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The effects of extrinsic stress on somatic markers and behavior are dependent on animal housing conditions

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HIGHLIGHTS

- A socially-enriched environment (SEE) prevents stress-induced anxiety.
- Rats in a SEE make less mistakes in an operant task.
- A SEE changes somatic markers suggestive of stress.
- In SEEs, stressed rats display less aggressive behaviors.

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ABSTRACT

Properties of the environment play an important role in animal wellbeing and may modulate the effects of external threats. Whereas stressors can affect emotion and impair cognition, environmental enrichment may prevent the occurrence of such negative sequelae. Animals exposed to semi-natural group-housing experience a complex environment; whereas environmental enrichment might protect against stressors, a socially-enriched environment(SEE) could entail aggressive inter-male encounters with additive stress effects. In the present study, we investigated the effects of exposure to external stressors, footshocks and forced swimming, on adrenal gland and body weights as well as on behavior in rats housed under SEE or standard, non-enriched environment (NEE), conditions. We found that SEEs reduced the anxiogenic effects of stress. Moreover, SEEs improved the performance in an operant task and prevented the increase in impulsive behavior produced by external stressors on NEE animals. Whereas these findings are indicative of stress-buffering effects of SEEs, adrenal gland weights were increased while total body weights were decreased in SEE rats, suggesting that SEEs may simultaneously exacerbate physiological measurements of stress. Finally, in the SEE, total aggressive behaviors and body wounds were paradoxically reduced in animals that received external stressors in comparison to non-stressed controls. The consequences of the external stressors applied here are not uniform, varying according to the housing condition and the outcome considered.

1. Introduction

The environment can impact quality of life, modulating behaviors and social interactions and ultimately even playing a role in psychopathologies [1–5]. While a breadth of literature on the impact of the social and physical environment on physiology and behavior of individuals, communities, and societies exists [6–9], our understanding of the impact of interactions between the social environment and the individual in determining physiological and behavioral outcomes remains incomplete.

Animal experimentation has started to address this issue through the application of diverse forms of environmental enrichment procedures [10–12]. A common approach consists of physical environmental enrichment, involving increased size and/or complexity of the cage, thereby enhancing sensory inputs and often enabling a diversified interaction with the environment. In some instances, the environmental enrichment involves a social component, allowing interactions between various individuals. Interestingly, some studies suggest that the combination of complex inanimate stimulation and social stimulation is required in order to obtain optimal effects of enriched environments as compared to housing conditions enriched in either physical or social aspects alone [8,10–13].

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For that matter, semi-natural environments have been developed to recreate ecological but controllable laboratory setups [14,15]. They typically include socially-enriched environments (SEEs), combining both environmental and social enrichments. Previous studies have shown that a SEE has many effects on the brain and behavior, including increased neural plasticity [16,17], enhanced hippocampal neurogenesis [10,11,18,19], improved learning and memory [8,10,12,19–21] and decreased anxiety-like behavior [22]. In contrast, exposure to sustained stressors can lead to hippocampal atrophy, increased anxiety and impaired learning and memory [23–26].

Given that environmental enrichment and stress can affect the brain and behavior in opposite ways, one might predict that SEEs would negate the detrimental effects of stress on the brain and behavior. Indeed, whereas maternal separation-stress enhanced the corticosterone response and increased anxiety-like behaviors in adulthood [27], a SEE was found to reverse these effects [28]. Moreover, animals housed in SEEs exhibited resiliency to social defeat, as illustrated by decreased anxiety in the light–dark box and less immobility time in the forced swim test [29]. The effects of chronic stress on hippocampal morphology (dendritic hypotrophy) and function (spatial learning deficits in the radial arm maze) were attenuated in rats housed in SEEs during adulthood [30]. On the other hand, physiological parameters, such as plasma corticosterone, indicate that some forms of social housing (e.g. in the visible burrow system, VBS) induce stress, owing to the agonistic interactions between males competing for the females in the colony [31]. The stressful nature of VBS-housing is demonstrated by a reduction in body weight, enlarged adrenal glands and increased basal levels of plasma corticosterone [32,33].

We hypothesized that SEEs would have additive effects on somatic markers (adrenal gland weight and body weight) suggestive of stress exposure, owing to the possible occurrence of intermale aggressive encounters. Here we questioned whether this somatic stress-inducing environmental manipulation is accompanied by corresponding perturbations in, social, cognitive, and anxiety-like behaviors.

2. Methods

2.1. Animals

Long–Evans males (n = 48) and females (n = 18) were used in this experiment. Males and females were housed in a 12 h reversed day–night cycle (lights off at 8 am) with food and water available ad libitum and with controlled temperature (20–22 °C) and humidity (56–58%). Experimental procedures were approved by the Animal Research Ethics Committee of Concordia University, and followed the guidelines of the Canadian Council on Animal Care (CCAC).

2.2. Housing conditions and experimental groups

Males arrived at postnatal day 21 (P21) from the local breeder (Charles-River, Saint-Constant, Quebec, Canada) in groups of four. After 1 day, males were pair-housed in standard Plexiglas cages (l × w × h: 45 × 20 × 25 cm). In order to habituate them to the experimental manipulations, animals were handled 5 days/week in the 3 weeks preceding the experiment. Handling consisted of either holding the rat for 1 min in the housing room, tail marking (Liquid Tip, Sanford®) or weighing. At P53, rats were randomly assigned to their housing condition: non-enriched environment (NEE, n = 12) or social enriched environment (SEE, n = 36). SEE males were weight-matched (<8% difference between any male in a group) and introduced to their behavioral setup (starting time: day 1) in groups of four. Males were fur-marked allowing identification during social interactions. NEE rats were socially isolated in standard cages and submitted to the same schedule as SEE rats, except that fur-marking was absent. Additionally, from the outset, NEE and SEE rats were split into subgroups and kept under either control (ctrl) or external stress conditions: NEE-ctrl (n = 6), NEE-stress (n = 6), SEE-ctrl (4 SEE cages, n = 16) and SEE-stress (5 SEE cages, n = 20).

2.2.1. Females and hormonal injections for estrus induction

Two females were added to each SEE in order to provide further social enrichment which has been previously shown to increase competition and agonistic behaviors between males [34]. In order to avoid pregnancy and yet maintain a natural estrous cycle, ovariectomized females (6–8 months old at the start of experimentation; kindly provided by Professor J. Pfaus, Concordia University) were made sexually receptive every 4 days (including days 1 and 5) by inducing estrus with a standard protocol: subcutaneous injection of estrogen (estradiol benzoate: 10 μg in 0.1 ml of sesame oil) and progesterone (500 μg in 0.1 ml of sesame oil), respectively administered 48 h and 4 h before heat induction [35]. Females were introduced into the SEE cages a few minutes after the males on day 1 and were present throughout the remainder of the experiment. On the periods when rats were handled, males were kept singly housed and females were pair-housed in standard cages in the housing room of NEE rats (adjacent to the SEE room).

2.2.2. Socially-enriched environments (SEEs)

The SEE setup (Fig. 1) consisted of a large cage (l × w × h: 144 × 62 × 90 cm) with 3 sides of wire mesh and 1 long side made of transparent Plexiglas to permit viewing and video-recording. SEEs were divided into three floors, and rats were free to move across them using vertical paths on the sides of the cage. The top floor was covered with woodchip bedding (Sani-chips, Harlan®) and equipped with a food dispenser and a water bottle. The middle floor was separated into two distinct, similar-sized compartments separated by a wall with a 15-cm swinging doorway at the bottom. The left chamber floor was covered with gravel and the right chamber with woodchip bedding. One metallic shelter was present in each chamber. The bottom floor was covered with corncob bedding (Harlan®). A food dispenser and a water bottle were placed on this floor, along with a 50-cm long T-shaped PVC pipe (20 cm diameter). In all floors several cardboard pieces and some pieces of wood were placed as chewing material and regularly renewed. During behavioral testing for anxiety or cognition, cleaning and weighing, SEE rats were individually placed in standard cages in another housing room.

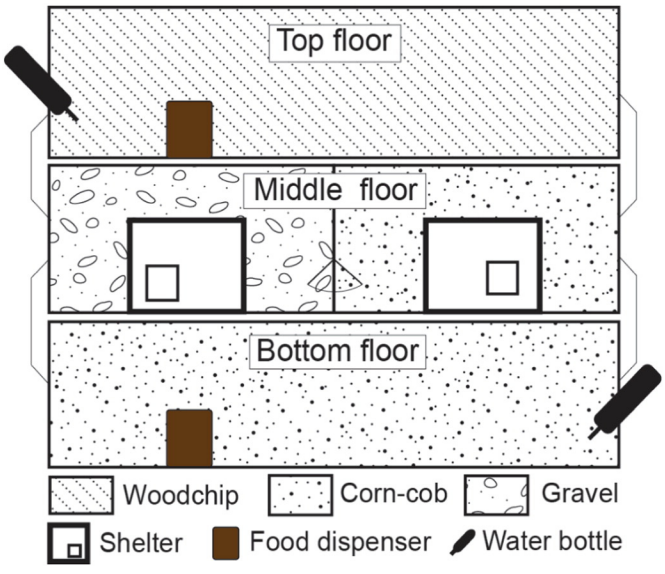

Fig. 1. Schematic diagram of the enriched environment. Adapted from http://www-psychology.concordia.ca/fac/mumby/research_topics_enrichment.html.

2.3. Stress procedure

Rats in the stress subgroups were submitted to stressors on days 1–4 of SEEs or NEEs between 10 and 12 am (Fig. 2). Two stressors were used: footshocks and the forced-swim-stress (FSS). On days 1–2, rats were placed in special acoustic chambers and exposed to fifteen unpredictable footshocks (0.6 mA) (Med. Associates Inc. chambers) during 10 min followed by 10 min without shocks. On days 3–4, stressed animals were submitted to the FSS. The swim tank was made of opaque plastic (44 cm height, ∅ = 30 cm) and filled with water (23–25 °C) to 10 cm from the top. Animals were individually placed in the water-filled tank and the sessions lasted 5 min.

2.4. Behavioral tasks

2.4.1. Circular open-field test

The circular open-field (∅ = 1 m, dark plastic walls of 70 cm height) was used to assess the effects of stress and enrichment conditions on anxiety-like behavior [36] and general locomotor activity. Rats were introduced to the center of the field and allowed to explore for 5 min. A virtual inner circle of 40 cm in diameter was drawn at the center and another virtual circle was used to delineate the outer part of the open-field (∅ = 60 cm). Behavior was video-recorded and movements and time spent in different zones (center and outer) were determined by tracking software. The percentage of time spent in the center is suggested to be inversely proportional to the anxiety-like tendencies of the rats [36].

2.4.2. Social interactions

Two hours of social interactions by SEE rats were video-recorded on days 1–5. Analyses of the social behaviors were conducted using The Observer XT (Noldus, The Nederland). The behaviors assessed included: male agonistic behaviors toward males and females (lateral threat, chasing, rat pinning its opponent, offensive-upright and biting), resting behavior (lying still on the bedding in the absence of overt social interaction) and mounting behavior. The number and location of visible wounds were evaluated during animal handling on days 10 and 25 of the enrichment session. Prior to social interaction, rats were housed in individual cages for 2–3 h. Animals submitted to the stressors during days 1–4 were allowed to recover for 1 h and fur-marked before being introduced simultaneously in the SEE setups for social interaction recording.

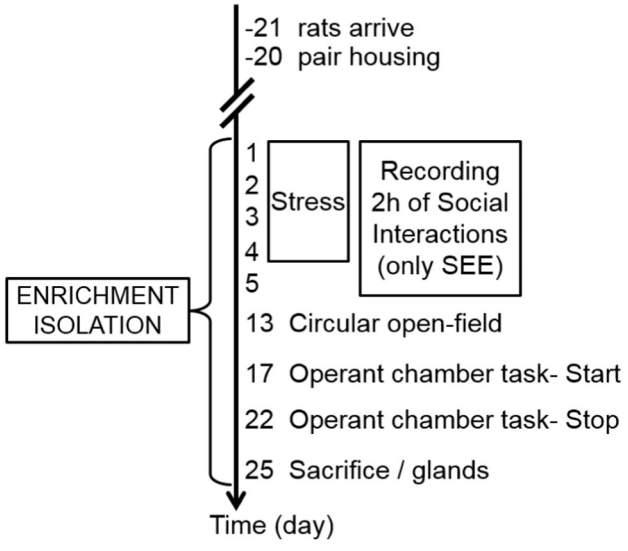

Fig. 2. Outline of the experimental procedures. Rats arrived in the animal facility 21 days prior to experimental manipulations. On day 1, rats were housed in the social enriched environment (SEE) or socially-isolated. The stress procedure (days 1–4) consisted of unpredictable footshocks (days 1–2) and forced-swim sessions (days 3–4). Social interactions were recorded and scored for 2 h/day from days 1–5. The open-field test was performed 12 days after the start of the enrichment/isolation of the rats. The operant chamber task was performed from days 17–22. Rats were sacrificed and glands were extracted after 25 days of enrichment/isolation.

In order to examine the possibility that individual variability within SEE subjects relating to the establishment of their social status may affect the outcomes investigated, we tested for the potential association of those outcomes with 'aggressive behavior reciprocity', an index of social status formulated as follows: (Aggressive behaviors toward cagemates − Aggression received) / (Aggressive behaviors toward cagemates + Aggression received). This formula was proposed to account for the asymmetry of agonistic behaviors between dominant and subordinate rats in semi-natural environments that was used as a marker of social status [37], the range of this index being +1 to −1, bounded by non-reciprocated relations, in opposite directions. Specifically, a rat with an index of +1 would be a rat that aggressed but did not receive any aggression from other males; conversely, an index of −1 denotes a rat that did not aggress his cagemates, but was the target of their aggression. Thus, in each SEE, the rat with the higher score was classified as dominant (R1) and the rat with the lower index as subordinate (R4), with two intermediate rats (R2 and R3). Next, the rank-closeness was calculated using the rank index of the most dominant animals (R1 and R2) subtracted by the rank index of the most subordinate animals in the SEE (R3 and R4). A big difference in rank-closeness indicates an important disparity between the dominant and subordinates, reflecting highly aggressive dominants and minimally aggressive, mostly submissive subordinates.

2.4.3. Operant chamber task

Operant test chambers (l × w × h: 30 × 25 × 30 cm, Coulbourn Instruments, Allentown, PA) were used to study the effects of stress and housing on cognitive integrity on days 17–22. Rats could earn sucrose pellets (45 mg sucrose pellets, chocolate flavor, BioServ®) by learning to appropriately lever press. Rats were first habituated to the pellets during handling, weighing, and animal marking. Each chamber was located within sound-attenuating isolation cubicles and contained a light (2.8 W) placed 20 cm above the grid floor in the center of the box. A pellet dispenser was located ~4 cm above the floor. The chamber also contained two levers on either end of the food dispenser. On day 17, rats were habituated to the operant chamber for 1 h with ~20 sucrose pellets readily available in the food dispenser. The day following habituation, animals were trained for 3 sessions lasting 30 min each, during which animals were shaped to press the active lever (either left or right) by spreading some sucrose pellet powder on the correct lever. Pressing the active (correct) lever released a sucrose pellet into the dispenser while simultaneously the light was turned off for 1 s as learning reinforcement. Pressing the inactive (incorrect) lever did not result in pellet delivery and light remained switched on. The number of lever presses on the active and inactive levers, the number of nose pokes in the food magazine and the total number of pellets distributed were recorded (Graphic State® 3.0, Coulbourn Instruments). Statistical analysis was performed on the averaged results from the 4 subsequent testing sessions.

2.5. Dissection and organ collection

At the end of experimentation on day 25 (P77), rats were weighed and the fur condition was assessed. Adrenal glands and testes were extracted and weighed using a high precision scale (AG-245, Mettler Toledo). As animals' body weight was affected by housing conditions, we report the gland weights without adjusting for body weight.

2.6. Statistical analyses

Data are presented as mean ± SEM (standard error of the mean). Statistical analyses were carried out using SPSS (IBM®, version 17.0). Statistical tests included two-way 2 × 2 ANOVAs [stress (control vs.

stress) × housing (SEE vs. NEE)] or repeated-measures ANOVA when appropriate with Fisher's least significant difference correction for multiple comparisons. For the SEE rats, correlational analyses were also conducted to examine the relationships of aggression and rank index with the behavioral and somatic outcomes. Aggressive behaviors during social interactions violated the assumption of normality of distribution and were appropriately transformed for using a square root transformation prior to analysis. The results of these analyses and the supporting graphs are presented with non-transformed data for ease of interpretation. Statistical tests were conducted using a significance level of 5%.

3. Results

3.1. Locomotion and anxiety-related behavior

Locomotor activity and anxiety-like behaviors were examined using the circular open-field test. We found that general locomotor activity (Fig. 3A) did not depend on either housing (SEE = 4.45 ± 0.47 m vs. NEE = 4.28 ± 0.63 m, $F_{1,44} = 1.038$, $p = 0.314$), stress condition (ctrl = 4.46 ± 0.58 m vs. stress = 4.36 ± 0.46 m, $F_{1,44} < 1$) or their interaction ($F_{1,44} = 1.021$, $p = 0.318$).

Enrichment appeared to produce a buffering effect on stress-induced anxiety. SEE rats exhibited less anxiety-like behavior than NEE rats, as suggested by an overall higher percentage of time spent in the central zone of the circular field (Fig. 3B, left part: SEE = 7.08 ± 0.48% vs. NEE = 4.53 ± 0.83%, $F_{1,44} = 7.03$, $p = 0.011$). Although stress did not overall affect the time that rats spent in the center of the open field (ctrl = 6.31 ± 0.69% vs. stress = 5.30 ± 0.67%, $F_{1,44} = 1.09$, $p = 0.301$), an interaction between housing and stress treatment on the time spent in the central zone approached significance ($F_{1,44} = 3.96$, $p = 0.053$). Simple effects analyses revealed that NEE-stress rats spent less time in the center of the field than SEE-stress rats (NEE-stress vs. SEE-stress = 6.62 ± 0.72%, $F_{1,44} = 11.09$, $p = 0.002$), with a similar tendency compared to NEE-ctrl rats ($F_{1,44} = 3.08$, $p = 0.086$), suggesting that environmental enrichment may buffer the effects of stress on anxiety. Regarding time spent in the less threatening, peripheral zone of the open-field (Fig. 3B, right part), there was an interaction between housing and stress ($F_{1,44} = 6.65$, $p = 0.013$), a main effect of stress (stress = 74.29 ± 1.92% vs. ctrl = 68.12 ± 1.97%, $F_{1,44} = 5.04$, $p = 0.030$), but no main effect of housing ($F_{1,44} < 1$). Post-hoc analyses confirmed an increase of time spent in the peripheral zone for stressed animals only in the NEE condition ($F_{1,44} = 4.76$, $p = 0.008$). Moreover, NEE-stress rats spent more time in the peripheral zone than SEE-stress animals ($F_{1,44} = 5.89$, $p = 0.019$).

3.2. Operant chamber and lever pressing task

During the four testing sessions of the operant task, there was no significant effect on the number of correct lever presses of housing condition (Fig. 4A: SEE = 56.5 ± 6.5 vs. NEE = 69.6 ± 11.2, $F_{1,44} = 1.02$, $p = 0.318$), stress (ctrl = 66.8 ± 9.3 vs. stress = 59.2 ± 9, $F < 1$) or their interaction ($F_{1,44} < 1$). However, SEE-animals committed fewer errors, pressing the incorrect lever less often (Fig. 4B: NEE = 6.4 ± 0.89 vs. SEE = 1.65 ± 0.52, $F_{1,44} = 20.96$, $p = 0.00004$). Stress did not affect the number of incorrect presses (ctrl = 4.17 ± 0.74 vs. stress = 3.85 ± 0.72, $F_{1,44} < 1$) and there was no interaction between stress and housing conditions for this variable ($F_{1,44} < 1$). In addition, we found that the number of magazine nose-pokes during the testing sessions (Fig. 4C) was affected by both housing (SEE = 164.2 ± 12.6 vs. NEE = 219.6 ± 21.7, $F_{1,44} = 4.85$, $p = 0.033$) and stress (ctrl = 165.7 ± 18 vs. stress = 218 ± 17.5, $F_{1,44} = 4.32$, $p = 0.043$), with a tendency for an interaction between these factors ($F_{1,44} = 3.35$, $p = 0.074$). Post-hoc analyses revealed that NEE-stress rats made more nose-pokes in the food dispenser than both SEE-stress ($F_{1,44} = 8.37$, $p = 0.006$) and NEE-ctrl ($F_{1,44} = 5.11$, $p = 0.029$) rats.

3.3. Body and organ weight measurements

Body weight was affected by the housing conditions (SEE = 306.6 ± 3.3 g, NEE = 334.7 ± 5.6 g, $F_{1,44} = 18.65$, $p = 0.0001$; Fig. 5A) but not by the stress treatment (ctrl = 320.57 ± 4.66 g vs. stress = 320.67 ± 4.54 g, $F_{1,44} < 1$). There was an interaction between time and housing ($F_{1,44} = 17.35$, $p = 0.0001$) due to a lower weight gain in SEEs.

Adrenal glands seemed affected by housing condition. Two rats were excluded from analysis for both adrenal and testis glands because one rat had an excessively small left adrenal gland (from the SEE-ctrl group) and another rat had only one testis (from the SEE-stress group). Enriched rats tended to present heavier adrenal glands than non-enriched animals (Fig. 5B: SEE = 64.91 ± 1.99 mg, NEE = 57.39 ± 3.37 mg, $F_{1,43} = 3.7$, $p = 0.061$). Stress alone did not affect adrenal gland weight (ctrl = 58.46 ± 2.82 mg vs. stress = 63.85 ± 2.71 mg, $F_{1,43} = 1.90$, $p = 0.175$), and there was no interaction between housing and stress ($F_{1,43} = 2.25$, $p = 0.141$).

Group housing did not affect testis weight (Fig. 5C; SEE = 3.73 ± 0.06 g vs. NEE = 3.69 ± 0.10 g, $F_{1,43} = 0.111$, $p = 0.741$), but stress tended to decrease the weight of testes (ctrl = 3.81 ± 0.079 g vs. stress = 3.61 ± 0.078 g, $F_{1,43} = 3.048$, $p = 0.088$). There was no interaction between housing and stress factors ($F_{1,43} < 1$).

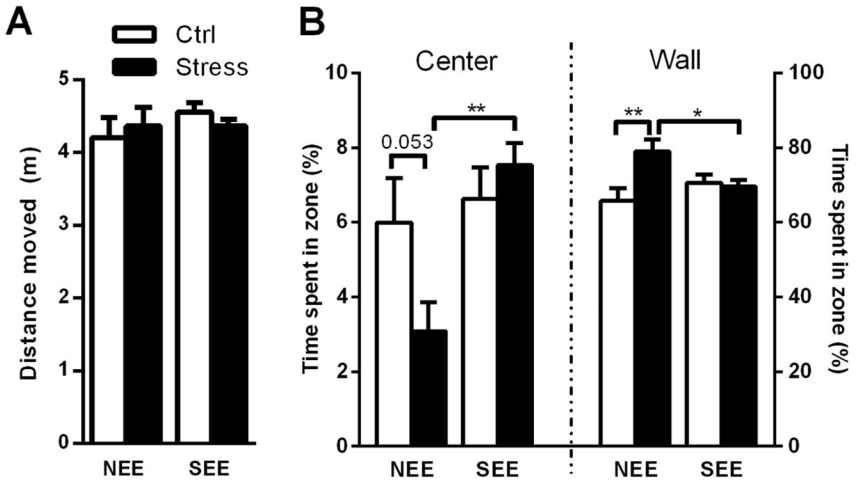

Fig. 3. Effects of stress and housing on locomotor activity and anxiety-like behaviors in the open-field task after 10 days of enrichment for NEE-ctrl (n = 6), NEE-stress (n = 6), SEE-ctrl (n = 16) and SEE-stress (n = 20). (A) Locomotor activity (m) during the open field test and (B) the time spent in different zones of the open field: center and wall. Trend for statistical significance between NEE-ctrl and NEE-stress is indicated in panel B;*p < 0.05 and **p < 0.01.

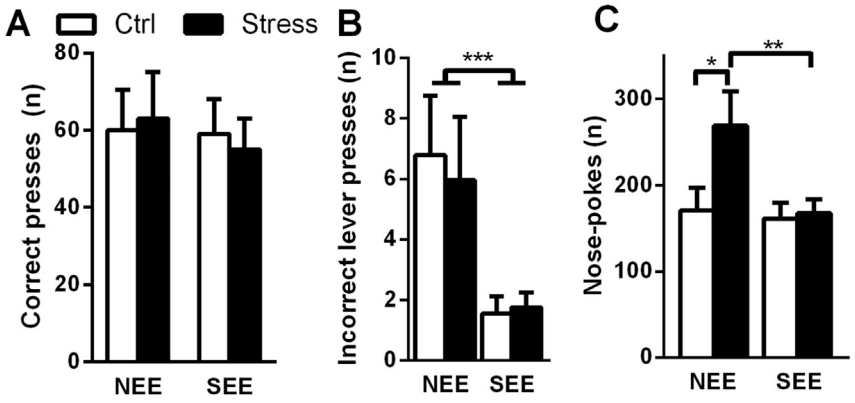

Fig. 4. Behavioral response to an operant conditioning (lever-pressing) task in NEE-ctrl (n = 6), NEE-stress (n = 6), SEEs-ctrl (n = 16) and SEE-stress (n = 20) rats. (A) Correct presses, (B) number of incorrect lever presses and (C) nose-pokes. *p < 0.05, **p < 0.01 and ***p < 0.001.

3.4. Social behavior in SEEs

A correlational analysis of the aggressive behavior and the rank index with the behavioral performance and somatic outcomes did not reveal any significant relationships (data not shown). The results of the analyses of variance are presented next.

We tested the effects of stress on social interactions obtained in the SEE-rats. A repeated-measures ANOVA across days 1–5 indicated that, as expected, overall, aggression (Fig. 6A) decreased throughout the testing days ($F_{1,34} = 2.79$, $p = 0.044$) but there was no difference due to stress treatment (ctrl = 3.625 ± 0.38 vs. stress = 3.41 ± 0.34, $F_{1,34} = 0.17$, $p = 0.682$). However, there was an interaction between time and stress factors ($F_{1,34} = 2.83$, $p = 0.041$). There was a time effect for the stress subgroup ($F_{4,31} = 5.9$, $p = 0.001$) but not for the control animals ($F_{4,31} = 0.337$, $p = 0.851$). Post-hoc analyses indicated that these divergent social dynamics were reflected in differences in aggressive behavior on day 5, with SEE-ctrl animals being more aggressive than SEE-stress rats ($F_{1,34} = 2.79$, $p = 0.001$).

In parallel, body wounds (Fig. 6B) increased over time (day 10 = 2.42 ± 0.51 vs. day 25 = 4.8 ± 0.8, $F_{1,34} = 21.42$, $p = 0.0001$). External stress also affected this measure of aggression, as SEE-stress rats tended to exhibit less body wounds than SEE-ctrl rats ($F_{1,34} = 4.01$, $p = 0.053$), without interaction between day and treatment ($F_{1,34} = 0.7$, $p = 0.407$).

Stress in a SEE-setting may not only reduce aggression but also interact with resting. Although overall SEE-stress rats spent more time resting (Fig. 6C) than controls (SEE-ctrl = 958.3 ± 140.5 vs. SEE-stress = 1587 ± 97.68, $F_{1,34} = 14.3$, $p = 0.0006$), a day effect ($F_{1,34} = 39.06$, $p < 0.0001$) was also accompanied by an interaction between day and stress on this behavioral measure ($F_{1,34} = 5.1$, $p = 0.003$). Post-hoc tests revealed that at day 1 SEE-ctrl rats were resting more than SEE-stress rats ($F_{1,34} = 5.93$, $p = 0.020$) but the effect was inverted for days 3–4 (day 3: $F_{1,34} = 13.48$, $p = 0.0008$; day 4: $F_{1,34} = 11.89$, $p = 0.0015$).

In addition to differences in aggressive and resting behaviors, non-aggressive social contact differed between control and stressed animals housed in enriched conditions on days 1–5 (Fig. 6D). While non-aggressive social interactions of cagemates decreased with time (d1 = 8.69 ± 0.75 vs. d5 = 5.89 ± 0.71, $F_{1,34} = 4.67$, $p = 0.034$), SEE-stress rats spent more time interacting with their conspecifics (SEE-ctrl = 5.98 ± 1.74 vs. SEE-stress = 8.35 ± 1.13, $F_{1,34} = 9.77$, $p = 0.004$). There was no interaction between time and stress for the non-aggressive social interactions ($p = 0.51$).

Finally, we found a significant interaction of extrinsic stress and time on the social rank distance between dominant and subordinate animals for the rank-closeness (Fig. 6E) on days 1–5 ($p = 0.019$) and no main effect of time (d1 = 0.602 ± 0.39 vs. d5 = 0.725 ± 0.001, $F_{1,7} = 0.93$, $p = 0.367$) or extrinsic stress treatment (SEE-ctrl = 0.469 ± 0.26 vs. SEE-stress = 0.858 ± 0.13, $F_{1,7} = 1.30$, $p = 0.291$). Follow-up simple effects analyses showed that on day 1 rank-closeness tended to be narrower for SEE-control animals than SEE-stress animals (d1: SEE-ctrl = 0.213 ± 0.27 vs. SEE-stress = 0.99 ± 0.16, $F_{1,14} = 2.133$, $p = 0.051$).

4. Discussion

Environmental enrichment has been suggested to be the functional inverse of stress [38] but SEEs can simultaneously parallel the

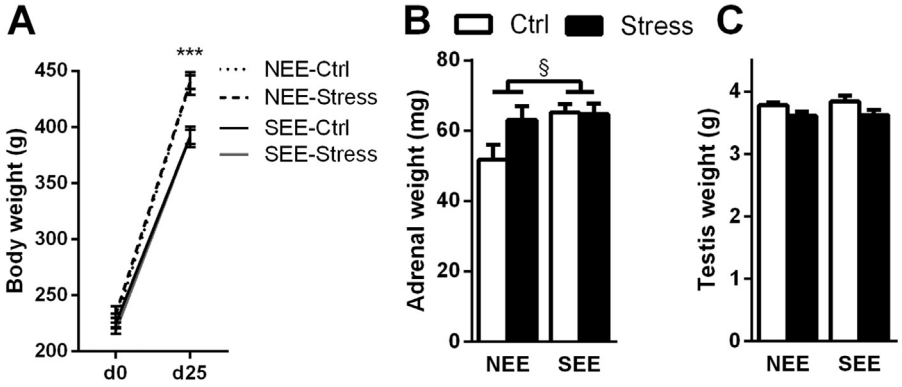

Fig. 5. Effects of stress and housing on body, adrenal gland and testis weights for NEE-ctrl (n = 6), NEE-stress (n = 6), SEE-ctrl (A, C: n = 16; B: n = 15) and SEE-stress (A, B: n = 20; C: n = 19) rats. Social housing affected body (A) and adrenal gland (B) but not testis (C) weight at the end of the enrichment session (day 25). §p = 0.061, ***p < 0.001.

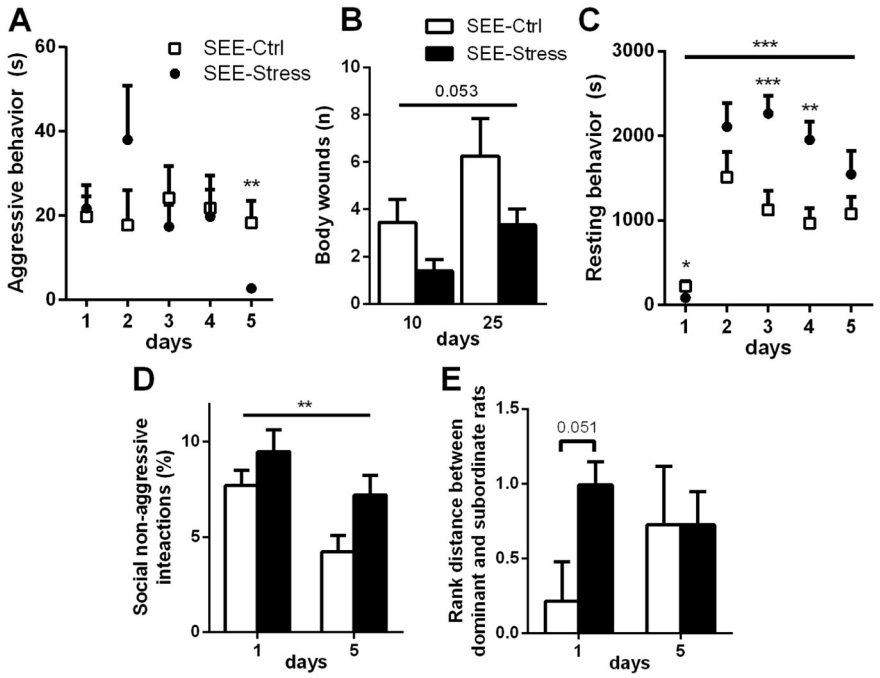

Fig. 6. Effects of stress on behaviors in environmental enrichments (SEE rats only): (A) Agonistic behaviors and the effect of stress during days 1–5 of social housing, (B) the number of body wounds at day 10 and day 25, (C) the amount of resting time during days 1–5, (D) the percentage of non-aggressive social interactions during 30 min of interaction at days 1–2 and (E) the dominancy rank distance between dominant (R1–2) and subordinate animals (R3–4). Trends for statistical significance are indicted on panels B (main effect of stress) and E (difference in rank-closeness between SEE-ctrl and SEE-stress on day 1); $^{**}p < 0.01$ and $^{***}p < 0.001$.

endocrine, neurobiological, behavioral and physiological aspects of stress [39]. Thus, it is not clear whether SEEs would intensify the effects of stress on behavior or whether this type of housing would buffer the effects of external stressors. Therefore, we investigated the effects of extrinsic stress and housing conditions on somatic markers of physiology, cognition, anxiety-like as well as social behaviors. We found that stress differentially affected behavior depending on the animals' housing background and the specific behavior scrutinized. Altogether, our findings with respect to anxiety and aggression are congruent with the recently postulated inoculation stress hypothesis [39]. This hypothesis claims that particular chronic mild stress, such as stress that originates from living in a complex social environment, can protect against subsequent stressors.

The external stressors applied in the current study increased anxiety-like behavior in the open-field task for NEE-, but not for SEE-housed animals. Thus, SEEs appeared to buffer the effects of such environmental stressors on anxiety-related behavior. These findings with non-social stressors are broadly in line with a study that showed that SEEs in mice reduced anxiety induced by social defeat stress [29]. Of note, the anxiolytic effects of SEEs are found in tests that include an exploratory component [40] but seem absent in other tests of anxiety-like behavior such as sucrose neophobia or the cold-stress defecation test [41]. Ignoring the extrinsic stress manipulation, in our hands, the type of housing per se did not affect anxiety-like behavior. This finding seems in contrast with earlier reports showing that individually housed rats exhibited increased anxiety-like behavior in comparison to group-housed animals [42,43]. Differences in strain (Wistar vs. Long–Evans rats), duration of social isolation, or experimental procedures (animals tested in the elevated-plus maze vs. open field) may underlie the differential outcome.

Importantly, our results on the open-field task were unaccompanied by potentially confounding abnormalities in locomotor activity. Although social isolation is reported to increase anxiety-like measurements on the elevated-plus-maze [44,45], this finding is not always confirmed [46]. In fact, group housing of mice during adolescence was reported to increase anxiety-like behavior in the light–dark test [47].

Regarding cognition, SEE-rats have been reported to outperform controls (isolated conspecifics, similar to the current study), as evidenced from a reduced latency to find a target platform in the water maze and an increased preference for the novel object in the recognition task [48]. We have used an operant conditioning task and found that while the number of correct lever presses did not differ between groups, SEE-animals made fewer mistakes as compared to NEE-animals, in agreement with that study. While stress, did not affect the number of correct/incorrect responses, it differentially affected the food retrieval behavior according to housing condition. The total number of nose-pokes (required to obtain the reward) was strongly increased for NEE-stress animals only. Since this surplus number of nose-pokes did not lead to increased food rewards, this behavior is reminiscent of wait-impulsivity [49] and SEE-rats seemed to be protected from stress-induced excessive nose-poking.

In order to assess the physiological effects of the external stressors and housing conditions, we measured adrenal gland, testes and total body weights. Although we did not see an effect of housing and stress on testicular weight, both adrenal gland weight and body weight were affected. The adrenal glands secrete glucocorticoids upon stressful stimuli and hyperplasia of these organs is therefore related to stress-exposure [50]. We observed that in NEE-animals the FST–footshock paradigm tended to increase adrenal weight, corroborating previous findings [51–53]. However, the adrenal weights were highest in SEE-animals with no additional effect of the stress-paradigm. This finding suggests that a SEE engages the hypothalamic–pituitary–adrenal axis, requires plasticity of the adrenal glands and may be viewed as a stressor in its own right. Enlarged adrenals in response to SEE conditions have also been reported by others [33,54,55]. Perhaps a ceiling effect prevented the existence of an appreciable *additional* effect of external stress on adrenal gland weights in SEE-animals. The duration of a stressor is an important consideration. While a SEE might pose a mild stressor to the animals, it lasted much longer (25 days) than the acute stressors

applied (FST and footshock exposure for 4 days). Thus, it seems likely that the SEE could have affected adrenal weight and total body weight because of its chronicity whereas the external stressors did not affect these stress-related measurements. The finding that repeated mild stress as well as SEEs decreased body weight [56,57] gives some credence to this line of thought.

Finally, we investigated the effects of stress on aggression in SEE animals. Exogenous stress reduced aggression during social housing leading to a significant difference on day 5. The relevance of this stress-induced reduction of social aggression is evident from the decreased number of bite wounds in SEE-stress animals. At a first glance, this finding seems at odds with literature as stressors have frequently been reported to increase aggression [2,58,59]. However, the stress-induced effects on aggressive behavior are also known to be context-dependent: in a social stress paradigm, stress only increases aggression for dominant animals [7,33,60]. Since footshocks are known to reduce social rank [61], it is thus conceivable that the external stressors altered the dynamics of social hierarchy formation, thereby affecting the amount of continued aggressive behavior. There is evidence that under certain conditions aggression levels may be uncoupled from hierarchical changes; for example, environmental enrichment was shown to decrease aggression without seemingly affecting the hierarchy [62]. Yet, our results suggest that an extrinsic stress manipulation may have produced a more pronounced dispute for the establishment of the social hierarchy on day 1 (Fig. 6E). Arguably, this seems to have led to enhanced social stability, as suggested by a reduction in subsequent aggressive behavior (see reduced aggression on day 5 in the extrinsic stress condition, Fig. 6A), which might reflect a reduction in rank assertion and/or challenge under this condition.

There are some limitations to the current study. First, a SEE is a complex environment that includes novelty, social and exercise aspects. Therefore, the present protocol precludes any claims on these specific SEE-aspects in relation to their effects on behavior and somatic markers involved in animals' physiology. On the other hand, no single variable can account for all effects of enrichment [10]. As the SEE components may act in a synergistic manner, a challenge for future studies would be the isolation of their separate contributions. In considering this novel combination of extrinsic stress with a joint social and environmental enrichment paradigm, future endeavors could also include the measurement of stress hormones (e.g. glucocorticoids) to enlighten the interpretation of the physiological impact of the different manipulations. In order to study the hierarchy establishment dynamics under the influence of external stress and the putative interacting effects of SEEs, the onset of SEEs and the induction of the external stress paradigm took place simultaneously. The timing of the external stress may be a factor also worth further investigating.

Our findings add to a growing body of research on the beneficial effects of environmental enrichment on non-social behaviors. We showed that while a SEE leads to alterations at a somatic level, suggestive of a stress-response, it also improved cognitive performance. Furthermore, anxiety-like and impulsive-like behaviors observed in NEE-rats were absent in SEE-rats. Thus, despite a degree of stress inherently produced by the SEE setup, we found beneficial effects on anxiety-like behavior and operant task learning. Thus, at least on these outcome parameters SEEs could be regarded as a 'eustress'. Therefore, we suggest that different stress-inducing conditions (the stress paradigm and SEE) may interact in a non-additive fashion and that SEEs may possibly promote resilience to the –otherwise adverse – influences of extrinsic stress.

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