups (Fig. 6a). Similar results were obtained by analysing time spent in the social quadrant (data not shown).

The time spent in the narrow area adjoining the socially primed and empty novel cages reflected

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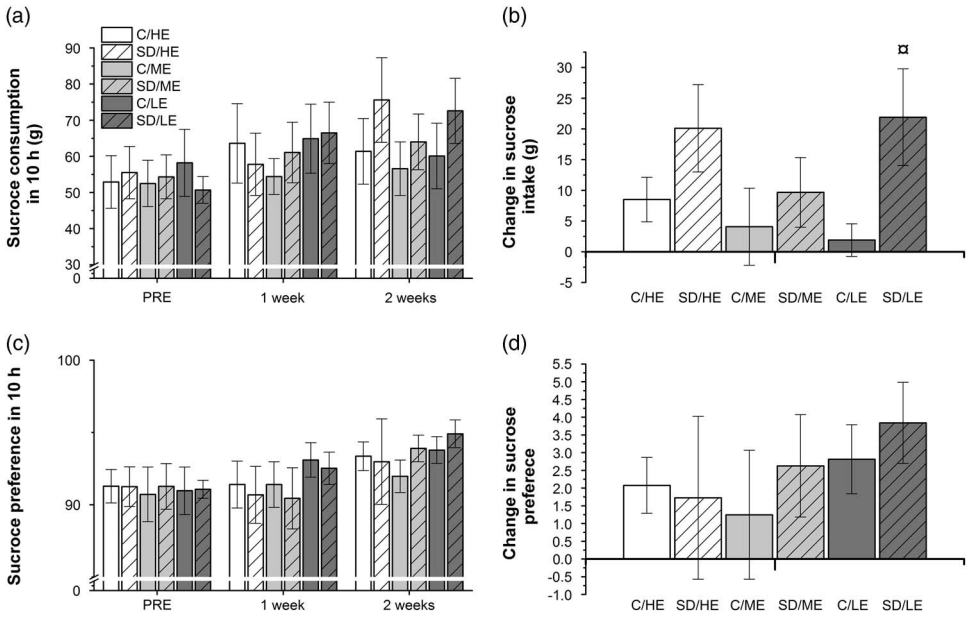

Fig. 4. (a) Ten-hour 1% sucrose water consumption (g) before and over 2 weeks of the social defeat (SD) regimen. (b) Change in 1% sucrose intake after 2 weeks of SD regimen (2 weeks minus PRE). (c) Ten-hour 1% sucrose water preference (% of total liquid consumed) before and over 2 weeks of the SD regimen. (d) Change in 1% sucrose water preference after 2 weeks of SD regimen (2 weeks minus PRE). ¤$p < 0.05$ versus respective Control (C). HE, high exploratory activity; LE, low exploratory activity; ME, medium exploratory activity.

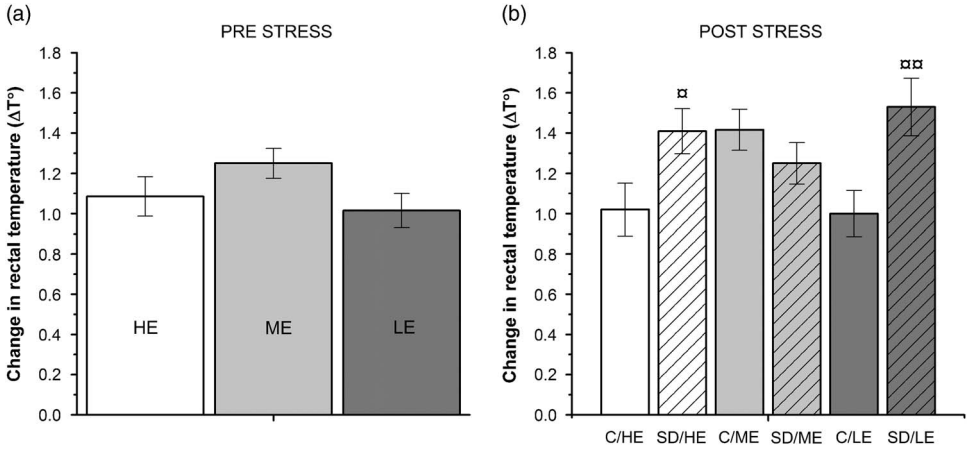

Fig. 5. Stress-induced hyperthermia ($\Delta T = T_{15} - T_0$) as measured before (a) and after 2 weeks of the social defeat (SD) regimen (b). ¤$p < 0.05$, ¤¤$p < 0.01$ versus respective Control (C). HE, high exploratory activity; LE, low exploratory activity; ME, medium exploratory activity.

a more focussed interest of tested animals towards novel stimuli. The time spent in the 'social' area revealed similar results to those exhibited in the quadrant with the social stimulus: both Stress [$F(1,58) = 4.7$, $p < 0.05$] and Exploration [$F(2,58) = 6.3$, $p < 0.01$] factors were statistically significant. Defeated LE-rats spent most time in the area around the social stimulus and differed significantly as compared with control LE, SD/ME, and SD/HE groups (Fig. 6b). Of the total time spent in the social quadrant, each animal remained on average 93.9% of time in the narrow area, herein the SD/LE again had the highest group score of 97.0%. In contrast, half of the SD/LE rats, as well as some members from other groups never entered the quadrant with the novel but vacant cage. For those animals who entered that quadrant, acquaintance with the novel stimulus was evidently still the predominant goal, as on average 88.5% of time was spent around the cage, with C/LE showing the highest preference of 91.5%. Overall, rats preferred to spend on average 59.6% of their test time in the

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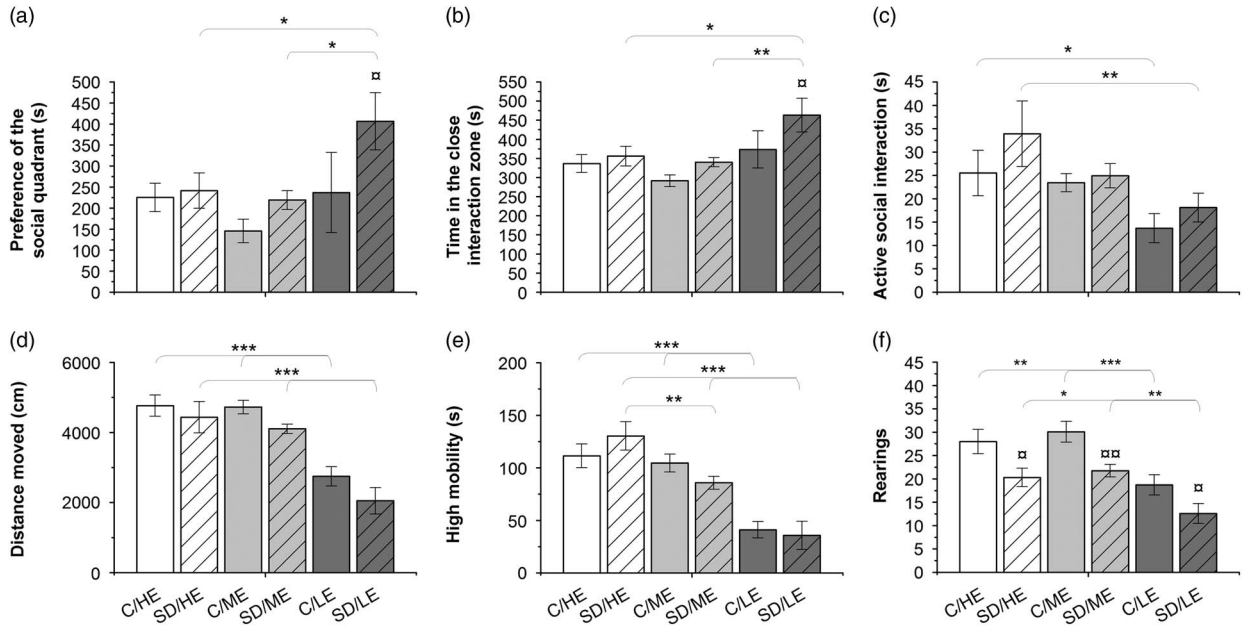

Fig. 6. Activity in the social preference test 5 days after the end of the social defeat (SD) regimen. (a) Preference of the social quadrant of the apparatus [time spent in the quadrant with a wire-mesh cage occupied by an unfamiliar conspecific minus time spent in the quadrant with a similar empty cage (s)]. (b) Time spent in the close interaction zone near the cage with an unfamiliar conspecific in it (s). (c) Social interaction with the unfamiliar stimulus rat (s). (d) Distance moved around in the social preference box (cm). (e) Time spent in 'highly mobile' state (s). (f) Rearings on hindpaws. ¤$p < 0.05$, ¤¤$p < 0.01$ versus respective Control (C); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. HE, high exploratory activity; LE, low exploratory activity; ME, medium exploratory activity.

narrow 'social' area. Among them, SD/LE rats were the most (77.2%) and C/ME rats the least (48.6%) social.

Social interaction between a rat undergoing testing and a previously unknown conspecific confined to the small cage was another indicator of a rat's social motivation (Fig. 6c). Its interpretation is not straightforward, as both rats have to be willing to interact. Percentage-wise, 7.1% was spent in the social interaction across all experimental groups. SD/LE rats spent the least time in the social interactions (4.4%), whereas SD/HE rats spent the most time (9.3%). The main effect of Exploration [$F(2,58) = 5.8$, $p < 0.01$] was present: C/HE rats were more active than the C/LE group, whereas SD/HE animals were more socially active than SD/LE conspecifics. Overall, HE-rats were more socially engaged than LE animals (29.7 ± 4.3 vs. 15.9 ± 2.2 s, $p < 0.01$). Therefore, while LE-rats spent more time in the socially primed quadrant, HE-rats were more engaged in the social interaction. HE-rats also exhibited higher levels of motor activity in the quadrant with the social stimulus as measured by the number of entrances to the narrow 'social' area [Exploration $F(2,58) = 24.4$, $p < 0.001$; HE 19.0 ± 1.5 vs. LE 8.3 ± 1.1 s, $p < 0.001$]. The higher locomotor activity of HE-rats was, however, not specific to the quadrant with the social stimulus, as the number of entrances in the narrow area adjoining the novel empty cage was also significantly higher in HE-rats [Exploration $F(2,58) = 23.4$, $p < 0.001$; HE 12.1 ± 1.0 vs. LE 4.6 ± 1.1 s, $p < 0.001$]. In the overall distance traversed in the test arena, both Stress [$F(1,58) = 5.3$, $p < 0.05$] and Exploration [$F(2,58) = 33.4$, $p < 0.001$] factors were significant (Fig. 6d). To analyse the locomotor activity in the entire test arena, the opposing conditions of high mobility and immobility were compared. Exploratory phenotype had a big impact on high mobility (Fig. 6e) [$F(2,57) = 32.5$] and, conversely, immobility [$F(2,57) = 11.8$, both $p < 0.001$]. HE-rats spend 120.9 ± 8.8 s in the highly mobile state and 225.5 ± 9.7 s being immobile, whereas LE animals were highly mobile for only 38.5 ± 7.4 s and immobile for 312.3 ± 22.8 s ($p < 0.001$ for both comparisons). ME-rats generally did not differ from the HE group in the presented scores of locomotor activity, but were less engaged in high mobility behaviours (95.2 ± 5.52 s) ($p < 0.05$). Both Stress [$F(1,58) = 18.8$, $p < 0.001$] and Exploration [$F(2,58) = 13.8$, $p < 0.001$] factors were significant for rearing activity (Fig. 6f). HE- (24.2 ± 1.8) and ME- (25.9 ± 1.5) rats recorded a significantly higher number of rearings than LE conspecifics (15.7 ± 1.6, $p < 0.001$ for both comparisons). Control condition animals also reared more often than SD rats (25.9 ± 1.6 vs. 18.4 ± 1.2, $p < 0.0001$). The number of rearings correlated highly with the total distance

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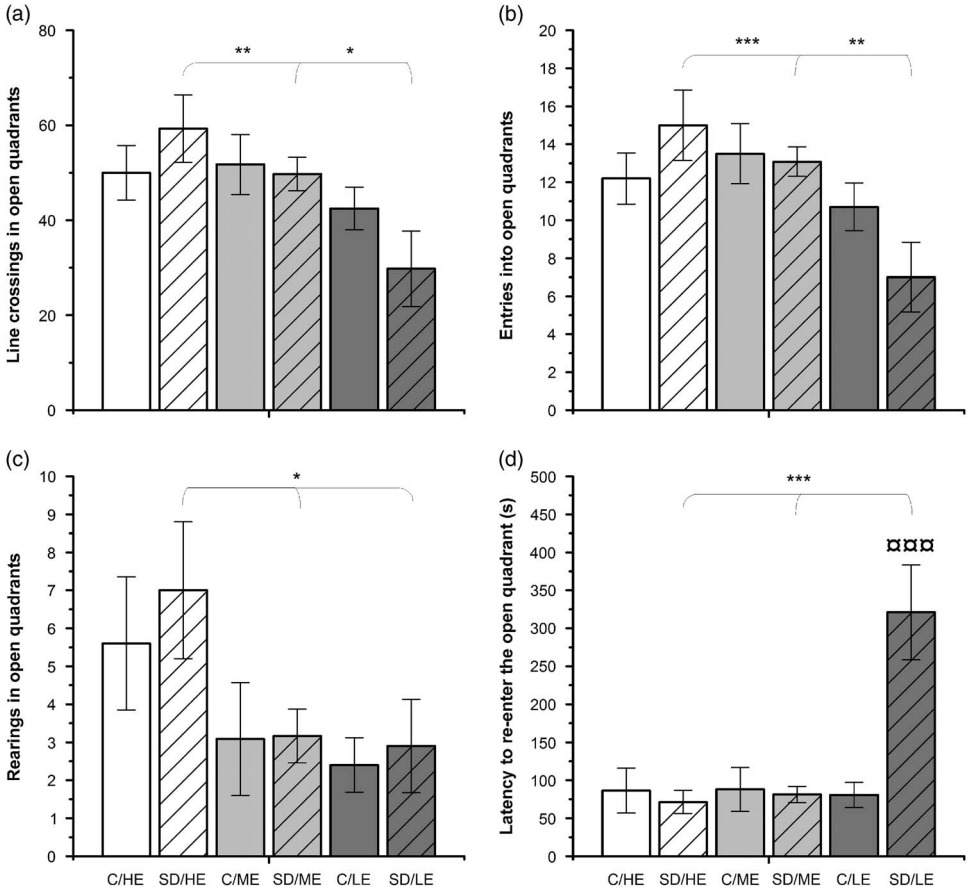

Fig. 7. Activity in an elevated zero maze 6 days after the end of the social defeat (SD) regimen. (a) Line crossings in open quadrants, (b) entries into open quadrants, (c) rearings in open quadrants, and (d) latency to re-enter the open quadrant (s). ¤¤¤0.001 versus respective Control (C); $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. HE, high exploratory activity; LE, low exploratory activity; ME, medium exploratory activity.

travelled by the animal in the test apparatus ($r = 0.78$, $p < 0.001$). However, it was not correlated with the time of social interaction.

In conclusion, SD/LE rats preferred to spend time in the vicinity of the unknown conspecific, but rarely actively engaged it in the social interaction. HE- and ME-rats were mostly similar in their behaviour, characterised by active exploration of the entire test apparatus. Stress was associated with the small increase of passive social preference.

EZM

Rats spent on average 41.2% of their time in the open part of the apparatus. Among them, stressed animals of all three exploration levels spend more time on the open quadrants than respective control animals, but the difference was rather trivial (43.3% vs. 39.0%). For the number of line crossings, the Exploration factor was significant [$F(2,58) = 5.1$, $p < 0.01$]. LE-rats crossed fewer lines than either the ME or HE conspecifics (36.2 ± 4.7 vs. 50.8 ± 3.5 and 54.7 ± 4.6, respectively, both $p < 0.05$) (Fig. 7a). In *post hoc* comparisons, the difference between SD/LE versus SD/HE and SD/ME groups was significant (29.8 ± 7.9 vs. 59.3 ± 7.1 and 49.8 ± 3.5, respectively, $p < 0.05$ for both comparisons). Similar results were obtained for the number of entrances into the open quadrants (Fig. 7b). Exploration [$F(2,58) = 6.3$, $p < 0.01$] was the only factor of statistical significance. ME (13.3 ± 0.9) and HE (13.6 ± 1.2) rats had a very similar activity and both differed significantly from the LE counterparts (8.9 ± 1.2, $p < 0.05$ for both comparisons). SD/LE group had a particularly low activity and differed significantly from SD/HE and SD/ME conspecifics. Exploration factor was statistically significant for the number of rearing [$F(2,58) = 4.2$, $p < 0.05$], but herein the ME-rats clustered with LE-rats: HE group's average was 6.3 ± 1.2 rearings versus 3.1 ± 0.8 for ME and 2.7 ± 0.7 for LE-rats, respectively, $p < 0.05$ in both cases (Fig. 7c). SD/HE rats exhibited higher scores than either SD/ME or SD/LE animals. Head dips were not significantly different between groups.

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Both Stress [$F(1,58) = 8.2$, $p < 0.01$] and Exploration [$F(2,58) = 9.4$, $p < 0.001$] factors contributed to the observed group differences in the initial latency of entrance into an open section [interaction of Stress and Exploration $F(2,58) = 10.5$, $p < 0.001$] (Fig. 7d). LE-rats were significantly slower to emerge into the open than HE and ME animals (200.9 ± 41.8 vs. 78.9 ± 16.2 and 84.6 ± 15.1 s, both $p < 0.01$). Likewise, stress group animals were slower than the control group counterparts (153.1 ± 28.3 vs. 85.2 ± 14.7 s, $p < 0.05$). In *post hoc* comparisons, the SD/LE group strongly differed from all others. As at the beginning of the zero maze test rats were placed in the open quadrant, the latency of the first entrance into the walled segment was also measured. Herein only the effect of Exploration was statistically significant [$F(2,58) = 4.5$, $p < 0.05$]. LE-rats (94.7 ± 23.7 s) exhibited a significantly higher latency of entrance to the walled quadrant than either ME (39.7 ± 4.8 s) or HE (45.4 ± 11.7 s) groups ($p < 0.05$ for both comparisons). The higher latency scores of LE-rats were mostly due to the SD/LE group (134.5 ± 41.7 s), which differed significantly from all five groups, including C/LE (54.9 ± 16.6 s), SD/ME (42.3 ± 5.7 s), and SD/HE (38.3 ± 5.7 s) animals ($p < 0.01$ for all comparisons). In conclusion, behaviour of the SD/LE animals was clearly different. LE-rats submitted to the SD regimen were slower to change quadrants, exhibited less exploratory activity, and seemed to engage in passive coping strategies throughout the test.

OF

Regarding locomotor activity the results in the OF resembled those of the social preference and the EZM tests. Only the Exploration factor was significant in the ANOVA [$F(2,58) = 5.2$, $p < 0.01$], the SD/LE group was specifically less ambulatory (Fig. 8a). When the total distance travelled by each rat was divided into 10 bins of 1 min of duration each, and the repeated measures ANOVA was applied, the Time factor [$F(9,522) = 53.7$, $p < 0.001$] and Time × Stress interaction [$F(9,522) = 2.1$, $p < 0.05$] were significant. Rats of all groups were highly active in the initial couple of minutes and their locomotion was decelerating throughout the trial (Fig. 8b). Control rats covered more distance in the beginning of the test than SD animals; however, this difference vanished from about the midpoint of the trial. The statistical significance of Time × Stress interaction was mostly owing to the low locomotor activity of the SD/LE group during the first 3 min of the test, which differed in *post hoc* comparisons from all other groups ($p < 0.05$). In the analysis of thigmotaxis (Fig. 8c), again, only Exploration was significant [$F(2,58) = 9.3$, $p < 0.001$], although Stress

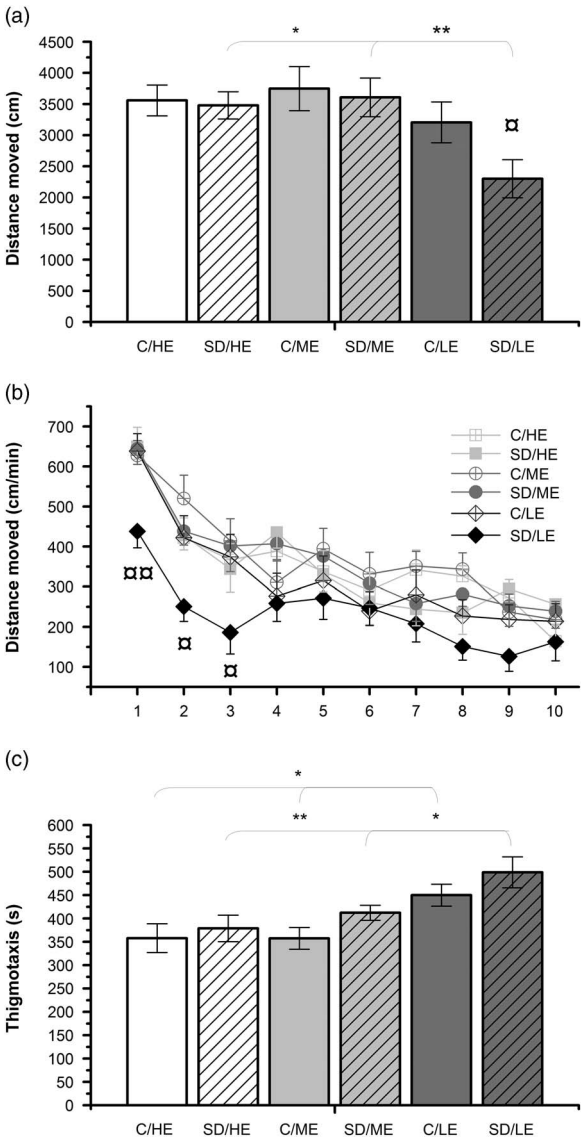

Fig. 8. Activity on an open field 7 days after the end of social defeat (SD) regimen. (a) Distance travelled (cm), (b) distance covered in 1-min time bins, and (c) time spent near the walls of the apparatus (s). ¤$p < 0.05$ versus respective Control (C); *$p < 0.05$, **$p < 0.01$. HE, high exploratory activity; LE, low exploratory activity; ME, medium exploratory activity.

tended to increase the time spent near the walls [$F(1,58) = 3.9$, $p = 0.054$]. The *post hoc* comparisons between exploratory phenotypes confirmed that LE-rats (474.4 ± 20.5 s) have spent longer time near the walls of the test enclosure than both HE (368.3 ± 20.6 s) and ME conspecifics (384.7 ± 15.0 s, $p < 0.001$ for both comparisons). Socially stressed LE animals still exhibited the highest thigmotaxis.

FST

There was an interaction between Exploration and Stress in struggling [$F(2,57) = 3.3$, $p < 0.05$] on the

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first test day, but no significant group differences emerged in *post hoc* comparisons. Hence, FST test did not differentiate between experimental conditions (data not shown).

Behavioural stability between tests

We conducted the correlation analysis between similarly measured behavioural outputs in several tests. Rearings were the type of behaviour measured most often across tests. Rearings measured in EB on the 2nd day, OF, EZM, and social preference tests were all significantly but moderately intercorrelated. The strongest relationship was that between the rearings in EB and social preference test ($r = 0.45$) and the weakest between rearings in social preference test and EZM ($r = 0.29$, all p values at least <0.05). High mobility and immobility were measured with the same algorithm in the social preference test and OF. High mobility behaviours were well correlated ($r = 0.49$, $p < 0.001$). So was the immobility ($r = 0.48$, $p < 0.001$). Another cluster of behaviours can be generalised as a distance covered in the open parts of a test apparatus. We included in the analysis the number of square crossings in EB and EZM, as well as the total distance measured in centimetre in OF. Here again, moderate correlations were observed between all measures ($r = 0.33$–0.41, all p values at least <0.01).

Monoamine levels *ex vivo*

Social stress [$F(1,51) = 6.4$, $p < 0.05$] turned out to be the significant factor for NA levels in amygdala and hippocampus. Overall, SD rats exhibited higher NA levels in amygdala (4.7 ± 0.2) than controls (4.2 ± 0.1, $p < 0.05$). Similarly to the results found in amygdala, NA levels in hippocampus were higher in SD rats [Stress $F(1,56) = 6.7$, $p < 0.05$]. Here exploration factor also had significant impact [$F(2,56) = 8.4$, $p < 0.01$]: ME-rats had significantly lower NA levels (3.3 ± 0.1) than both HE- (3.7 ± 0.1, $p < 0.05$) and LE- (3.8 ± 0.1, $p < 0.001$) rats (Table 1). Group-wise, the SD/LE rats were found to have significantly higher NA levels (4.2 ± 0.2) than all other groups. In frontal cortex, there was also an overall effect of Stress on HVA [$F(1,33) = 4.2$, $p < 0.05$]: control animals had higher HVA levels than socially stressed rats (0.09 ± 0.01 vs. 0.06 ± 0.01).

For serotonin turnover in amygdala, the interaction of Stress and Exploration was noted [$F(2,53) = 4.0$, $p < 0.05$]. This effect was mainly due to the opposing results in ME-rats: *post hoc* tests indicated the significantly lower serotonin turnover in SD/ME group as compared with C/ME rats. Another significant effect was found in the hippocampal serotonin levels, in the interaction between Stress and Exploration [$F(2,54) = 5.4$, $p < 0.01$]. *Post hoc* comparisons showed that C/ME rats exhibited lower serotonin levels than either C/LE, C/HE, or SD/ME groups. A similar effect was also found in serotonin turnover [Stress × Exploration $F(2,54) = 5.4$, $p < 0.01$]: C/ME rats had higher serotonin turnover than C/LE, C/HE, and SD/ME. In addition, the SD/HE group demonstrated higher 5-HT turnover in comparison with C/HE.

Discussion

Rats submitted to SD exhibited lower weight gain, higher sucrose consumption, showed larger SIH, had lower levels of HVA in the frontal cortex, and higher levels of NA in the amygdala and the hippocampus. OF, EZM, and social preference tests revealed the interaction between stress and phenotype, as only LE-rats were further inhibited by SD. ME-rats exhibited the least reactivity to stress in terms of changes in body weight, SIH, and sucrose intake.

We will consider the following three themes: (1) How successful was SD stress in affecting rat behaviour?, (2) What role did exploratory phenotype play in the sensitivity to the effects of SD stress?, and (3) What biological mechanisms could underlie differential sensitivity to SD stress?

SD stress

The resident–intruder paradigm was initially developed to study natural aggressive behaviour in rats (32,33). Over time research focussed on the prolonged effects of defeat on the intruder yielding the animal model of social stress (34). SD stress has been shown to elicit many physiological changes, such as tachycardia and cardiac arrhythmias, increased hyperthermia in response to acute stressor, suppression of the circadian rhythmicity, and changes in reactivity of the HPA axis (35–38). Behaviourally, it tends to suppress exploratory and social activity, increase anxiety-like behaviour, promote passive coping strategies in the FST, and reduce preference for sweetened water (39–42). Experimental results in SD paradigms have been summarised in several recent reviews (13–15,43).

In general, rats submitted to SD regimen in this study exhibited lower weight gain, drank larger amount of sucrose water, showed bigger amplitude in the SIH, had lower levels of the DA metabolite HVA in the frontal cortex, and higher levels of NA in the amygdala and the hippocampus. OF, EZM, and social preference tests did not reveal any sweeping SD effect, but rather the interaction between stress

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Table 1. Biogenic amines, their principal metabolites, and turnover ratios in the frontal cortex, the amygdala, and the hippocampus

	Control			Social defeat		
	LE	ME	HE	LE	ME	HE
Frontal cortex						
NA	2.99 ± 0.15	3.07 ± 0.13	2.87 ± 0.12	3.46 ± 0.32	3.03 ± 0.13	3.02 ± 0.13
NMN	2.49 ± 0.16	2.71 ± 0.15	2.42 ± 0.17	2.69 ± 0.21	2.36 ± 0.15	2.52 ± 0.16
DA	0.43 ± 0.04	0.41 ± 0.03	0.38 ± 0.02	0.42 ± 0.04	0.40 ± 0.03	0.40 ± 0.01
DOPAC	0.16 ± 0.02	0.14 ± 0.02	0.11 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.13 ± 0.01
HVA	0.11 ± 0.03	0.10 ± 0.03	0.07 ± 0.01	0.08 ± 0.02	0.05 ± 0.01	0.06 ± 0.01
3-MT	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.02	0.16 ± 0.02	0.16 ± 0.01	0.14 ± 0.02
5-HT	3.37 ± 0.14	2.96 ± 0.15	3.26 ± 0.14	3.07 ± 0.20	3.27 ± 0.11	3.36 ± 0.17
5-HIAA	1.47 ± 0.08	1.52 ± 0.06	1.43 ± 0.09	1.62 ± 0.11	1.37 ± 0.04	1.54 ± 0.07
DOPAC/DA	0.42 ± 0.09	0.34 ± 0.03	0.29 ± 0.02	0.32 ± 0.02	0.37 ± 0.03	0.33 ± 0.03
NMN/NA	1.22 ± 0.05	1.15 ± 0.04	1.21 ± 0.04	1.29 ± 0.06	1.30 ± 0.04	1.23 ± 0.06
5-HIAA/5-HT	0.47 ± 0.04	0.52 ± 0.03	0.44 ± 0.01	0.48 ± 0.03	0.42 ± 0.02††	0.46 ± 0.02
Amygdala						
NA	4.53 ± 0.26	3.81 ± 0.16	4.33 ± 0.22	4.81 ± 0.31	4.44 ± 0.26	4.82 ± 0.29
NMN	2.42 ± 0.14	2.30 ± 0.12	2.27 ± 0.08	2.20 ± 0.12	2.30 ± 0.07	2.44 ± 0.12
DA	1.78 ± 0.19	1.66 ± 0.42	2.04 ± 0.45	1.92 ± 0.57	1.53 ± 0.23	1.15 ± 0.38
DOPAC	0.23 ± 0.03	0.30 ± 0.09	0.21 ± 0.04	0.28 ± 0.06	0.23 ± 0.03	0.26 ± 0.07
3-MT	1.03 ± 0.66	1.12 ± 0.61	0.87 ± 0.62	1.77 ± 0.73	0.90 ± 0.45	1.12 ± 0.44
5-HT	4.71 ± 0.39	3.24 ± 0.27$^{\Delta\Delta}$	4.62 ± 0.29	4.09 ± 0.39	4.34 ± 0.37†	3.67 ± 0.35
5-HIAA	2.39 ± 0.29	2.62 ± 0.20	2.30 ± 0.17	2.97 ± 0.34	2.35 ± 0.20	2.77 ± 0.18
DOPAC/DA	0.13 ± 0.02	0.21 ± 0.03	0.13 ± 0.02	0.18 ± 0.04	0.17 ± 0.03	0.37 ± 0.16
NMN/NA	2.00 ± 0.16	1.75 ± 0.10	1.91 ± 0.09	2.19 ± 0.11	2.00 ± 0.16	2.02 ± 0.15
5-HIAA/5-HT	0.53 ± 0.08	0.87 ± 0.08$^{\Delta\Delta}$	0.53 ± 0.06	0.77 ± 0.09	0.61 ± 0.08†	0.82 ± 0.09†
Hippocampus						
NA	3.50 ± 0.10	3.24 ± 0.14	3.62 ± 0.14	4.23 ± 0.20†††	3.34 ± 0.13$^{\Delta\Delta\Delta}$	3.70 ± 0.15
DA	0.08 ± 0.01	0.07 ± 0.01	0.11 ± 0.03	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.005
DOPAC	0.15 ± 0.04	0.15 ± 0.04	0.14 ± 0.04	0.22 ± 0.05	0.13 ± 0.01	0.12 ± 0.01
3-MT	0.05 ± 0.005	0.04 ± 0.004	0.06 ± 0.01	0.04 ± 0.004	0.05 ± 0.01	0.04 ± 0.01
5-HT	2.58 ± 0.35	2.28 ± 0.21	2.85 ± 0.53	2.87 ± 0.46	2.35 ± 0.10	2.26 ± 0.13
5-HIAA	1.90 ± 0.14	1.78 ± 0.07	1.86 ± 0.09	1.94 ± 0.11	1.71 ± 0.05	1.95 ± 0.10
DOPAC/DA	1.98 ± 0.41	2.68 ± 0.70	1.71 ± 0.50	2.66 ± 0.43	1.97 ± 0.31	1.55 ± 0.20
5-HIAA/5-HT	0.81 ± 0.08	0.85 ± 0.10	0.80 ± 0.10	0.80 ± 0.11	0.74 ± 0.03	0.89 ± 0.07

3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HE, high exploratory activity; HVA, homovanillic acid; LE, low exploratory activity, ME, medium exploratory activity; NA, noradrenaline; NMN, normetanephrine.

Levels of monoamines and their metabolites are expressed as pmol/mg of brain tissue ± SEM. Turnover of DA, NA, and serotonin is expressed as ratios to the concentrations of one of their major metabolites.

Post hoc least significant difference significance levels are indicated as follows: $\dagger p < 0.05$, $\dagger\dagger p < 0.01$, $\dagger\dagger\dagger p < 0.001$ versus respective control group of the same exploratory phenotype; $p < 0.05$, $p < 0.01$ versus respective HE group of the same stress condition; $\Delta\Delta p < 0.01$, $\Delta\Delta\Delta p < 0.001$ versus respective LE group of the same stress condition.

and exploratory phenotype, as only socially defeated LE-rats were inhibited in their behaviour.

Thus, our SD regimen produced a typical weight gain-lowering effect, but somewhat atypically also increase in sucrose intake. Several previous studies have not found the SD effect on sucrose preference (13,44,45). Increased intake of sucrose solution may be part of the coping response to the chronic SD stress, potentially potentiated by the acute separation from cage mates, as our rats were housed individually for the duration of the sucrose preference testing. We have previously observed increased sucrose intake after chronic variable stress in rats that had a partial lesion of the serotonergic nerve terminal elicited by a low dose parachloroamphetamine treatment resulting in the 20–30% reduction of serotonin levels (46). This effect resembles the carbohydrate-craving state related to low serotonin in depression (47) as chronic administration of citalopram prevented its development (48). As no systematic change in 5-HT was observed in stressed rats in the present study, the parallelism at the behavioural level may not be related to a similar neurochemical substrate, but this can only be settled by *in vivo* neurochemical measurements. Presumably, the increased activation of HPA axis and decelerated weight gain may also produce a shift in the preference towards more energy dense or 'comforting' food options (49). Hyperthermia is a marker of the sympathetic activation and hence a reliable physiological correlate of stress. Increases in

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magnitude of acute hyperthermia in response to a stressor (SIH) as well as in chronic core body temperature have previously been recorded in the SD rats (34,50) and confirm the efficacy of SD in the present study.

Nevertheless, the impact of our SD regimen was comparably less drastic than is often published. One reason may be the choice of residents. Wistar rats are rather non-aggressive by nature (8), therefore it is customary to employ more aggressive feral or Long Evans rats as residents (8,40). However, Wistar rats of larger body size were used as residents and their aggressiveness was potentiated by the daily subcutaneous injections of apomorphine. Apomorphine produces a reliable increase in aggressiveness even in tame strains of rats such as Wistar. However, the elicited aggressive behaviour is somewhat different qualitatively than is naturally observed in aggressive rat strains. Residents under apomorphine influence prefer to assume the upright threatening posture and engage in sham boxing, while biting is rare (51) so our resident–intruder stress paradigm may be perceived as less stressful by intruders. The second important factor in mediating the effects of SD was the social housing of our intruders. Rats are colonial animals that get along quite well in social housing. Individual housing during SD has been shown to potentiate the effect of SD by reducing the locomotion in the OF and time spent on the open arms in EPM, as well as lead to higher activity of the HPA axis, whereas socially housed rats show significant attenuation in stress-related indicators (42,52).

Exploratory phenotype and response to defeat

Exploratory phenotype differentially influenced sensitivity to SD, as reflected in the social interaction, EZM, and OF tests in case of the LE-rats and in SIH in case of ME-rats. Behaviour in both EZM and OF tests reflects anxiety, but also locomotor activity and exploration. Socially defeated LE-rats were significantly inhibited, whereas ME- and HE-rats were little influenced by stress. EPM and EZM are conceptually similar test apparatuses best suited to measure the anxiety in rodents. EZM, however, produces more locomotor behaviour than EPM by promoting the smooth locomotion in the same direction, whereas the abrupt endings of arms in EPM and the central crossroads introduce more uncertainty and behavioural choice (29). Previous studies in the rat SD paradigm have been conducted exclusively on EPM and showed anxiogenic effects of SD. For example, a single defeat session was sufficient to decrease the time spent in the open arms of EPM in Wistar rats (53). Results in the OF type environments have been more equivocal: some studies find no reduction in the non-social exploratory activity after SD (44,45), whereas others note the decreases in locomotion and in such exploratory acts as sniffing and rearing (13,41). Results from the current experiment demonstrate that low exploring animals become further behaviourally inhibited after SD stress, whereas rats with higher basal exploration levels are not affected in their non-social exploration by the social stress regimen.

Another phenotypic model connected to EB has been used in the SD paradigm. Low and high responders (LR and HR, respectively) were initially selected based on their level of locomotion (HR-rats cover about two-thirds to one-third more distance than LR-rats) in a novel circular corridor (54). Interestingly, it was soon discovered that HR-rats are also more sensitive to stress as they exhibit a prolonged elevated corticosterone response in novel environments (55,56). It appears that HR animals show more sensitivity to repeated SD than LR-rats in some behavioural tests. They demonstrate lower weight gain, sucrose and social preference, as well as higher immobility in FST and corticosterone secretion (57,58). Interestingly, the LR-rats display significantly higher levels of passive–submissive behaviour in their encounters with aggressive residents (58). LR- and HR-rats respond similarly to the repeated SD when tested in the OF or on their long-term contextual fear memory (57). Parenthetically, it should be mentioned that in these studies the Sprague–Dawley rat strain was used as intruders and rats were pair-housed in random phenotype combinations during and after the repeated SD regimen (57).

In the social preference test that combines exploratory possibilities of the exploration box with an opportunity of social engagement, the SD/LE group rats again showed the most distinct behaviour: they moved little across the arena, instead preferring to stay in the vicinity of the cage with a stranger rat; however, they rarely engaged in the social interaction with the stranger. In comparison with previously published studies (13,39), we did not find the suppressive effect of SD on the social interactions, whereas the passive social preference was rather promoted by stress.

If a case can be made that LE-rats were the most sensitive to the effects of SD, it is the ME-rats who were the most resilient. The ME-rats were the least susceptible to the stress-induced increased intake of sucrose and to the deceleration of the weight gain. Their reactivity to the acute act of measuring the core body temperature did not change after SD either. In the tests of non-social and social exploration and anxiety, ME-rats behaved rather similarly to their HE

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counterparts (except for rearing in EZM), as both groups were largely unaffected by the SD regimen, whereas LE-rats were clearly retarded in their activity levels. The mechanisms behind this stress resilience remain to be studied. Correlational analysis between similarly defined behavioural constructs across behavioural tests yielded moderate to high correlations. This finding provides further confirmation that the behaviour of animals was rather stable in time and between different test environments. However, these correlations have to be interpreted cautiously, as low activity levels of LE-rats biased the centre of gravity of measured variance towards lower scores, hence in many cases data deviates from Gaussian distribution.

SD, resilience, and monoamine levels

Levels of serotonin were lower and turnover higher in the frontal cortex and the amygdala of control ME-rats. This finding may be related to the lesser stress reactivity of ME-rats, but requires further verification. Biochemically, the most prominent effect of the SD regimen was associated with the increase in NA tissue levels in the amygdala and the hippocampus. Noradrenergic neurocircuitry plays prominent role in the reactivity to acute stress. Noradrenergic neurons in the locus coeruleus are activated by stressors (59,60) and increase in NA release occurs [e.g. in the hippocampus (61) and the amygdala (62)] in stressful conditions. NA transporter knockout mice that have significantly reduced tissue levels of NA were resistant to the effects of chronic SD and restraint stress (63). It is noteworthy that in the hippocampus ME-rats had the lowest NA levels, whereas SD/LE rats the highest, which accords well with their putative rank in stress sensitivity. HVA is a major catecholamine metabolite and is used as an index of DA release. In the current study control rats had higher HVA levels in the frontal cortex than stressed animals. Fittingly, human depression is associated with the dopaminergic hypofunction (60). In rats, DA tissue levels have been extensively studied in connection to the chronic mild stress paradigm. Findings are equivocal, as numerous studies have found the decrease in levels of DA and its primary metabolites in the frontal cortex, whereas equally numerous studies found no effect (64), and while dopaminergic neurotransmission also responds to SD, this occurs in a complex temporal and context-dependent pattern (65).

This study has a number of limitations. Habituation to the repeated presentation of the same stressor is an important concern that pushes researchers to deploy more unpredictable and variable stress regimens. The flip side of the increased complexity in the delivery of stressors is the reduced replication of experimental designs both within the same lab and between research groups. Even in the relatively narrow confines of the resident–intruder stress paradigm many variations on the timing and duration of antagonistic social encounters, as well as delivery of stressors and experimental endpoints have been used (14). This fact makes comparison between studies problematic. In this study we decided to include rats of moderate exploratory preference as an internal control group [similar in logic to (66)]. Limited basic neurochemical information on only three brain regions is available and this prevents any advanced conclusions about stress response. We have recently found by integrated analysis of a number of depression models, including SD, that while vulnerability is rather systematically associated with lower oxidative metabolism across the brain, the stress response has at least three distinct regional patterns in diathesis-stress analysis (67). Lastly, only male rats have been studied here, whereas in humans females have higher incidence of mood disorders (68). However, female rats show little territorial aggressiveness.

In conclusion, the exploration box test reveals large and stable variation in EB. It was found that LE behaviour predict passive coping strategies in response to chronic stress, but also that high levels of novelty-related behaviour may be detrimental to stress resilience.

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Conflicts of Interest

None.

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