Social stress: the good, the bad, and the neurotrophic factor

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DOI:
10.33612/diss.98795800

Chapter 5

Short- but no long-term effect of social stress in rodent depressive-like behavior is not affected by chronic treatment with Harmine

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Abstract

Introduction: Depression is characterized by behavioral, cognitive and physiological changes, imposing a major burden to the overall wellbeing of the patient. Some evidence indicates that social stress, changes in growth factors, like brain-derived neurotrophic factor (BDNF) and neuroinflammation, are involved in the development and progression of the disease, suggesting a potential role for anti-inflammatory drugs. The monoamine oxidase A inhibitor drug harmine was suggested to have both antidepressant and anti-inflammatory properties and may, therefore, be a potential candidate for treatment of depression. **Aim:** The goal of this study was to assess the effects of harmine on depressive-like behavior, brain BDNF levels and microglial activation in a rat model of social stress. **Material and methods:** Rats were submitted to 5 consecutive days of repeated social defeat (RSD) or control conditions. Animals were treated daily with harmine (15mg/kg) or vehicle from day 3 until the end of the experiment. To assess the effects of RSD and harmine treatment on behavior, the sucrose preference test (SPT) was performed on day -1, 6 and 15, the open field test (OFT) on days 6 and 14 and the novel object recognition test (NOR) on day 16. Brain microgliosis was assessed using [^{11}C] PBR-28 PET on day 17. Animals were terminated on day 17 and BDNF protein concentrations in the hippocampus and frontal cortex were analyzed using ELISA. **Results:** Both RSD and harmine treatment caused a significant reduction in bodyweight gain. The OFT and SPT showed that RSD significantly increased respectively anxiety and anhedonia related parameters on day 6, but these effects were not observed anymore on day 14/15. Harmine treatment induced anhedonia in the SPT on day 6 and significantly reduced the mobility and exploratory behavior of the animals in the OFT. PET imaging and the NOR test did not show any significant effects on microglia activation and memory, respectively. BDNF protein concentrations in the hippocampus and frontal cortex were not altered by either RSD or harmine treatment. **Discussion:** The main finding of the study was the short- but not long-term effect of RSD on anxiety and depressive-like behavior, and the long-term effect of harmine on the general locomotion and weight of the animals. RSD stress was not strong enough to induce a long-term effect on the behavior of the animals. Additionally, there was no difference between groups in the [^{11}C] PBR28 uptake and BDNF protein concentration. Thus, the long-term effect of treatment with harmine could not be properly evaluated in this model, and therefore, stronger stressful stimuli are needed in order to draw further conclusion on the effectiveness of harmine as an antidepressant and anti-inflammatory drug.

Keywords: Major depressive disorder; neuroinflammation; harmine; monoamine oxidase inhibitors; behavior; PET imaging

Introduction

Major depressive disorder (MDD) is a psychiatric disorder that affects the daily life of millions of people and poses a burden to healthcare systems worldwide [1]. Depression is mainly characterized by the loss of willingness to perform activities, sleeping and eating problems, sadness and social isolation. Clinical and preclinical research indicates that decreased neurotransmitter and growth factor activation, microgliosis and astrocytosis are involved in the pathogenesis of depression [2–4]. Neuroinflammation was suggested to play a major role in stress response to internal and external challenges, and increased inflammatory markers have been reported in MDD patients [5,6], leading to the hypothesis of neuroinflammation-derived depression. Although the mechanisms are not completely understood, it is possible that brain inflammation may be caused by severe or prolonged stressful events and in turn, cause some of the symptoms associated with MDD.

The involvement of neuroinflammation in depression was corroborated by the results of imaging studies. Positron emission tomography (PET) using [^{11}C]PBR28 or [^{11}C]PK11195 as a microgliosis marker has been used to assess neuroinflammatory processes throughout the brain in a non-invasive manner [7]. Both tracers bind to the mitochondrial receptor 18kDa translocator protein (TSPO), which is expressed mainly in endothelial cells, glial cells and astrocytes [8]. Although baseline expression of TSPO in these cells is low, there is a strong increase in the expression of this protein when an inflammatory challenge to the brain occurs [9–11]. PET studies showed increased TSPO binding in the brain of MDD patients [5,12], although another study failed to show any changes [13]. A few preclinical studies in animal models of depression have also shown increased binding of TSPO radiotracers [14,15]. These results suggest that neuroinflammatory processes may be associated with depressive behavior, which opens the possibility for the use of anti-inflammatory drugs as therapeutic candidates for mitigation of MDD symptomatology, either as mono-therapy or in combination with conventional antidepressants.

Monoamine oxidase-A (MAO-A) inhibitors have been used as treatment for MDD and other mood disorders for a long time. In the brain, the main function of MAO-A is the degradation of neurotransmitters, such as serotonin (5-HT), dopamine and norepinephrine, and blocking their release into the synaptic cleft [16]. Like many other interventions used for depression, however, there is a large variability of treatment efficacy of MAO-A inhibitors, with a large percentage of MDD patients showing partial or no remission of symptomatology [17]. Harmine is a β-carboline alkaloid derived from *B. caapi* (Malpighiaceae) found mainly in the Amazon rainforest of South America. Its main mechanism of action is through reversible inhibition of MAO-A [18]. Harmine may be an

interesting candidate drug as it shows not only antidepressant [19–21], but also anti-inflammatory properties [22,23].

The goal of this study is to assess the anti-inflammatory and antidepressant effects of the MAO-A inhibitor harmine in rats submitted to repeated social defeat (RSD). RSD is considered a model of MDD for its ability to emulate psychosocial stressors of human depression in an animal model by using territoriality and hierarchical status as motivators. To verify the effect of RSD and harmine treatment on the animals, anhedonia, explorative behavior, anxiety, and memory were measured with the sucrose preference test (SPT), the open field test (OFT) and the novel object recognition test (NOR) respectively. [^{11}C]PBR28 PET of the brain was performed to assess stress-induced neuroinflammation in various brain regions and the modulating effect of harmine thereon. To further understand the effect of harmine on the brain, we also assessed the concentration of brain-derived neurotrophic factor (BDNF) – a protein associated with neuronal survival and maintenance. BDNF was shown to be decreased in MDD patients [24,25] and animal models of depression [26,27] and models of neuroinflammation [28,29]. BDNF concentration was quantified *ex vivo* in the frontal cortex and hippocampus – two of the main brain structures associated with the cognitive outcome of depressive-like behavior.

Material and Methods

Animals and drug

The study protocol complied to European Directive 2010/63/EU and the Law on Animal Experiments of The Netherlands; it was approved by the Central Committee on Animal Experiments of The Netherlands (The Hague, license no. AVD1050020171706) and the Institutional Animal Care and Use Committee of the University of Groningen (IvD 171706-01-006). Male Wistar rats (HsdCpb:WU, 8 weeks old – Envigo, The Netherlands) were housed individually at the Central Animal Facility (CDP) of the University Medical Center Groningen (UMCG). Prior to the experiments animals were habituated to the facility for at least 7 days. Animals were maintained in rooms with controlled temperature (21±2 °C) and humidity in a 12/12 hours cycle (lights off at 08:00 P.M.), with food and water provided *ad libitum*. After acclimatization, animals were randomly divided according to harmine treatment (harmine or vehicle: n=10 per group) and social defeat protocol (RSD or control: n=10 per group). Harmine hydrochloride (Santa Cruz biotechnology; sc-295136B) was diluted in saline water to the desired concentration of 15 mg/kg in a volume of 1ml. The solution was then

heated up to 50 °C and stirred with ultrasound until it became a clear solution (about 10 minutes). When injected in the animals, the solution was at room temperature.

Study design

A summary of the experiment is presented in figure 1. Five days before the beginning of the RSD animals were daily trained for the SPT for 1h (SPT – training). The first SPT (day 0) was performed on the night before the first day of RSD. Animals were then submitted to the RSD protocol daily for 5 days. The second SPT and the first OFT were performed one day after the last RSD trial (day 6). On the third day of RSD, harmine or vehicle administration was started, which lasted until the end of the experimental phase (day 3-17). Nine days after RSD (day 14), animals were submitted to the second OFT. On day 15, the third SPT test and the training for the NOR test were performed. On day 16, the NOR test was done. and finally, on day 17, a [^{11}C]PBR28 PET scan was acquired before termination of the animals and collection of brain tissue for further analysis. Animals were weighed daily from day 1 to 17, always before the drug administration.

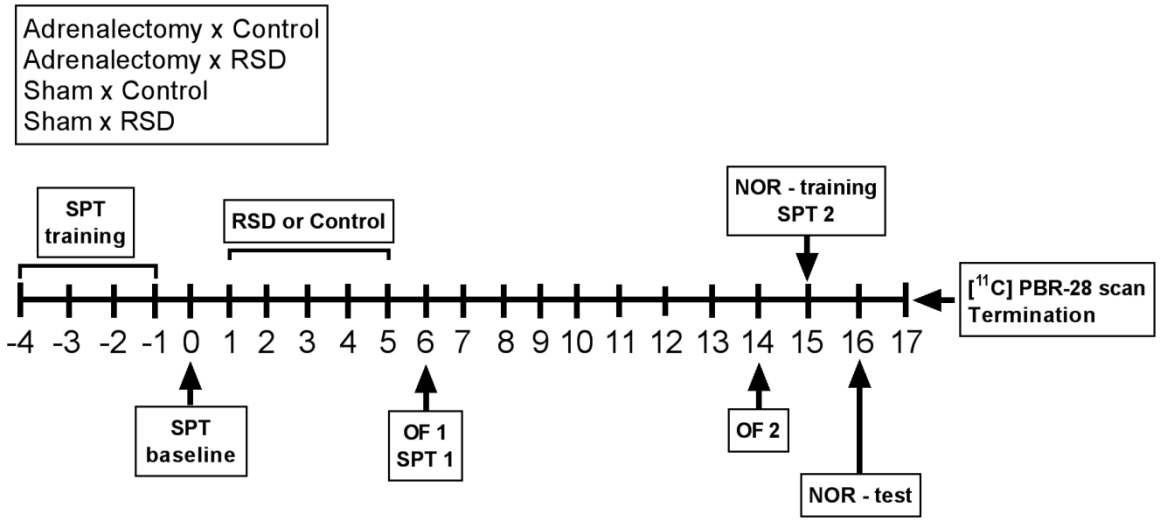

Figure 1: Design of the study. Abbreviations: SPT: Sucrose Preference Test; RSD: Repeated Social Defeat; OF: Open Field test; NOR: Novel Object Recognition

Repeated social defeat

For the social defeat, 12-weeks old male Long-Evans (residents: HsdBlu:LE – Harlan, The Netherlands; n=6; weight: 450 to 500 grams at the beginning of RSD protocol) were paired with females of the same age in a large wooden cage (80x50x40 cm) with a plastic lid. This setup allowed the resident to develop territorial behavior over a large area. The Long-Evans rats were submitted to a training social defeat protocol to allow for selection of their aggressiveness prior to the beginning of the first RSD protocol. Animals that showed an attack latency (i.e.: time to initiate the first attack)

of 60 seconds or less and no signs of violent behavior (i.e.: attack latency of fewer than three seconds without threatening behavior before the first attack) during the five days of training were selected for the study. Long-Evans rats that showed non-aggressive or over-aggressive behavior were excluded from the study.

One hour before the beginning of RSD, the female rats were removed from the resident cage. Then the experimental animals (intruders, Wistar rats) were placed in the resident cage to begin the defeat protocol. Attack latency and submission time (i.e.: time the intruder takes to show a submissive posture for at least three seconds) were measured. After the intruder displayed a submissive posture, it was placed in a wire mesh cage (40x20x20 cm) inside the resident cage for 60 minutes. By placing the intruder in a wire mesh, there is no physical contact between intruder and resident anymore, but the intruder is still aware of the presence of the aggressive resident. After 60 minutes, the intruder is removed from the RSD cage and placed back to its home cage, and the female is placed back in the resident cage. Control animals were placed in a large, plastic cage for 10 minutes without resident and subsequently in the wire mesh cage for 60 minutes. Then the animals were placed back to their home cages. This protocol was repeated on five consecutive days, and the intruder was always introduced to a different resident.

Drug administration

From day 3 to 17, defeated and controls submitted to a daily intraperitoneal injection of either harmine (15mg/kg) or vehicle solution. Harmine caused slight tremors in the animals one minute after injection, as was previously described in the literature [30]. In our study, the effect lasted for 45-60 minutes, and the behavior of the animals returned to normal after this period. To avoid the motor effects on the RSD or behavioral parameters, all interventions were performed at least one hour after the injections.

Sucrose preference test (SPT)

Animals were habituated to the SPT protocol by replacing their water bottle for a bottle containing 1% sucrose for 1h on 4 consecutive days. For the test SPT, 2 identical bottles– one containing drinking water and the other containing 1% sucrose solution – were placed in the cage of the rat and left overnight (placement of bottles at 03:00-04:00 P.M.). The next day, the bottles were removed (at 10:00 A.M.) and weighed to estimate the amount of fluid consumed by the animal. The percentage of sucrose intake was calculated from the weight difference of the 1% sucrose bottle divided by the sum of the weight differences of both bottles.

Open field test (OFT)

OFT were performed on day 6 and 14 to observe the acute and delayed effects of RSD and harmine treatment on the animals. To avoid habituation effect, two different arenas were used for the trials. For the first OFT, a round wooden arena of 80 cm diameter was used, whereas a square arena of 50x50 cm^2 was used for the second trial. For both tests, the animal was placed in the room 1h before the experiment and left alone during this period. After 1h, the investigator placed the animal in the arena facing the wall and started recording its exploratory behavior for 6 minutes, after which the animal was placed back into its home cage. The arena was cleaned with ethanol 70% and wiped with dry paper after each test. Analysis of the total distance the animal moved, its velocity, the time spent in the center and in the periphery of the arena, and time moving was performed using Ethovision XT 14.0 software (Noldus, The Netherlands). The number of times the animal explored the environment (rearing), the number of times the animal spent grooming and the time the animal spent immobile (freezing) were measured manually by the investigator.

Novel object recognition test (NOR)

The NOR test was performed in the circular OFT arena. The test was performed on day 15 (training) and 16 (Long-term memory – LTM). For training, two identical objects (A and A' – plastic cylinders) were placed 20 cm from the wall and 20 cm from the center on opposite sides of the field. Thus, the animal had plenty of space to explore the environment and interact with the objects separately. For training, the animals were placed in the arena and allowed to explore the objects. When the animal had explored both objects for 30 seconds, the training protocol was ended, and the animal was returned to its cage. If the animal did not reach the exploration criteria after 8 minutes, the training protocol was also ended, and the animal was returned to its cage.

For the long-term memory test, the animals are placed back into the arena 24 hours after training, but with one object being replaced by an object with a different shape and color (A' replaced by B – piled Lego bricks). The animal was placed on the corner of the arena facing towards the objects and left to explore freely, during which the animal was recorded. After 6 minutes, the animal was retrieved and placed back in its home cage. After each trial the objects and apparatus are cleaned with 70% Ethanol and wiped dry with paper. Analysis of the time spent exploring objects A and B were analyzed automatically with Ethovision XT 14.0. The recognition index (RI) was defined as the time spent exploring object B divided by the total amount of time exploring both A and B. Animals that explored the objects for less than five seconds were excluded from data analysis.

Positron emission tomography (PET)

[^{11}C]PBR28 was performed on small animal PET scanner (Focus 220, Siemens Medical Solutions, USA) with constant monitoring of the animal's heart rate and blood oxygen levels. Anesthesia was induced with 5% isoflurane and maintained with 2% isoflurane. After anesthesia induction, a cannula was inserted in the lateral tail vein for tracer injection. [^{11}C]PBR28 (49.62 ± 3.33 MBq) was injected as a bolus and the animal was placed in their home cage for 30 minutes. Then the animals were anesthetized again and a transmission scan with a Co-57 source was performed for the correction of attenuation and scatter. A 30-minute emission scan was started 45 minutes after tracer injection.

Images were iteratively reconstructed (OSEM2D, 4 iterations and 16 subsets) after correction for attenuation and radioactive decay. The reconstructed PET images were automatically co-registered to a [^{11}C]PBR28 rat brain template using PMOD software (PMOD Technologies LLC, Switzerland). Regions of interest (ROI's) were delineated for the following regions: amygdala, cerebellum, corpus callosum, midbrain, frontal cortex, temporal cortex, dorsal cortex, hippocampus, hypothalamus, brainstem, olfactory nucleus, thalamus, and striatum. The average uptake in the ROI's (in kBq/cc) was corrected for the injected tracer dose and the bodyweight of the animals and expressed as standard uptake value (SUV).

BDNF analysis

After the PET scan, the animals were transcardially perfused with cold phosphate-buffered saline pH 7.4 (PBS) and the brain was removed for tissue extraction. The frontal cortex and hippocampus were excised from the brain, placed in ice-cold PBS solution, snap-frozen in liquid nitrogen and stored at -80 °C until further analysis. RIPA buffer (Sigma-Aldrich, R0278 – containing 150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0) was added to the brain tissue (50 µl/mg tissue) and cooled on ice. The tissue was pounded until no solid fragments were visible anymore. The homogenized tissue was centrifuged at 12000 rpm for 15 minutes. The supernatant was collected for total protein quantification by the bicinchoninic acid assay (BCA) using bovine serum albumin as a standard. Then, BDNF was measured with ELISA (Cloud-clone, SEA011Ra) according to the manufacturer instructions. Intra-assay precision was <10%. Tissue lysate was diluted 1:5 in PBS (five samples were diluted 1:6 due to low amount of lysate). Samples were read at 450nm and corrected for the total amount of protein.

Statistical analysis

Analyses were performed using the two-way generalized linear model (GLM) with RSD and harmine treatment as factors. A within-subject factor (time) was added to the SPT analysis. The main effects of RSD and harmine were evaluated, as well as the interaction between both factors and time, whenever needed. For all tests, $p<0.05$ was considered statistically significant. SPSS 23 (IBM, United States) was used for all statistical analyses.

Results

Social defeat and harmine treatment decrease bodyweight gain

Figure 2 depicts the effect of RSD and harmine on bodyweight gain over time. As expected, there was a significant effect of time on bodyweight within animals ($F=11.380$, $p<0.001$). Additionally, there was a significant effect of RSD and harmine treatment on bodyweight gain (RSD: $F=3.275$, $p=0.040$. Harmine: $F=0.192$, $p<0.001$), but no interaction between RSD and harmine treatment ($p>0.05$). RDS induced a significant reduction in bodyweight gain compared to the control group ($F_{(1,25)}=12.123$, $p=0.002$), and also harmine treatment caused a significant reduction in bodyweight gain when compared to vehicle-treated controls ($F_{(1.25)}=28.624$, $p<0.001$).

The significant reduction in bodyweight induced by RSD lasted until day 12 ($p<0.05$), after which the effect of RSD had resolved. Harmine treatment seemed to have a stronger effect on bodyweight, as harmine-treated animals showed a significant difference when compared to vehicle-treated animals until the end of the experiment (day 17 – $p<0.05$ at all time points).

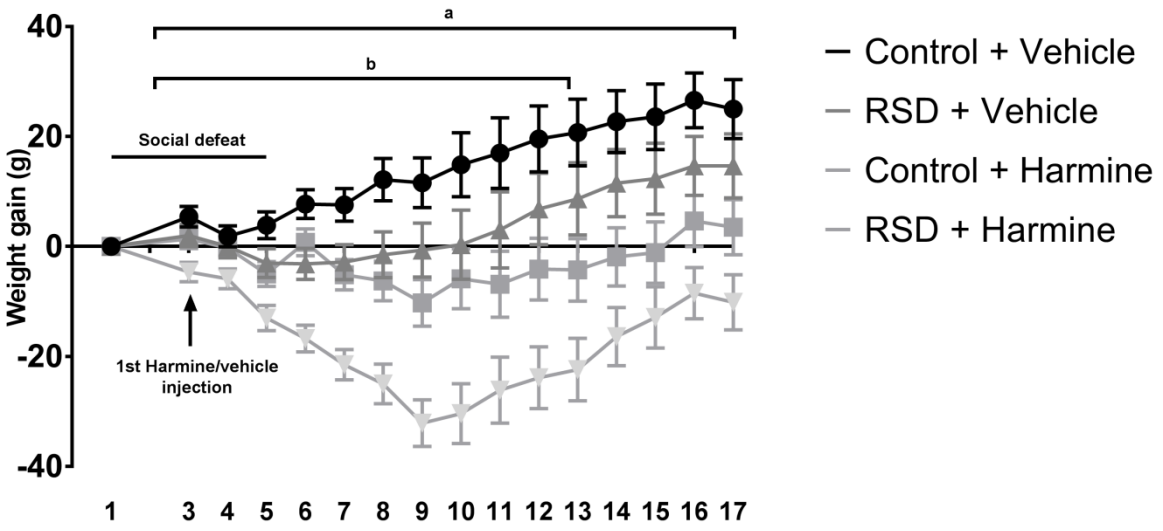

Figure 2: Bodyweight change over time. a: p<0.05 between harmine and vehicle treatment for each time point until day 17. b: p<0.05 between RSD and control for each time point until day 12. Points and whiskers represent mean ± SEM.

RSD affects sucrose intake on a short-, but not long-term

The sucrose preference test was performed on days -1, 6 and 15. There was a main effect of time ($F_{(2,54)}$=17.270, p<0.001) and a main effect of RSD (F=3.797, p=0.036) on SPT. The between-group analysis, however, did not show any significant differences at any time point (p >0.05).

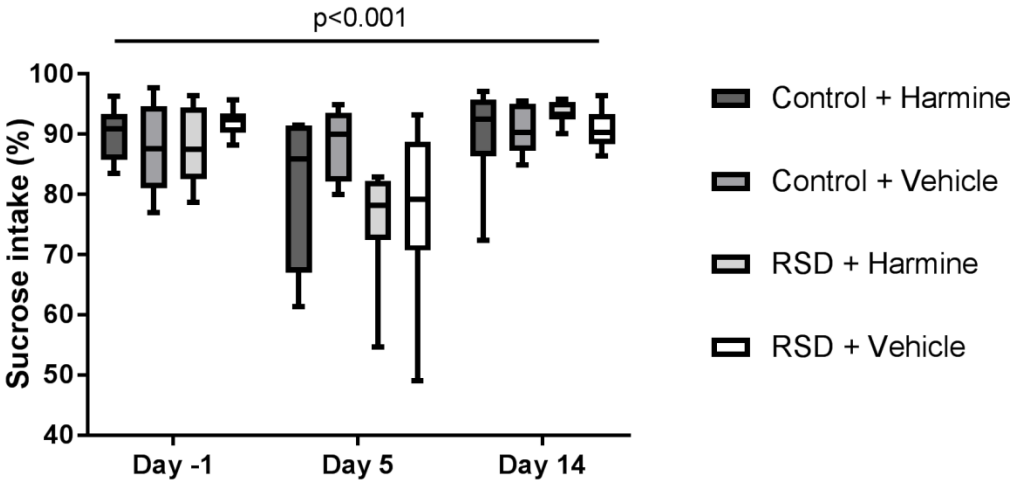

Figure 3: Sucrose preference at different time points. Baseline: 1 day before RSD; SPT 1: 1 day after RSD; SPT 2: 10 days after RSD. Horizontal lines and whiskers represent median ± 95% CI, respectively.

Transient effect of RSD on anxiety-like behavior

The effect of RSD and harmine treatment on anxiety was assessed by the time the animal spent in the center of the arena during the OFT. The OFT showed a significant main effect of RSD on

the time the animal spent in the center of the arena in the OFT performed on day 6 (F=4.747, p=0.038 – Figure 4), with defeated animals spending less time in the center when compared with control ones. However, this effect of RSD was not observed anymore on day 14.

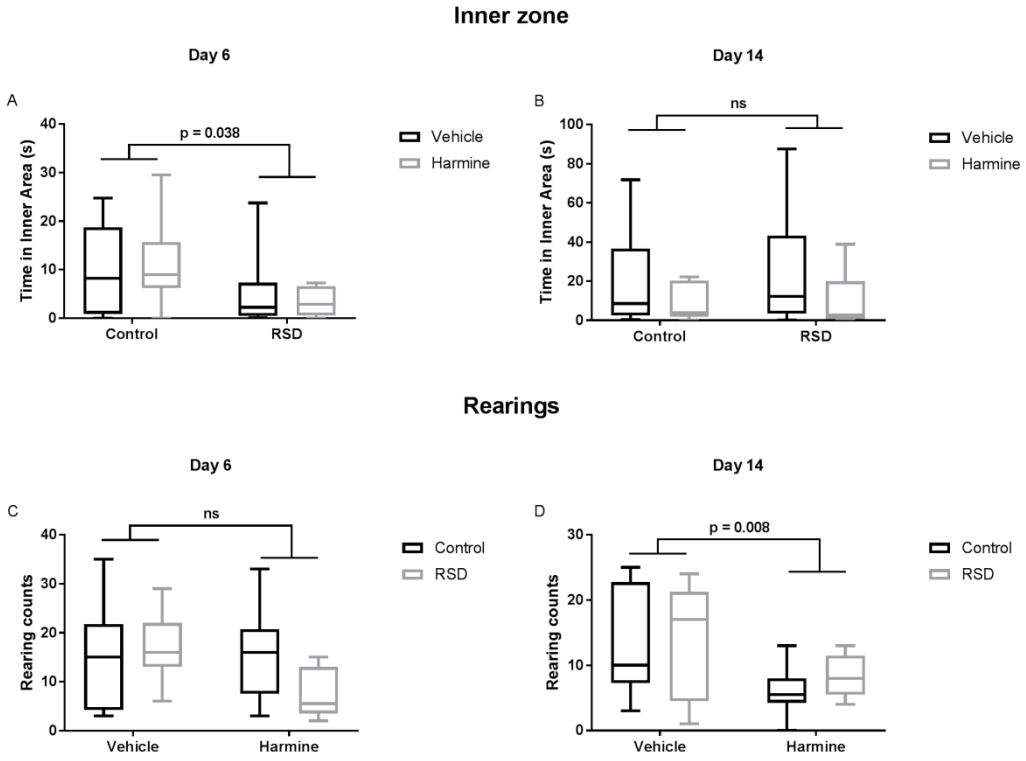

Figure 4: Effect of social defeat on the time spent in the inner zone of the arena (A-B) and the number of rearings (C-D) in the OFT on day 6 (left panel) and day 14 (right panel). Horizontal lines and whiskers indicate median ± 95% CI, respectively; sample size: 7-8.

Long-term effect of harmine on mobility

Harmine treatment significantly reduced the time the animal spent moving in the OFT on day 6 (F=6.356, p=0.018) and day 14 (F=7.283, p=0.012 – Figure 5). Likewise, the total distance moved by harmine treated animals was significantly smaller than the distance traveled by vehicle-treated animals (F=7.283, p=0.012). RSD did not have any effect on mobility neither on day 6 nor on day 14.

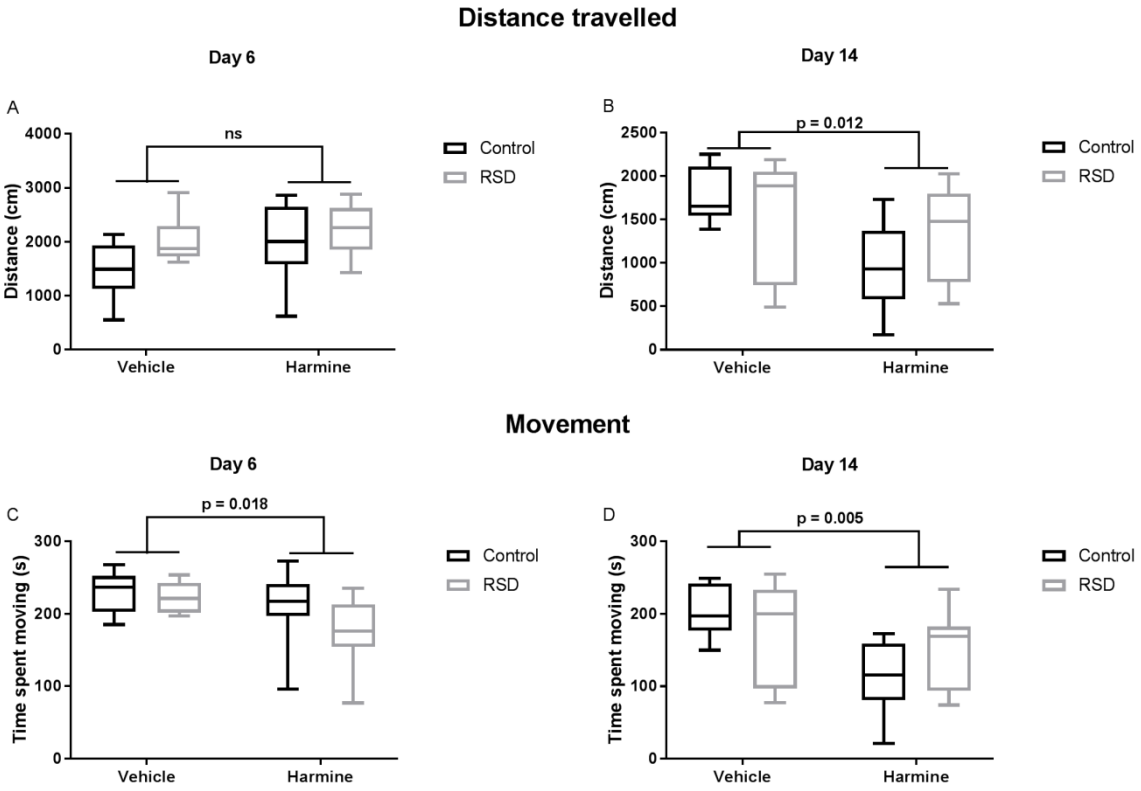

Figure 5: Short- (left panel) and long-term (right panel) effect of harmine treatment on the distance traveled (A-B) and time on movement (C-D). Horizontal lines and whiskers indicate median ± 95% CI, respectively; sample size: 7-8 animals per group.

Additionally, there was a significant effect of harmine treatment on rearing (exploratory behavior). Animals administered with harmine display less frequently a rearing posture ($F=4.475$, $p=0.012$).

No chronic effect of treatments on long-term memory

The NOR test did not show any effect of harmine or RSD on long-term memory, as no significant differences in recognition index between groups were observed ($p>0.05$).

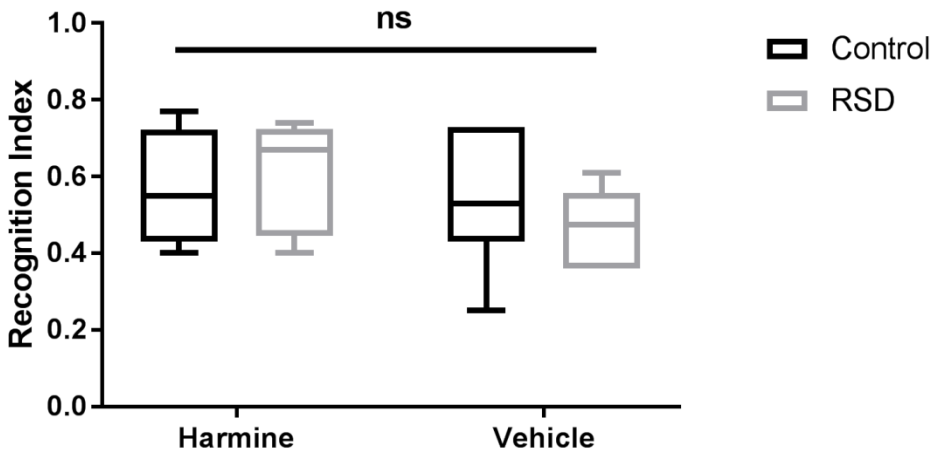

Figure 6: Results of the NOR test on day 16, showing no effect of RSD or harmine treatments on long-term memory. Horizontal lines and whiskers indicate median ± 95% CI, respectively; sample size: 6-7 animals per group.

No chronic effect of treatments on neuroinflammation

For all groups, [^{11}C]PBR28 PET showed the highest tracer uptake in olfactory nucleus, frontal and dorsal cortex and cerebellum. However, stress-induced neuroinflammation could not be detected on day 17, as there was no significant effect of RSD or treatment with harmine on the uptake (SUV) of [^{11}C]PBR28 in any of the brain regions assessed (all $p>0.05$ – figure 7).

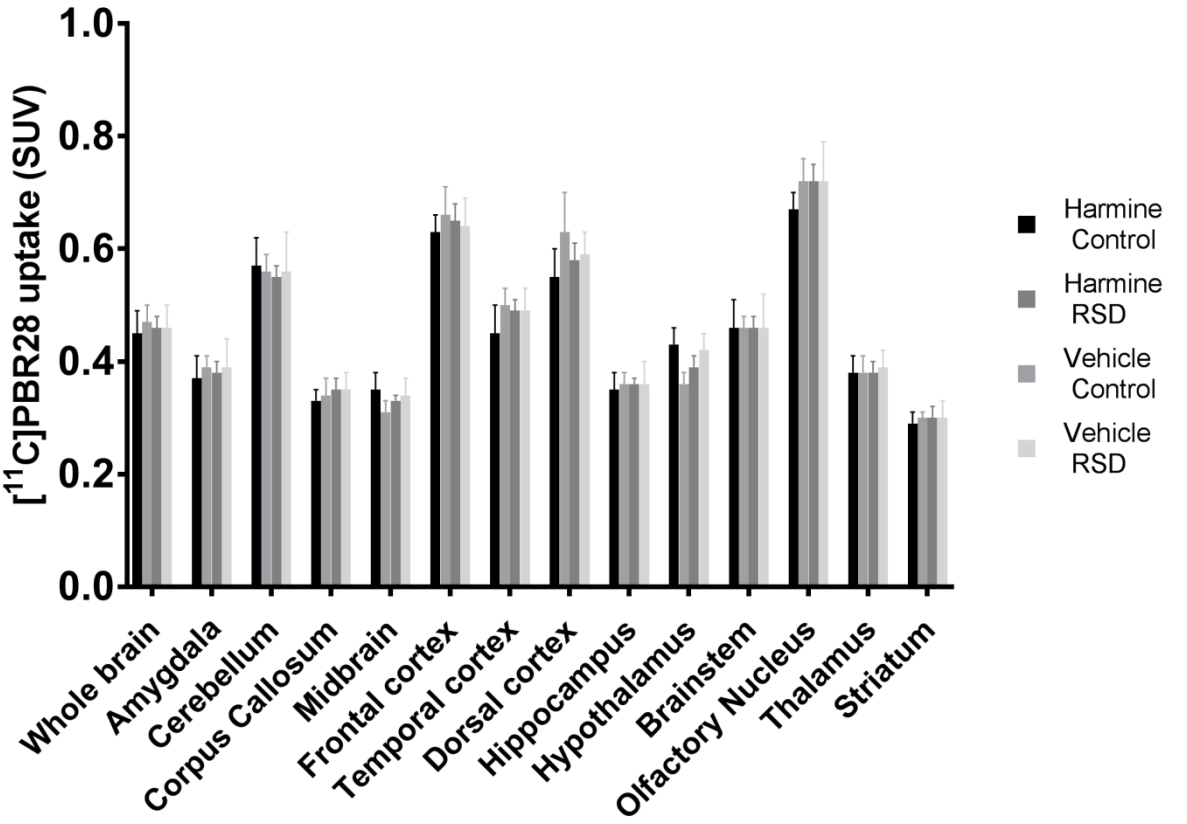

Figure 7: Results of [11C]PBR28 PET, showing no significant effect of RSD or harmine treatment on tracer uptake (SUV) in any brain region of interest. Bars and error bars represent mean and SEM, respectively; sample size: 7-8 animals per group.

No chronic effect of treatments on BDNF concentration

There was no significant main effect of RSD or treatment with harmine on the BDNF concentration in the hippocampus or frontal cortex ($p>0.05$ – figure 8). As BDNF is highly correlated with cognitive parameters, we also assessed if there was a significant relationship between the memory and the concentration of BDNF in either brain region using linear regression.

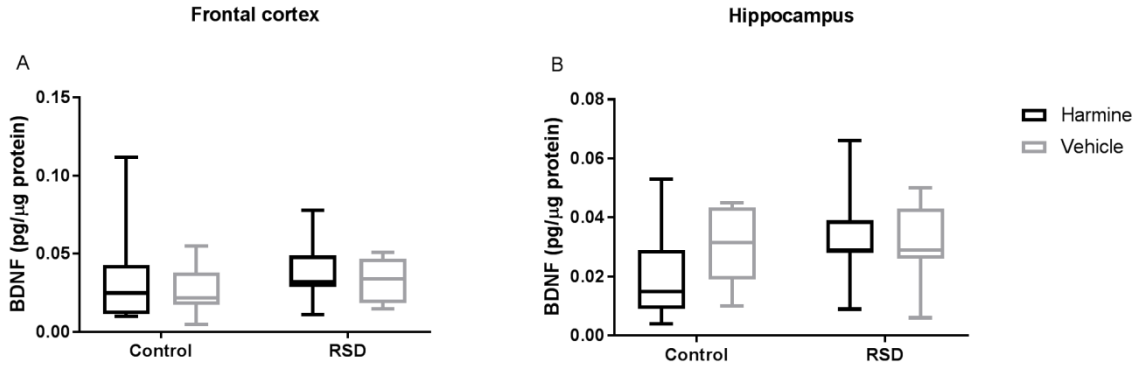

Figure 8: BDNF concentration in the hippocampus and frontal cortex on day 17, showing no significant effects of RSD or harmine treatment. BDNF corrected for total concentration of protein. Horizontal lines and error bars represent mean ± 95% CI, respectively; sample size: 5-8 samples per group

Discussion

This study aimed to assess the antidepressant and anti-inflammatory properties of harmine in rats submitted to RDS. We found that RSD was able to induce a transient depressive-like state in defeated animals. Harmine, on the other hand, negatively affected the bodyweight gain, general movement and explorative behavior of the animals. No delayed effects of both RSD or harmine on the memory performance, BDNF levels or the neuroinflammation were observed, probably due to the transient nature of the five-day RSD protocol.

RSD and harmine reduce bodyweight

Both RSD and harmine treatment caused a reduction in bodyweight. The effect of RSD is in line with previous literature describing that animals submitted to the RSD show a transient weight loss – or a lower weight gain – during the period of such protocol [15,31]. Stress can increase brown adipose tissue thermogenesis and hyperthermia and thus cause a reduction in bodyweight [32]. The effect of harmine treatment on bodyweight might be due to a similar mechanism, as harmine is able to induce adipose tissue thermogenesis by blocking *Ucp1* gene inhibition by chromodomain helicase DNA binding protein 4 (CHD4) [33].

Harmine reduces locomotion

Our findings show that harmine significantly decreased the general movement of the animals, as seen by their immobility time on days 6 and 14. Unlike our study, others have not observed any differences in locomotion after acute or chronic administration of harmine for 12 days [20,34]. However,

another study showed that the harmine analogs, harmane and norharmane, induced a significant decrease in the distance traveled by the animals, but no differences in anxiety or motor coordination outcomes [35]. Harmine has been suggested as a potential metabolite of harmane [36].

Previous research has shown that acute administration of harmine causes tremorgenic effects on rats [30]. In this study, we also found that harmine administration caused transient tremors, which lasted for approximately 60 minutes (data not shown). Although the tremors were not visible anymore after 1 h, it may have had some lingering effect on the general locomotion. Indeed, one of the main side-effects of monoamine oxidase inhibitors is movement impairment due to increased serotonin neurotransmission (i.e.: serotonin syndrome) [37]. Likewise, one could speculate that the reduced mobility induced by the administration of harmine in our study could be the cumulative effect of the daily treatment on serotonin neurotransmission. However, further investigation of the mechanisms for the effect of harmine on general locomotion is required.

RSD transiently induces anxiety and depressive-like behavior

Animals submitted to RSD showed more anxiety (time spent in the center of the open field arena) and depressive-like behavior (preference of sucrose solution over water) than controls. These results are supported by literature showing that several stressors can induce anxiety and depression-associated parameters in animals [21,38,39 40,41]. Although our results show an acute increase of anxiety and depression-associated measures in animals submitted to RSD, this effect did not last until 9 days after RSD. This transient effect has previously been observed in various RSD protocols, using different species, number of defeats, RSD duration and evaluation period. Kopschina Feltes and colleagues observed in Wistar rats that the effect of a similar RSD protocol was observed one week after, but was resolved after 90 days [15]. Martin and colleagues used a modified 10-day RSD protocol on C57BL mice and found that the transient effect of RSD had normalized after 18 days [42]. We found that the effect of RSD was already gone 9 days after the last RSD protocol, suggesting that the effect of RSD as a stressor in our study might be lower than expected. Thus, a priming effect (e.g. adding another RSD trial before the behavioral paradigm) might be needed in order to obtain a stronger effect in future studies.

Our study shows that acute administration of harmine was unable to improve the acute depressive-like state of the animals subjected to RSD. Although there are no studies on the therapeutic effect of harmine after social stress, it is known that mid- to long-term administration of harmine improves depressive symptomatology in animals submitted to a chronic unpredictable stress protocol [43]. One study reported that chronic administration of harmine prior to application of

chronic unpredictable stress was able to mitigate the stress-induced depressive-like behavior [21]. Another study investigating the therapeutic effect of chronic harmine administration for 1 week in chronically stressed rats showed similar results, using the preference for sugary food as outcome measure [44]. Both studies showed the effect of harmine after chronic administration of the drug, whilst in our study the effect of RSD on anhedonia was observed only 1 day after completion of the RSD protocol. Consequently the treatment period may have been too short for harmine to become effective. Unfortunately, the anhedonia effect of RSD had already resolved 9 days after RSD and therefore the effect of chronic harmine treatment could not be assessed in our study.

RSD and harmine do not affect long-term memory

There was no significant effect of harmine or RSD on the long-term memory 11 days after RSD. This is similar to what was found previously in our laboratory [15]. Other studies, however, showed that different subtypes of memory are affected by RSD. McKim and colleagues found that a six-days RSD protocol was able to impair spatial memory recall, as assessed with the Morris and Barnes mazes [45]. Wohleb and colleagues found that this effect lasts for up to eight days, suggesting a subchronic effect of RSD [41]. It is worth noting that memory tests can pose stressful environments, the NOR test is considered a substantially less stressful event than the Barnes maze, or Morris water maze; comparison between the tests is therefore difficult.

To the best of our knowledge, this is the first attempt to assess the effects of harmine on memory performance of socially defeated animals. A similar study using chronic unpredictable stress for 40 days also did not show any significant effect of harmine on memory [43]. In our study, however, the effect of the stressor seemed to be transient and on the day of the memory test the effect of the social stressor likely already had resolved. Consequently, no conclusion can be drawn on the protective effect of harmine. However, harmine by itself was unable to modify – positively or negatively – the long-term memory. Further studies are needed with stressors that are able to induce long-term cognitive impairment to assess the effect of harmine administration on stress-induced cognitive impairment.

RSD and harmine do not affect glial activation

[^{11}C]PBR28 PET imaging showed no significant effect of the social stress protocol or harmine treatment on glial activation. It is known that microglial activation starts hours after exposure to the stressor and can last for several days or even weeks, decreasing gradually as the resolution of neuroinflammation begins [46]. Kopschina Feltes and colleagues found a significant effect of RSD protocol on [^{11}C] PK11195 6 days after RSD in several key regions associated with depressive

behavior (e.g. medial prefrontal cortex, entorhinal cortex, and insular cortex), but this effect was not observed anymore 3 weeks after RSD [15]. In our study apparently either the neuroinflammatory process was not severe enough to be shown by PET imaging or the neuroinflammatory response had already resolved 11 days after the last RSD trial. The latter option is in line with the results of the behavioral studies, which also did not elicit long-term changes in behavioral parameters associated with depressive-like behavior. [^{11}C]PBR28 PET also did not show any significant effect of harmine treatment on tracer uptake, neither in controls nor in defeated animals. The latter is most likely due to the lack of an effect of RSD glial activation at the time of the PET scan.

RSD and harmine do not affect BDNF concentration

Reduced levels of BDNF protein in specific regions of the brain have been associated with cognitive impairment [47–51]. Treatment of brain disorders is generally accompanied by an alteration – usually an increase – in BDNF levels [24,52,53]. In this study, there was no effect of RSD or treatment with harmine on BDNF concentration in frontal cortex or hippocampus. The absence of an effect of RSD on BDNF levels might be explained by the transient effect of the stressor used in this study, resulting in a normalization of BDNF levels at the time of assessment. Other studies suggest that assessment at an earlier time point may have shown changes in BDNF concentration [54]. However, earlier assessment may potentially have obscured the effect of harmine treatment, as treatment with antidepressant drugs often take at least one week to induce behavioral changes.

Conclusion

RSD generated a fluctuation on a short-term (here observed by the behavioral outcome of the first OF and the SPT after RSD), but not a long-term effect, as seen by the lack of difference in uptake of [^{11}C] PBR28 between RSD and control groups, together with unaffected long-term behavioral alterations on anxiety and depressive-like behavior. The changes of RSD or harmine did not alter BDNF concentration in the frontal cortex or hippocampus, regions that are key for stress regulation and further brain homeostasis. With the results reported in this study, it is not possible to infer the effect of harmine as an antidepressant and anti-inflammatory drug, only to conclude that the treatment had no long-lasting harm towards the organism. To better assess the influence of harmine in depression and its effect as an antidepressant, there is a need for further studies using different stressors or longer time RSD protocols in order to induce a chronic stress response in the organism, thus allowing a more efficient analysis of how harmine could act under such conditions.

Acknowledgments

The authors thank the staff of the animal facility (CDP) at the University Medical Center Groningen. Also, the staff responsible for the production, synthesis and quality control of the radiotracer. This paper would not be possible without their contribution.

E. Bromberg has a CNPq research fellowship.

This research was supported by the Stichting de Cock-Hadders grant (grant number: 681717)

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