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Short communication

Stress triggers anhedonia in rats bred for learned helplessness

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ABSTRACT

Congenitally helpless (cLH) rats, a well-accepted model for depression, show reduced consumption of sweet solutions only under single-housing conditions, indicating anhedonia under stress. We investigated if anhedonic-like behaviour, measured by a reduction of sweetened-condensed milk (SCM) intake and the pleasure-attenuated startle response (PAS), could be induced by an electric foot-shock stress challenge in group-housed rats. After foot-shock stress, reduced SCM intake was observed in cLH rats compared to non-helpless (cNLH) rats. Furthermore, cLH rats also showed a decreased PAS, indicating deficient reward perception. In summary, we demonstrate that a predisposition for learned helplessness interacts with stress to trigger anhedonic-like behaviour in cLH rats. These findings further add to the validity of congenitally learned helplessness as an animal model of depression, since gene-environment interactions are considered to play a role in the etiology of this disorder.

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Depression, a chronic, recurring and often life-threatening disorder affects up to 20% of the population across the globe and genetic–environmental interactions seem to play a major role in inducing the illness [1].

One well-validated animal model of depression is learned helplessness, in which a depressive-like state in rats is induced by uncontrollable and unpredictable electrical foot-shock stress [2,3]. By selectively breeding helpless and non-helpless animals, two different rat lines could be established: rats that congenitally exhibit helpless behavior (cLH rats) and rats that show relative resistance to learned helplessness (cNLH rats). These strains differ in neurochemical and behavioral parameters that are related to depression [4,5].

One core feature of a depressive episode is the reduced ability to experience pleasure (anhedonia) [6]. In animals, hedonic-like behavior is commonly assessed by evaluating their consummatory response to highly palatable rewards [7]. Indeed, rats of the cLH strain show reduced consumption of a sweet solution, when they are kept under single-housing conditions [8]. However, when measuring consummatory behavior in a cohort of group-housed rats for a pilot study, we could not observe such a deficit in cLH rats. Since isolating social animals constitutes a major stressor, we suggest that single-housing serves as an environmental factor that induces anhedonic-like behavior in genetically predisposed cLH rats. In the present study we therefore aimed to investigate if anhedonic-like behavior could be induced by stress in group-housed rats. Although, a reduction in consumption of palatable liquids or food is generally considered to represent anhedonia, a mere reduction in intake might not necessarily reflect an alteration in the hedonic state of an animal, but might also be confounded by different additional factors such as taste perception, locomotor activity or novelty reponse and anxiety. Therefore, to assess differences in the hedonic perception of a food reward more directly between cLH and cNLH rats, we additionally used the pleasure-attenuated startle (PAS) paradigm [9]. The amplitude of the acoustic startle response (ASR) decreases if elicited in a pleasant emotional context. PAS probes the incentive properties of a conditioned cue by attenuating an unconditioned reflex, thereby making it a well-suited measure of hedonic affective states in rats [9,10].

In this study 32 cLH and 30 cNLH rats from the 66th generation, aged 9–13 weeks at the beginning of the experiment, were used. The origin and selective breeding of both strains have been described previously [2,4]. All rats were group-housed in standard macrolon cages (type IV) and kept under standard conditions (12 h light/dark cycle, lights on at 08:00 am). Water and food were available *ad libitum* except for testing days where SCM was available. Here animals were maintained on a mild food restriction schedule of 15 g lab chow/rat/day. Since SCM is highly caloric we only observed a minimal reduction of 1–2% of bodyweight compared to free-feeding conditions.

The experimental protocols used in this study were in line with national and international ethical guidelines, conducted in compli-

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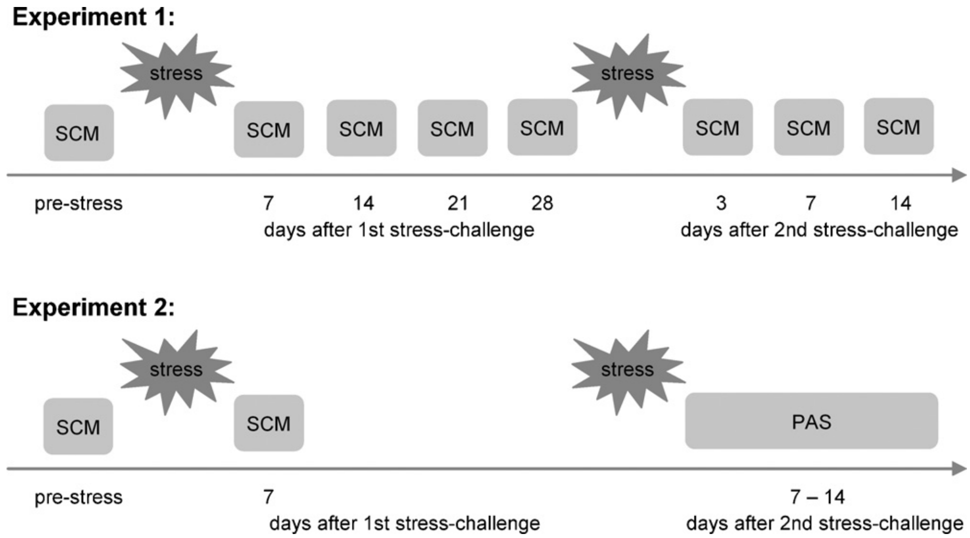

Fig. 1. Schematical overview on the study design. Experiment 1 investigated SCM consumption in congenitally helpless (cLH) and non-helpless cNLH rats at different time points before and after exposure to electric foot-shock stress. Experiment 2 investigated sweetened-condensed milk (SCM) consumption before and after stress to validate the phenotype and additionally, pleasure-attenuated startle (PAS) was tested in cLH and cNLH rats after the second stress exposure.

ance with the German Animal Welfare Act and approved by the Regierungspräsidium Karlsruhe.

For the present study two experiments were performed as depicted in Fig. 1.

By testing SCM intake repeatedly before and after foot-shock exposure, experiment 1 investigated whether stress would differentially affect reward sensitivity in cLH ($n = 9$) and cNLH ($n = 8$) rats and determined the time course of a putative stress effect. Experiment 2 aimed to assure that differences seen in consummatory behavior are related to the hedonic perception of the reward. Therefore, cLH ($n = 13$) and cNLH ($n = 13$) rats were exposed twice to foot-shock stress and were tested for SCM intake and PAS. SCM intake was measured before and 7 days after the first stress exposure to validate the findings from experiment 1. PAS was measured in the same animals 7 days after the second foot-shock stress exposure. In addition, a sham-training procedure was performed in cLH ($n = 10$) and cNLH rats ($n = 9$).

For the foot-shock stress procedure electric shocks were applied in modified skinner boxes equipped with a grid floor consisting of 24 electrifiable steel rods and electrifiable walls (TSE Systems, Bad Homburg, Germany); levers were retracted. After 3 min of habituation, 4 unsignalled electric shocks (2 s, 0.8 mA, pulsed) were delivered at a variable interstimulus interval (60 ± 30 s). 1 min after the last shock, rats were returned to the home cage.

For the measurement of limited access SCM intake rats were separated in small cages (type 3) to which they had been habituated 48 h before the first testing. After 5 min of habituation rats had access to the SCM solution for 15 min. The amount of liquid consumed was recorded for each rat and was calculated in relation to the individual body weight [ml/kg] [11].

In the second experiment of the study animals were tested twice for their ASR in startle chambers (SR-LAB; San Diego Instruments, San Diego, USA) in the presence of an odor cue (orange, essential oils, Primavera Life, Sulzberg, Germany), once before (=ASR baseline; ASR1) and again 24 h after 5 days of odor-reward association training (ASR2) as described in detail before [9]. Shortly, during odor training, which lasted 90 min in total, rats were placed in single cages (Eurostandard type III) and experienced 3 odor-reward presentations at random time points. Odor-reward presentations involved the introduction of the odor in a petri dish (orange, 15 μl) followed 5 s later by the presentation of the bottle containing the SCM. After free access to the reward for 5 min the odor and the SCM were removed. The ASR test program consisted of 35 startle pulses (white noise of 100 dB sound pressure level; 40 ms duration; inter-trial interval 10–20 s). PAS was calculated as mean percent decrease over baseline ASR amplitude [$100 - (100 \times$ mean ASR2 amplitude/mean ASR1 (baseline) amplitude)]. In order to assure that ASR attenuation was due to the training procedure and was not affected by a different ASR habituation or sensitization in both strains, an additional sham-training experiment was performed. Control animals underwent a sham-training procedure where they received the same odor and reward presentations as conditioned animals but the cues were presented in an explicit unpaired fashion as described before [8].

For statistical analysis repeated measurement of SCM intake was evaluated using a two-way repeated measure ANOVA, followed by separate unpaired t-tests. Differences in startle amplitude and PAS between cLH and cNLH rats were assessed by unpaired t-tests. An alpha value of 0.05 was considered to represent a significant effect.

In experiment 1 statistical analysis revealed that SCM intake differed significantly between cLH and cNLH rats (Fig. 2A) depending on the test session (phenotype × session RM ANOVA: $F_{7,105} = 5.3$; $p = 0.0002$). Both groups consumed similar amounts of SCM in the pre-stress test session ($p = 0.15$), but cLH rats drank significantly less SCM when tested 7 days ($p = 0.012$) and 14 days ($p = 0.009$) after the first stress. This effect was still present as a statistical trend 21 days after stress ($p = 0.084$) but was absent on test day 28 ($p = 0.52$). The second stress challenge reinstated lower SCM intake in cLH rats when tested 3 days ($p = 0.001$), 7 days ($p = 0.010$) and 14 days ($p = 0.008$) later.

In experiment 2 a significant interaction effect was detected for the SCM intake measured pre- and post-stress between cLH and cNLH rats (ANOVA: $F_{1,24} = 5.5$; $p = 0.027$) (Fig. 2B). Both groups did not differ in their SCM intake prior to the first stress exposure ($p = 0.27$). After stress cLH rats showed a significant lower SCM intake compared to cNLH rats ($p = 0.049$) and to their pre-stress measurement ($p = 0.001$), confirming the results from experiment 1.

Note that initial pre-stress SCM intake was higher than in experiment 1 due to the fact that those rats were young adults at the beginning of behavioral testing (9 weeks), whereas animals from the second cohort were already 13 weeks old. However, when rats in experiment 1 were 13 weeks old (28 days after first stress), SCM consumption was similar between cohorts.

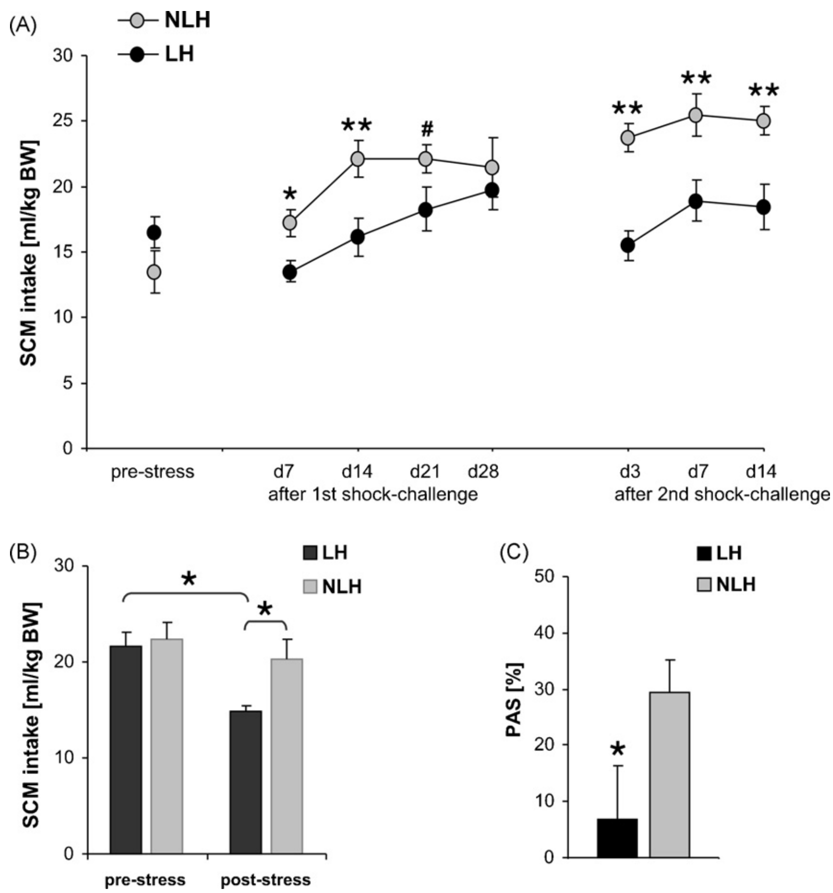

Fig. 2. (A) Experiment 1: stress differentially affects sweetened-condensed milk (SCM) intake in congenitally helpless (cLH) and non-helpless cNLH rats. While initially SCM intake was similar in both strains, cLH rats consumed significantly less SCM than cNLH rats after stress exposure for almost 3 weeks. After consumption had normalized (28 days post-stress), a second stress exposure reinstated lower SCM intake in cLH rats. (B) Experiment 2: stress-induced decrease in SCM intake was verified in a second cohort of cLH rats prior to PAS measurement. (C) Experiment 2: pleasure-attenuated startle (PAS) in cLH and cNLH rats measured after the second stress exposure. PAS indicates the percentage reduction in baseline ASR by the odour presentation. PAS was significantly reduced in cLH compared to cNLH rats. $^{\#}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$; data are presented as means ± S.E.M.

Percentage PAS, measured after the second stress exposure, differed significantly between cLH and cNLH rats (Fig. 2C). A significantly lower PAS response was observed in cLH rats compared to cNLH controls (Student's *t*-test; $p = 0.037$). No differences in the basal ASR of both groups were detected (Student's *t*-test; $p > 0.05$) (basal ASR values: cLH: 451 ± 117; cNLH: 416 ± 123). No significant differences in startle attenuation between cLH and cNLH rats were detected in the sham-training conditions (percentage ASR reduction; values: cLH: 1 ± 10.9 S.E.M.; cNLH: 2.73 ± 11 S.E.M.)

Taken together the present study shows that anhedonic-like behavior can be triggered by electric foot-shock stress in rats congenitally exhibiting a depressive-like phenotype. While initially showing similar consummatory behavior as cNLH rats in a SCM limited access task, cLH rats consumed significantly less SCM in the same task after a short stress challenge. This decrease was temporary and normalized after 3 weeks. An even more pronounced reduction in SCM intake could be reinstated by a second stress challenge. Decreased consumption of a sweet reward is thought to be a correlate of anhedonia, which represents a key symptom of depression. In addition, the repeated stress challenge induced a significantly reduced PAS response for SCM in cLH rats, confirming that the decrease in consummatory behavior observed in the present study was definitely due to a lower hedonic perception of SCM in cLH animals, compared to cNLH controls. The PAS paradigm is a valid psychophysiological measure of positive affective states since it measures the reduction of an aversive reflex, instead of reinforcing or increasing operant behavior [9,10]. Our data indicate that beside the measurement of positive hedonic states, the PAS paradigm is also suitable for detecting anhedonic states in rats, as shown for the cLH rats in the present study after stress exposure. The use of sham-trained control groups in the present study demonstrates that a higher PAS in cNLH rats was not due to differences between both strains in long-term habituation of the ASR as a result of repeated testing. Only few studies have so far investigated the neurobiological pathways underlying PAS. PAS is not impaired by blockade of accumbal D1 and D2 dopamine receptors, indicating that dopamine in the nucleus accumbens is not necessary for the evaluation of the hedonic properties of rewarding stimuli [12]. More recently we could show that the endogenous opioid system seems to be an important modulator of the hedonic attenuation of the startle reflex in a pleasant context, since the opioid antagonist naloxone dose-dependently inhibited PAS [11].

Depressive episodes are thought to be consequences of the interaction of genetic predispositions with environmental risk factors, such as stress [1]. Although rats from the cLH strain show some behavioral abnormalities related to depression even without being subjected to a particularly stressful event [4,13], some depressive-like symptoms, like anhedonia in this study, seem to require an additional external stressor to manifest. In this respect, our results further validate the usefulness of a genetic learned helplessness model of depression.

Current concepts of reward processing emphasize a role for abnormal dopamine, cannabinoid and opioid actions in anhedonia and depression, with dopamine rather coding for the preparatory aspects of behavior, while endocannabinoids and brain opioids seem to mediate the perception of the hedonic properties of rewards [6,14–17]. In line with this, stimulation of the dopaminergic, endocannabinoid and endogenous opioid systems have been linked to anti-depressant effects [18,19]. Experimental manipulations of these systems in our genetic learned helplessness model combined with PAS measurements might be a useful approach to further study the neurobiological underpinnings of anhedonia.

Conflicts of interest

Rainer Spanagel received research and consultant contracts with Abbott, GSK, Organon, Solvay and XenoPort. Authors declare no competing financial interests.

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