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Repeated testing modulates chronic unpredictable mild stress effects in male rats

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ABSTRACT

Depression is a highly prevalent, debilitating mental disorder. Chronic unpredictable mild stress (CUMS) is the most widely applied model to study this affliction in rodents. While studies incorporating CUMS prior to an intervention often require long-lasting stress effects that persist after exposure is ceased, the longevity of these effects is rarely studied. Additionally, it is unclear whether behavioural assessments can be performed before and after interventions without repeated testing effects. In rats, we investigated CUMS effects on components of depressive-like behaviour both acutely after stress cessation and after a recovery period, as well as effects of repeated testing. We observed acute disruptions of the circadian locomotor rhythm and a reduced sucrose preference immediately after CUMS exposure. While circadian locomotor rhythm effects persisted up until four weeks after stress cessation, independently of repeated testing, sucrose preference effects did not. Interestingly, CUMS animals tested once after a recovery period of four weeks showed reduced anxiety-like behaviour in the open field and elevated plus maze compared to their control group and repeatedly-tested CUMS animals. These findings suggest that distinct CUMS-induced components of depressive-like behaviour are affected differentially by recovery time and repeated testing; these aspects should be considered carefully in future study designs.

1. Introduction

Depression is among the most prevalent leading causes of disability worldwide with about 350 million people suffering from major depressive disorders (MDD) [1]. Besides its detrimental effects on quality of life of patients and their families, MDD also has a great economic impact that extends to society in general, making this illness a global social burden [2]. Yet, while it is known that depression causes a wide variety of symptoms and is associated with temporal periods of recovery and relapse that occur in about 60% of patients [3,4], much about its pathology is not well understood.

In susceptible people, negative environmental stimuli critically contribute to the onset of the pathology as well as to disease relapse. Accordingly, exposure to chronic stress is the most studied and characterized environmental factor at the roots of the illness [5]. In order to better understand the impact of stress exposure on brain function, different preclinical animal models have been developed and characterized over the years. Chronic unpredictable mild stress (CUMS) in rodents is one of the most employed preclinical models used to understand the onset and the progression of MDD [6]. Indeed, the CUMS paradigm has been reported to induce several features of the human pathology, such as alteration of the circadian rhythm, anhedonia, increases in anxiety and behavioural despair (a delay or cessation in attempts to escape foot shocks in response to prior exposure to unescapable shocks, which has been interpreted as a theoretical analogue of learned helplessness), as well as social deficits in rodents [7–13], confirming its translational value to study MDD pathology and potential new treatment options. The vast majority of CUMS studies is focused on the effects of CUMS while the stressors are still present [14]. However, studies investigating the efficacy of any type of intervention (i.e. drug, diet, medical device, behavioural training, surgical procedure) in the treatment of depression often require cessation of stress exposure during the intervention period. On top of that, these studies often demand assessment of behavioural symptoms in the long term. The latter poses experimental challenges, as one would ideally first confirm the immediate effects of CUMS prior to treatment initiation, and later assess

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whether its long-term consequences are ameliorated by treatment. However, behavioural testing includes exposure to new environments, often individually, which is an additional source of stress [15]. Moreover, repeated behavioural testing could induce learning effects [16,17], potentially confounding the long-term assessment of CUMS-induced symptomatology.

Here, we set out to test whether CUMS induces long-term effects on the different behavioural symptoms observed in MDD, i.e., disturbed circadian rhythm, anhedonia, increased anxiety, reduced sociability, and increased behavioural despair. In order to do this, behavioural assessments that have been validated either in our lab [18,19] or in the literature [20,21] were used. More specifically, we tested whether assessment immediately following CUMS affected the presence of these long-term effects. After initial exposure, we tested groups of CUMS and control animals both acutely and a second time after two weeks of recovery. Additionally, other groups of CUMS and control animals were only tested once after a four-week recovery period after CUMS; these test series coincided in time with the second test series for the previous groups. We expected that (1) exposure to CUMS would cause the animals to display various components of depressive-like behaviour, present both immediately after stress exposure and after a rest period, and that (2) exposing animals to the same behavioural tests twice might lead to habituation to these tests, in particular the anxiety tests, leading to the double-tested animals showing less depressive-like symptoms than the single late-tested animals for both CUMS and control groups.

2. Materials and methods

2.1. Animals

A total of 72 adult (68–70 postnatal days), male wild-type Wistar rats were obtained from Charles River (Cologne, Germany). The animals were housed in pairs in standard type III cages upon arrival, and given a minimum of 10 days to habituate and adjust to a reversed light/dark cycle (12:12 h, lights on at 1900 or 2000 h). Animals were housed on corncob bedding in temperature-controlled rooms (22 ± 1 °C), with ad libitum access to rodent chow (ssniff Spezialdiäten, Soest, Germany) and water. Drinking water was supplemented with 0.1% saccharin, as the current study was a pilot for another experiment in which this was required. Clean cages were provided once a week. All animals were handled by the experimenter during the five days leading up to the experiment, to habituate them to being handled.

All experiments were approved by the Central Committee on Animal Experiments (Centrale Commissie Dierproeven, CCD, The Hague, The Netherlands), and carried out in compliance with European Union Directive 2010/63/EU. All efforts were made to minimize animal suffering and to reduce the number of animals.

2.2. Experimental outline

Cages containing two animals each were randomly assigned to one of four experimental groups (each n = 18) (Fig. 1). Two groups were exposed to CUMS for 14 days at the start of the experiment, while the other two control groups were housed regularly during this time. Immediately after stress exposure, one control group (Control – Double-tested) and one CUMS group (CUMS – Double-tested) were subjected to a battery of behavioural tests designed to assess different components of depressive-like behaviour immediately after stress cessation (i.e., acute stress effects). The two other groups (Control- and CUMS - Single late-tested) were housed regularly during this time. Upon finishing the series of behavioural tests, animals in the double-tested groups were housed regularly without interference for a duration of 14 days. After this time period, the same series of behavioural tests was performed in all four groups (i.e., long-term stress effects). Animals from different experimental groups were not housed together in the same cages, in order to prevent stress effects to carry over to control animals and to prevent isolation stress during the first test series. During periods of regular housing, animals were handled by the experimenter weekly. No additional handling sessions were performed as substitute placeholders during the first test point for the single-tested animals.

Each of the four experimental groups was divided into three cohorts of animals that entered the experiment separated by intervals of several days. Each cohort (n = 8) consisted of animals coming from one of the experimental groups (except for the final cohort that needed to complete the group sizes (4x n = 2 per experimental group)). The different experimental groups were counterbalanced in the order of cohorts entering the experiment, in order to correct for possible cohort or time effects.

Originally, we aimed for making comparisons between groups at different time points to determine the stability and longevity of the stress-induced behavioural alterations, which is why we chose to keep the contexts constant. However, as this experiment was originally intended as a pilot study for the optimization of parameters, we did make adjustments in the exact task settings between time points in some cases, in order to optimize testing parameters. We therefore chose to refrain from making direct comparisons in behaviour between the two time points. Rather, to assess the acute effects of CUMS we compared the double-tested groups at test point I, while we investigated prolonged CUMS effects and how these effects were affected by repeated testing by comparing double-tested and single late-tested CUMS groups to their corresponding control groups, and double-tested CUMS and control groups to their corresponding single late-tested groups at test point II.

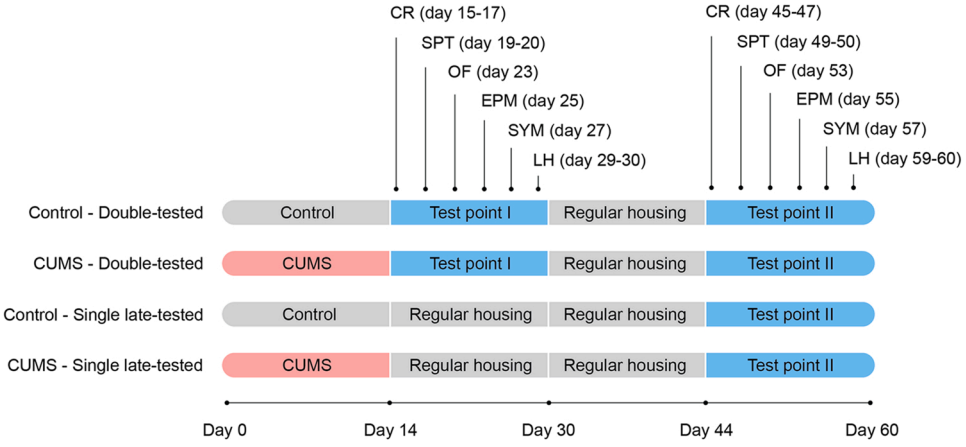

Fig. 1. General study design for assessing the behavioural consequences of CUMS. Animals were divided into four groups, which were either exposed to CUMS or control for the first 14 days. From both conditions, one group underwent two series of behavioural tests, while the other only participated in the second test series and was otherwise left undisturbed. Abbreviations: CR: circadian rhythm; SPT: sucrose preference test; OF: open field; EPM: elevated plus maze; SYM: social Y maze; LH: learned helplessness.

2.3. Chronic unpredictable mild stress exposure

Animals were exposed to 7 unpredictable mild stressors for a duration of 14 days (Table 1). The order of stressor application was randomized, and each stressor was presented twice. As the experimental groups were divided into several cohorts that entered the experiment separated by intervals of several days, the exact timing of the different stressors differed across experimental cohorts. However, total stress exposure and predictability of the stressors was equal across all cohorts.

2.4. Behavioural assessments

2.4.1. Circadian rhythm

Animals were placed in Phenotyper units (Noldus) of 45 × 45 cm, simulating a home cage environment, and their positions tracked through Ethovision® software (Noldus Information Technology). The first 24 h were considered the habituation period, after which two day-night cycles (48 h) of locomotor activity was recorded. Distances moved during the dark and light period (12:12 h) were tracked for each animal, and analyses were performed on the averages of these readouts across the two test days.

2.4.2. Sucrose preference

The animals were habituated to drinking from two bottles in the Phenotyper units and their home cage afterwards, by presenting them with two identical drinking bottles filled with their standard drinking solution. This habituation period comprised the three days they were housed in the Phenotyper units, as well as two days in their home cage. After these two days, the test days started. During the test days, the animals were presented with one bottle containing their standard drinking water and one bottle containing a sweeter sucrose solution. Consumption of both solutions was measured after 8 h, from which sucrose preference was calculated as follows: (sucrose consumption / (water consumption + sucrose consumption)) x 100%. Bottle locations were counterbalanced between animals, and switched every day. During time point I, animals were exposed for 3 days to sucrose of varying concentrations (+2% compared to standard for the first two days, and +1% for the third day), and during time point II for 2 days (+1% sucrose compared to standard).

2.4.3. Open field

Animals were placed in a corner of a 100 (w) x 100 (l) x 40 (h) cm dark gray PVC arena. They were allowed to explore freely for a duration of 5 (time point I) or 10 min (time point II). Animals were tested under red light (time point I) or white light at 5 lux (time point II). The centre of the open field was defined as the 50 × 50 cm region in the middle of the arena. Distance moved, time spent in the centre, frequency visiting the centre, and latency to first visit of the centre were tracked in Ethovision® (Noldus Information Technology).

2.4.4. Elevated plus maze

Animals were placed in the centre of an elevated plus maze (raised 46.5 cm above the ground) with two open arms (all arms: 48.5 ×10 cm) and two closed arms facing an open arm, and were allowed to explore freely for a duration of 5 min. Animals were tested under red light, in order to ensure proper sight for the experimenters while causing minimal disturbance of the animals. Distance moved and time spent in the open arms were tracked in in Ethovision® (Noldus Information Technology), whereas frequency visiting the open arms and latency to first visit the open arms were scored by an observer blinded to the experimental group by means of The Observer® software (Noldus Information Technology).

Table 1
Incorporated stressors and their duration.

Stressor	Duration
Light/dark cycle reversal	24 h
Wet bedding	8 h
Social isolation	8 h
Cage tilt (45°)	8 h
White noise (90 dB)	3 x 1 h
Strobe light	3 x 1 h
Water deprivation	3 h (start dark phase)

2.4.5. Social Y maze

Four hours prior to the start of the test, animals were housed individually. The arena consisted of a modified Y maze where the ends of two arms (all arms: 50 ×15 x 40 cm) were closed off by a thin metal plate containing circular openings that allowed for nose pokes from either side. The test animals were first placed in the maze for 15 min to explore and habituate. After the habituation trial, the test animals underwent a 10-minute test trial in which they could choose to interact with other animals placed in the closed compartments. During this test trial, one compartment contained an unknown animal while the other compartment contained the cage mate of the test animal; locations were counterbalanced between animals. Each animal participated once as test subject, once as known animal, and once as unknown animal. Animals were tested under red light conditions. Active interaction time and interaction frequency were scored by an observer blinded to the experimental group by means of The Observer® software (Noldus Information Technology), and distance moved was tracked using Ethovision® (Noldus Information Technology).

2.4.6. Learned helplessness

Animals were placed in a shuttle box which was divided in two by a retractable door (ENV-010MD, Med Associates, St. Albans, VT, USA). The floor consisted of a metal grid, used to subject the animals to foot shocks. On day 1, the training day, after habituating for 5 min, the animals were exposed to 60 inescapable, unpredictable foot shocks (0.6 mA). Durations of shocks and intervals varied, adhering to a range of 8–14 s for the shocks, and 10–18 s for the intervals. Shocks were applied in both compartments, in order to prevent place preference effects. During the intervals when the shocks were not applied, the door was raised, allowing the animals to move between compartments. About 24 h after the first session, 10 test trials were performed in which the animals were able to escape the shocks by escaping through the door, as the door was now opened 1 s after shock onset, and current was only applied in the compartment in which the animals resided during the start of that specific trial. A new context was introduced on the test days by cleaning the shuttle boxes with a cleaning detergent with an unknown, novel smell (in addition to the standard 70% ethanol), in order to reduce any memory effects carrying over from the training trials. During the test trials, interval duration was fixed at 25 s, and shock duration had a maximum of 15 s. Escape latency was measured per trial. If rats failed to escape, the maximum of 15 s was registered as escape latency. The total number of escapes over the trials was also assessed.

2.5. Statistical analysis

The data obtained in these experiments were analyzed using Microsoft Office Excel 2016 and IBM SPSS Statistics 27, and visualized using GraphPad Prism 9.12. Outliers were identified based on z-scores > 3.00 and < -3.00. Normality was assessed through Shapiro-Wilk tests, and sphericity was assessed through Mauchly's tests where applicable. Results were analyzed by means of repeated measures/mixed model ANOVAs, and Mann-Whitney U tests in the case of non-normal data distributions. When interaction effects were found, (Welch's) t-tests and Mann-Whitney U tests were used to assess differences between groups. $P < 0.05$ was accepted as statistical significance. When multiple comparisons were made, a Bonferroni correction was applied; $\alpha = 0.05/4 = 0.0125$.

3. Results

3.1. Acute effects of chronic unpredictable mild stress

3.1.1. CUMS exposure acutely disrupts locomotor circadian rhythm

We assessed distances moved by the rats in the dark and light phases of the circadian cycle in order to investigate their locomotor circadian rhythm. This rhythm is often disturbed in depression [22,23]. A repeated measures ANOVA over diurnal phases revealed an interaction effect between CUMS exposure and diurnal phase ($F(1,34) = 69.729$, $p < 0.001$, Fig. 2). *Post hoc* tests revealed that CUMS exposed rats displayed lower distance moved in the dark phase compared to control animals ($p < 0.001$), whereas they moved increased distances during the light phase ($p < 0.001$). These data indicate that CUMS induced acute disruptions of the locomotor circadian rhythm.

3.1.2. CUMS acutely reduces preference for lowest sucrose concentration

In order to investigate the acute effects of CUMS exposure on anhedonia, another component of depressive-like behaviour, we assessed the rats' preferences for sucrose consumption (Fig. 3) [24,25]. To this end, we measured the consumption of a sucrose solution over the course of three consecutive days. The first 2 days, the animals could choose freely between 2% sucrose solution and normal drinking water. A repeated measures ANOVA revealed an overall decrease of sucrose preference on day 2 compared to day 1 ($F(1,33) = 23.744$, $p < 0.001$), but neither a CUMS x day interaction effect ($F(1,33) = 0.190$, $p = 0.666$) nor a main effect of CUMS ($F(1,33) = 1.269$, $p = 0.268$). However, when presented with a 1% sucrose instead of 2% sucrose solution on day 3, the CUMS group displayed a lower sucrose preference compared to the control group ($t(34) = 2.880$, $p = 0.009$). Thus, while CUMS did not affect preference for the 2% sucrose solution on day 1 and 2, it did affect the animals' preference for the 1% sucrose solution on test day 3, matching an anhedonic phenotype.

3.1.3. Anxiety-like behaviour largely unaffected in the acute aftermath of CUMS

We assessed anxiety-like behaviour, another frequent symptom of depression [26], in the open field and elevated plus maze tests (Fig. 4). In the open field, we detected no differences between groups in total distance moved ($t(34) = -0.033$, $p = 0.974$), time spent in the centre area ($t(34) = 0.108$, $p = 0.915$), frequency of visiting the centre area ($t(34) = -1.174$, $p = 0.249$), and latency to the first visit of the centre area (Mann-Whitney $U = 130.00$, $p = 0.323$). In the elevated plus maze the CUMS group displayed a higher latency to enter either of the open arms (Mann-Whitney $U = 98.50$, $p = 0.044$). However, there were no group specific differences in total distance moved ($t(33) = -0.024$, $p = 0.981$), time spent in the open arms ($t(34) = 0.400$, $p = 0.692$), and frequency of visiting the open arms ($t(34) = 1.514$, $p = 0.139$). As such, CUMS exposure did not consistently induce high levels of acute anxiety-like behaviour.

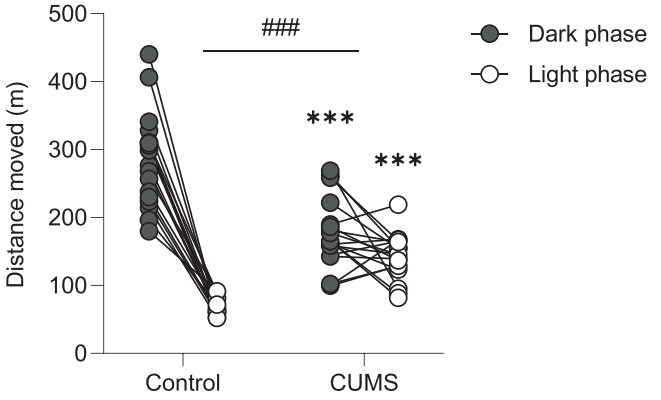

Fig. 2. Circadian rhythm as indicated by distance moved in the dark phase and light phase of the day (each n = 18). ###: $p < 0.001$, CUMS x diurnal phase effect. * ** : $p < 0.001$ compared to control group.

3.1.4. CUMS acutely decreases total distance moved in social Y maze

We investigated social behaviour and memory by means of a social Y maze (Fig. 5), since a withdrawal from social behaviour comprises another component of depressive-like behaviour [27]. During the test trial, the CUMS-exposed animals showed reduced exploration behaviour measured as distance moved compared to controls ($t(34) = 2.379$, $p = 0.023$). However, neither a main effect of CUMS was observed on the time spent interacting with either familiar or unfamiliar animals ($F(1,33) = 2.070$, $p = 0.160$), nor an interaction between CUMS and familiarity of the animal ($F(1,33) = 0.611$, $p = 0.440$) or a main effect of animal familiarity ($F(1,33) = 2.358$, $p = 0.134$). Also in the frequency of interaction with the familiar and unfamiliar animals, no significant interaction effect between CUMS and familiarity of the animal were detected ($F(1,33) = 3.713$, $p = 0.063$), nor main effects of CUMS ($F(1,33) = 0.231$, $p = 0.634$) or familiarity ($F(1,33) = 0.010$, $p = 0.921$). Overall, while CUMS exposure decreased exploratory behaviour in the social Y maze, we found no evidence supporting CUMS acutely affecting social behaviour with familiar and unfamiliar animals.

3.1.5. No acute effects of CUMS on learned helplessness

As a prevalent behavior in depression, we assessed learned helplessness behaviour [28] by measuring shock escape latencies and the number of successful escapes over a total of 10 trials (Fig. 6).

No significant effect of CUMS on average escape latency was found ($U = 160.500$, $p = 0.962$), nor was the total number of escapes affected by it ($U = 130.000$, $p = 0.283$). A repeated measures ANOVA revealed no main effect of trial ($F(5.541,188.406) = 1.605$, $p = 0.154$), nor CUMS x trial number interaction ($F(5.541,188.406) = 1.153$, $p = 0.334$), indicating that there was no improvement in escape responses over trials (Fig. S1).

3.2. Long-term effects of CUMS

After having investigated the acute effects of CUMS exposure, in a next set of experiments we tested whether the behavioural consequences of CUMS are longer lasting (i.e. persistent effects) and whether they may become affected by repeated behavioral testing. To this end we subjected the previously tested CUMS and control groups to the same behavioural paradigms a second time after two weeks of recovery; these groups will be referred to as double-tested. Additionally, additional CUMS and control groups were incorporated during the second test moment; these animals had been housed regularly for a period of four weeks after the CUMS exposure period. These groups will be referred to as single late-tested.

3.2.1. Long-term disturbance of circadian rhythm in both double-tested and single late-tested CUMS animals

Circadian rhythm, assessed by means of locomotor activity, revealed a CUMS exposure x diurnal phase interaction effect ($F(1,63) = 18.900$, $p < 0.001$), independent of the number of test series (i.e., repeated testing) ($F(1,63) = 0.851$, $p = 0.360$). Also, no interaction between diurnal phase and number of test series was observed ($F(1,63) = 0.009$, $p = 0.923$) (Fig. 7). In the dark phase, a main effect of stress was detected ($F(1,64) = 7.395$, $p = 0.008$) where CUMS animals moved less than controls. In the light phase, a main effect of CUMS was also present ($F(1,63) = 16.159$, $p < 0.001$); here, the CUMS animals moved more than controls. These data suggest that CUMS exposure disrupts the locomotor circadian rhythm persistently, and that this effect is not influenced by repeated testing.

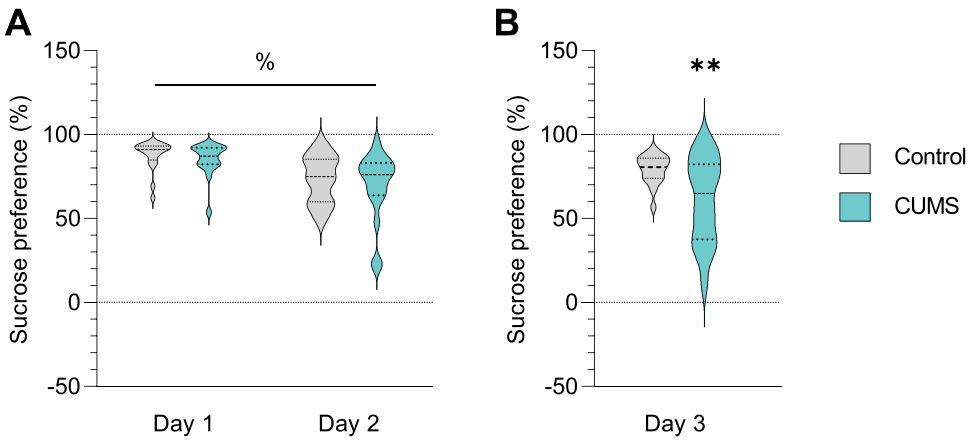

Fig. 3. Sucrose preference as measured over 3 consecutive days (n = 18). A. On days 1 and 2, sucrose solution contained 2% sucrose, for which no differences in preference were observed between groups. B. On day 3, the sucrose solution consisted of 1% sucrose, for which CUMS displayed reduced preference compared to control rats. %: $p < 0.05$, main effect of test day. * *: $p < 0.01$ compared to control group.

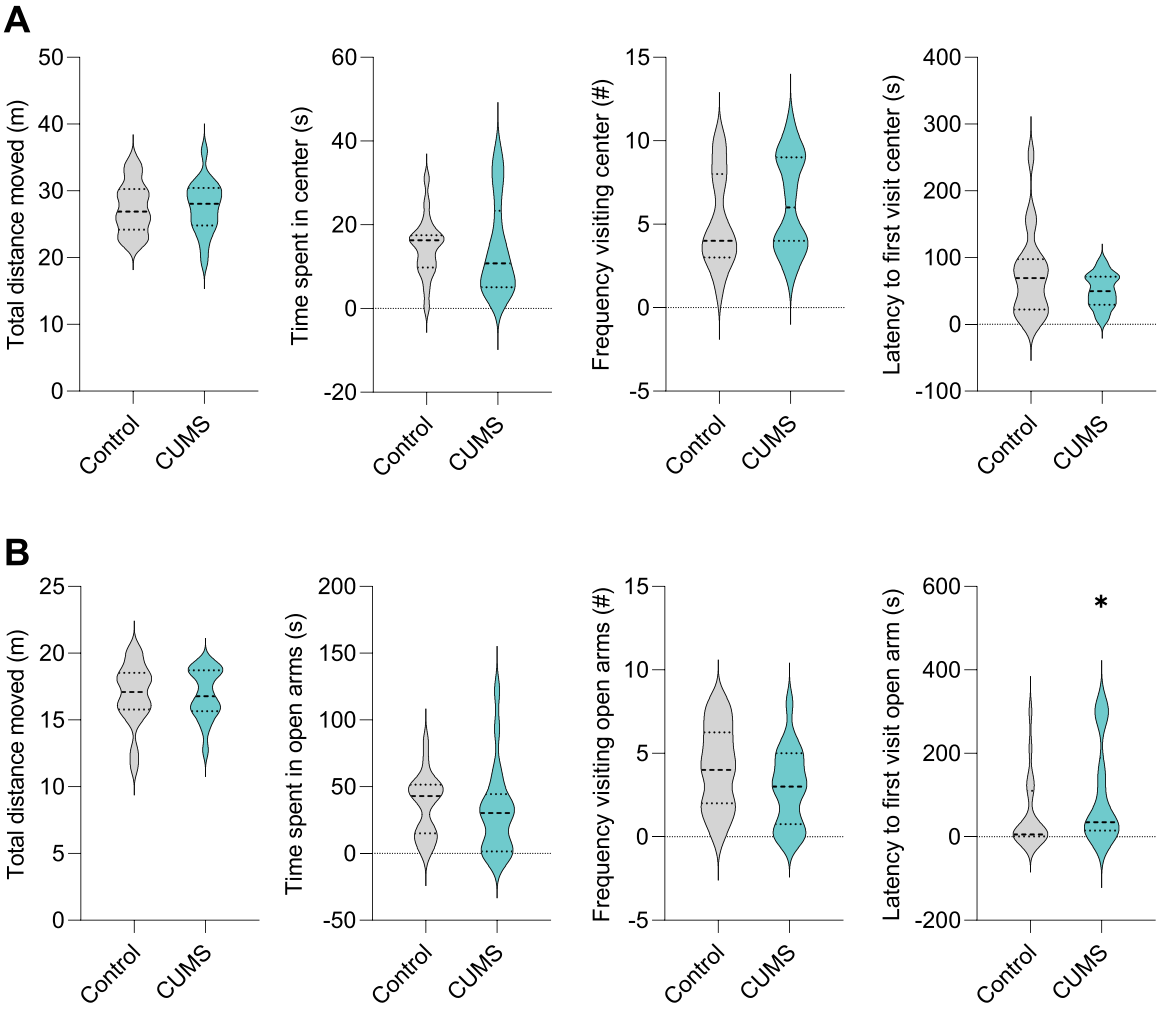

Fig. 4. Anxiety-like behaviour as measured in the open field and elevated plus maze (n = 18). A. Total distance moved, time spent in the centre area, frequency of visiting the centre area, and latency to first centre area visit in the open field were not affected by CUMS. B. Total distance moved, time spent in the open arms, frequency of visiting the open arms were not affected by CUMS, whereas CUMS exposed rats displayed a higher latency to first visit the open arms of the elevated plus maze. * : $p < 0.05$ compared to control group.

3.2.2. Sucrose preference was not persistently altered by CUMS nor depends on repeated testing

Sucrose preference decreased on day 2 compared to day 1 ($F(1,36) = 6.301$, $p = 0.017$), but did not display a main effect of CUMS ($F(1,36) = 0.828$, $p = 0.369$), repeated testing ($F(1,36) = 2.449$, $p = 0.126$), nor any interaction effect between these factors (all p's > 0.242) (Fig. 8).

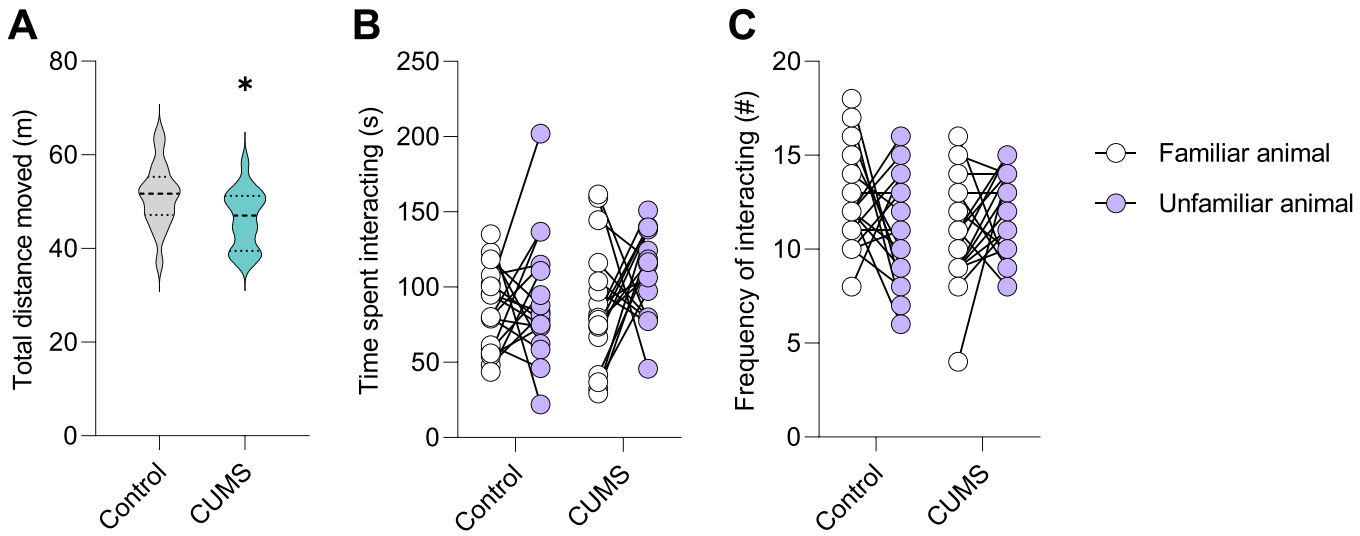

Fig. 5. Social behaviour as investigated in the social Y maze (n = 18). A. Total distance moved in the social Y maze was significantly lower in CUMS exposed rats compared to controls. B. Time spent actively interacting with a familiar and unfamiliar animal was not affected by CUMS. C. Frequency of interaction with a familiar and unfamiliar animal was not significantly different between groups. * : $p < 0.05$ compared to control group.

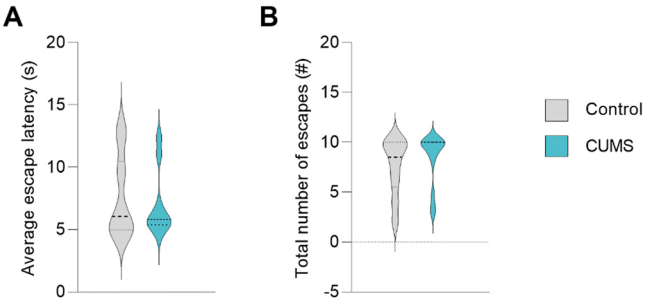

Fig. 6. Learned helplessness behaviour as indicated by escape latency and number of escapes (n = 18). A. The average escape latency over 10 trials was not significantly affected by CUMS. B. The total number of escapes across all trials was not affected by CUMS.

Overall, our results imply that CUMS exposure does not induce a persistent anhedonic phenotype, regardless of whether the animals were tested once or twice following CUMS.

3.2.3. Long-term effects of CUMS on anxiety-like behaviour are moderated by repeated testing

Persistent effects of CUMS on anxiety-like behaviour were significantly affected by the number of tests (Fig. 9A). When investigating the total distance moved in the open field, we observed an interaction effect between CUMS exposure and the number of test series to which the animals were subjected ($F(1,52) = 8.801$, $p = 0.005$). In case the animals were only tested once, i.e., only for the persistent consequences,

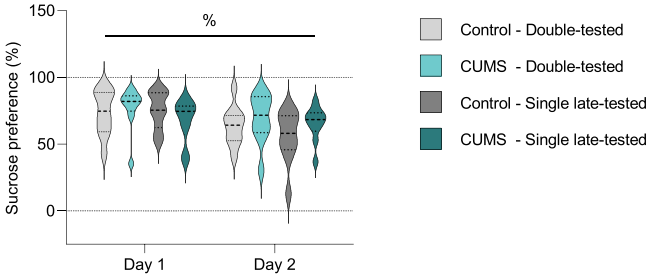

Fig. 8. Sucrose preference of a 1% sucrose solution over two days revealed a reduction over days and no effect of CUMS or repeated testing (n = 10). %: $p < 0.05$, main effect of test day.

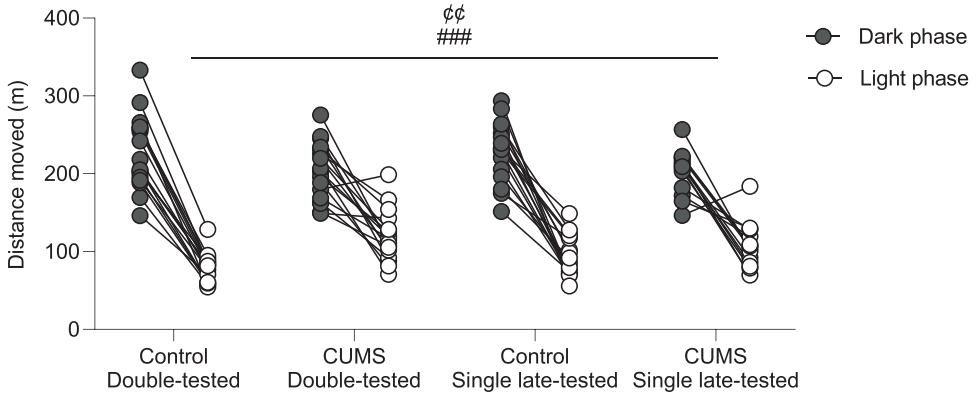

Fig. 7. Circadian rhythm as measured by distance moved in dark phase and light phase revealed that CUMS-exposed animals generally moved less during the dark phase, and more during the light phase when compared to control animals (n = 14–18). ###: $p < 0.001$, CUMS x phase interaction effect, ¢¢: $p < 0.01$, main effects of CUMS per phase.

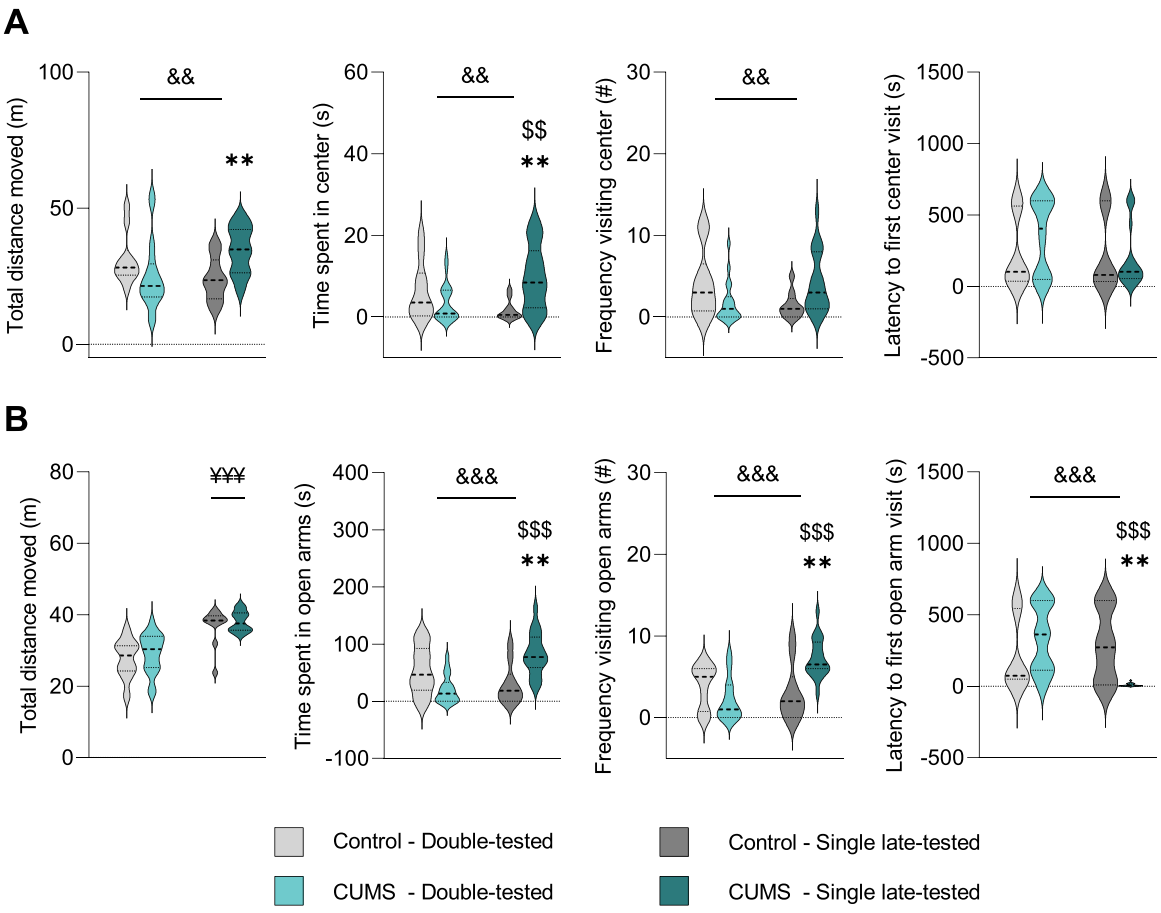

Fig. 9. Anxiety-like behaviour as measured in the open field and elevated plus maze (n = 10 for control groups, n = 18 for CUMS groups). A. CUMS x repeated testing interaction effects were found for total distance moved, time spent in the centre area, and frequency of visiting the centre area, but not for latency to first centre area visit in the open field. B. CUMS x repeated testing interaction effects were found for time spent in the open arms, frequency of visiting the open arms, and latency to first open arm visit in the elevated plus maze. A main effect of repeated testing was found for the total amount of distance moved. &&: $p < 0.01$, &&&: $p < 0.001$, CUMS x repeated testing interaction effect. ¥¥¥: $p < 0.001$, main effect of repeated testing. * *: $p < 0.01$ compared to respective control group. $$$: $p < 0.001$, $$: $p < 0.01$ compared to double-tested CUMS group.

CUMS resulted in a greater distance moved compared to control animals ($p = 0.004$). In contrast, CUMS animals that were repeatedly tested did not significantly differ from repeatedly tested controls ($p = 0.190$). Single late-tested CUMS animals also moved greater distance than double-tested CUMS rats ($p = 0.011$), whereas control groups did not differ significantly from one another ($p = 0.085$). When assessing time spent in the centre area, we found a similar CUMS x repeated testing interaction effect ($F(1,52) = 10.687$, $p = 0.002$). Here, single late-tested CUMS animals spent a significantly higher amount of time in the centre than both the single late-tested control group ($p = 0.001$) and the double-tested CUMS group ($p = 0.006$), whereas double-tested CUMS animals did not differ from the double-tested controls ($p = 0.196$). Control groups did not differ from one another in this readout either ($p = 0.070$). When assessing the frequency of visits to the centre area, we found another CUMS x repeated testing interaction effect ($F(1,52) = 8.062$, $p = 0.006$), again caused by a significant difference in the single late-tested CUMS group compared to the single late-tested control group ($p = 0.012$), and the double-tested CUMS group ($p = 0.032$), although the latter was not significant after applying the Bonferroni correction. Also here, the double-tested CUMS group did not significantly differ from the double-tested control group ($p = 0.084$), and both control groups did not differ from one another either ($p = 0.075$). The CUMS x repeated testing interaction effect was not found for the latency to the first centre area visit ($F(1,52) = 1.226$, $p = 0.273$), nor did we find main effects of CUMS ($F(1,52) = 0.514$, $p = 0.477$) or repeated testing ($F(1,52) = 1.430$, $p = 0.237$) on this parameter. Overall, the single late-tested CUMS group thus appeared less anxious than their corresponding control group; an effect that was not observed in animals that were subjected to repeated testing. Double-testing therefore seemed to increase anxiety in CUMS animals compared to single late-testing.

In the elevated plus maze (Fig. 9B), we observed similar CUMS x repeated testing interaction effects for the amount of time spent in the open arms ($F(1,52) = 20.739$, $p < 0.001$), the frequency of visits to the open arms ($F(1,52) = 15.535$, $p < 0.001$), and the latency to first visit either of the open arms ($F(1,52) = 12.697$, $p < 0.001$). The single late-tested CUMS animals spent more time in the open arms, showed a higher frequency of visits, and displayed a lower latency to visiting either of the open arms compared to the single late-tested control animals ($p = 0.001$, $p = 0.001$, $p = 0.006$, respectively) and the double-tested CUMS animals ($p < 0.001$, $p < 0.001$, $p = < 0.001$, respectively). For these three readouts, the double-tested CUMS group did not differ significantly from the double-tested control group ($p = 0.014$, $p = 0.116$, $p = 0.099$), and both control groups did not differ from one another ($p = 0.161$, $p = 0.522$, $p = 0.631$). For the total distance moved in the elevated plus maze, we did not observe a CUMS x repeated testing interaction effect ($F(1,52) = 0.010$, $p = 0.923$), nor a main effect of CUMS ($F(1,52) = 1.181$, $p = 0.282$), but we did observe a main effect of repeated testing ($F(1,52) = 47.084$, $p < 0.001$), with the single late-tested animals traveling a greater distance. Similar to the open field data, these results on the elevated plus maze indicate that CUMS reduced anxiety-like behaviour on the long-term when animals were tested once, but not when tested repeatedly. Double-testing moreover

increased anxiety-like behaviour in CUMS animals specifically, compared to single-testing.

3.2.4. General preference of unfamiliar animal over familiar animal for interaction time in the social Y maze

When assessing the total amount of distance moved in the social Y maze, we found no significant CUMS x repeated testing interaction effect ($F(1,68) = 3.308$, $p = 0.073$) (Fig. 10). Analyses of the interaction times with the familiar and unfamiliar animals revealed a general preference for the unfamiliar animal over the familiar animal ($F(1,67) = 7.113$, $p = 0.010$), but we did not find a main effect of CUMS ($F(1,67) = 0.339$, $p = 0.562$), repeated testing ($F(1,67) = 1.788$, $p = 0.186$) or interaction effects between these factors (all p's > 0.310). There was no main effect of animal familiarity on the frequency with which the animals interacted with their peers ($F(1,68) = 2.679$, $p = 0.106$), nor did we find main effects of CUMS ($F(1,68) = 1.937$, $p = 0.169$), repeated testing ($F(1,68) = 1.020$, $p = 0.316$), or any interaction effects (all p's > 0.576) of these three factors in this readout. Thus, CUMS did not seem to affect social behaviour and memory in a persistent manner, and repeated testing did not modulate this behaviour either.

3.2.5. Interaction effect of number of test series and escape behaviour over trials in learned helplessness task

In the learned helplessness task, we observed no significant effects of CUMS ($U = 600.500$, $p = 0.734$) or repeated testing ($U = 627.500$, $p = 0.977$) on the average escape latency (Fig. 11). We also did not observe any effects of CUMS ($U = 637.000$, 0.892) or repeated testing ($U = 637.500$, $p = 0.897$) on the number of escapes made by each animal. A repeated measures ANOVA over all groups combined did not reveal a significant effect of trial number ($F(7.143,471.469) = 1.563$, $p = 0.143$) or CUMS x trial number interaction ($F(7.143,471.469) = 0.923$, $p = 0.490$). However, we did observe an interaction effect between repeated testing and the number of trials ($F(7.143,471.469) = 3.015$, $p = 0.004$), in the absence of a CUMS x repeated testing x trial number interaction ($F(7.143,471.469) = 0.872$, $p = 0.530$) (Fig. S2). While there was no significant interaction effect between trial number and repeated testing ($F(5.891,194.413) = 1.678$, $p = 0.130$) for the control animals, we did observe such interaction for the CUMS animals ($F(9297) = 2.266$, $p = 0.018$). Neither single-tested nor double-tested CUMS groups showed a significant effect of trial number ($F(5.050,80.804) = 1.790$, $p = 0.124$, $F(4.704,79.963) = 2.062$, $p = 0.083$, respectively). CUMS groups did not differ significantly from one another during any trial (all p's > 0.055).

4. Discussion

In this study, we set out to study the effects of CUMS on a wide variety of behaviours resembling depressive-like symptoms immediately after the cessation of stress (acute effects), and after a recovery period (persistent effects). Moreover, we assessed whether the long-term effects of CUMS on these readouts were affected by prior assessment (repeated testing effects). Overall, CUMS induced acute changes in circadian rhythm and sucrose preference behaviours, inducing reduced amplitude of the circadian rhythm without affecting total activity and anhedonia, respectively. However, when assessing the effects of CUMS on the longer term, we mostly observed interaction effects between CUMS exposure and repeated testing in the readouts of anxiety-like behaviour. More specifically, single late-tested CUMS animals showed less anxiety-like

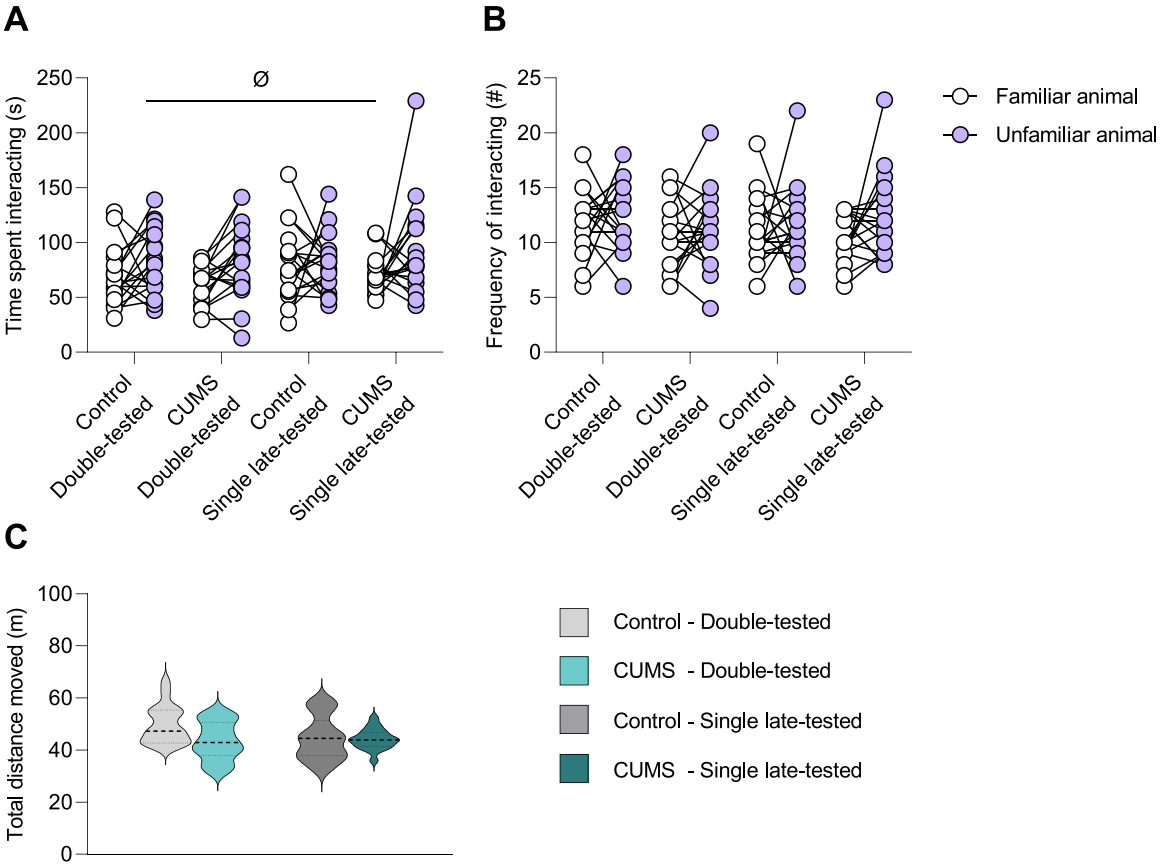

Fig. 10. Social behaviour as investigated in the social Y maze (n = 18). A. A main effect for familiarity of the animal was found for the time the animals spent interacting, in which the unknown animal was preferred. B. No main or interaction effects of CUMS and repeated testing were found for the frequency of interactions with known or unknown animals. C. No main or interaction effects of CUMS and repeated testing were found for total distance moved during the test trial. Ø: $p < 0.05$, main effect of familiarity animal.

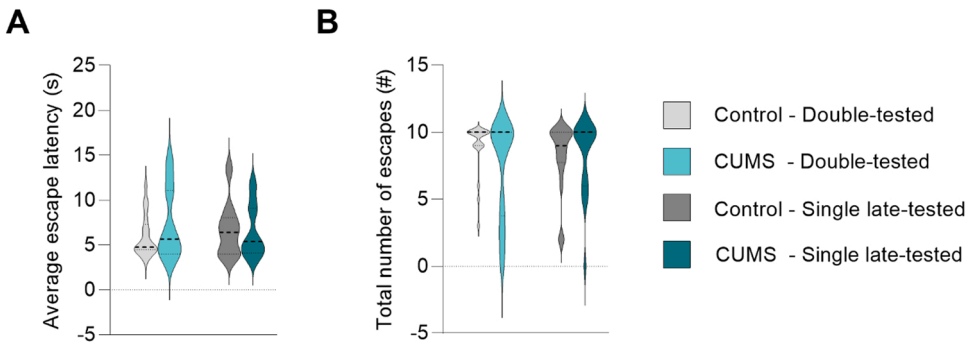

Fig. 11. Learned helplessness behaviour as indicated by escape latency per trial (n = 18). A. The average escape latency over 10 trials was not significantly affected by CUMS or repeated testing. B. The total number of escapes across all trials was also not affected by CUMS or repeated testing.

behaviour, which we did not observe when we subjected CUMS animals to behavioural testing repeatedly.

Currently, CUMS is the most widely applied and dependable model of depression used in rodents [14]. While previous animal models of depression included either surgical procedures or exposure to acutely stressful situations [29–31], chronic stress approaches seem to be more effective in inducing behavioural alterations similar to those observed in patients, such as anhedonia [32] and anxiety [33], but also changes in brain structure and synaptic function [34–36]. Additionally, chronic stress paradigms possess greater etiological validity, as they more closely resemble continuous daily life stress exposure in humans [37].

It is worth noting that studies incorporating chronic mild stress usually showcase the effects of these stress paradigms on late-adolescent to young-adult rodents, contrary to this study in fully adult animals. When directly comparing animals across ages, it has been shown that chronic variable stress can cause adult animals to present increased immobility in the forced swim test compared to adolescent animals, while the adolescent animals were more susceptible to somatic, HPA axis, and neuropeptide-related effects of chronic stress [38]. Moreover, 30 days of adult-onset (at postnatal day 60) unpredictable chronic stress has been shown to induce impairments in avoidance learning and memory in adulthood, whereas 60 days of juvenile-onset (at postnatal day 30) stress exposure failed to do so [39]. Therefore, it is important to keep in mind that age can influence the manner in which stress exposure affects organisms, including rats.

Although the rodent CUMS model is primarily known for its anhedonia-inducing potential, depression consists of a wide array of behavioural symptoms. In human subjects, one of the symptoms of depression encompasses sleep disturbances. Insomnia and sleep quality have been shown to be bidirectionally related to depression [40], and comorbidity of depression and insomnia has been associated with poorer outcomes for both conditions [41]. The circadian amplitude of several physiological readouts, e.g. body temperature, norepinephrine, thyroid-stimulating hormone, and melatonin levels, is blunted in depressed patients [42]. Moreover, altered expression patterns of canonical clock genes can be observed in postmortem gene expression analyses in the brains of MDD patients [43]. Here, in rats we observed acute effects of CUMS on locomotor activity during both dark and light phases, resulting in a lower circadian activity amplitude. These observations are in line with prior reports in mice, in which four weeks of CUMS exposure were shown to reduce the circadian amplitude of activity and body temperature, the severity of which correlated directly with depressive-like behaviour in the open field and forced swim test [20]. Whereas the decreased dark phase activity was persistent even when testing 3 weeks after CUMS exposure, light phase activity was increased acutely only. Prior work in rats exposed to CUMS also showed acutely decreased dark phase activity, yet light phase activity was unaffected [7]. Our data showed that alterations in circadian activity patterns can be found in double-tested as well as the single-late tested stress groups compared to their corresponding controls. Differences with prior literature could possibly relate to differential inclusion of exposure to a reversed 12:12 light/dark schedule within the CUMS protocol. However, we verified that acute circadian rhythm readouts did not directly correlate with the timing of this reversed cycle during the CUMS exposure across animals. Moreover, abnormalities remained present even at 4 weeks post CUMS exposure, suggesting that these alterations in rhythm are not mere washout effects of this stressor, but rather a persistent trait induced by CUMS. Repeated testing did not seem to have any influence on the circadian rhythm of locomotor activity during the second test series, indicating no habituation effects of the double-tested animals compared to the single late-tested animals. These data suggest that two weeks of CUMS exposure is sufficient for inducing long-lasting disruptions of the circadian locomotor rhythm.

As evaluation of the circadian locomotor rhythm was deemed the least stressful, this was the first assessment of each test series. The sucrose preference paradigm followed, as the two-bottle habituation could be performed during the circadian rhythm assessment. After that, open field, elevated plus maze, social Y maze, and learned helplessness tasks were performed in that order. By ordering the tasks from least to most stressful, we attempted to prevent stress effects of done tasks from affecting the following tasks.

Symptoms of anhedonia, the reduced ability to experience pleasure, are another core component of depressive-like behaviour. Studies have shown that the presence of anhedonia can be a predictor of poor treatment response in patients with MDD [44–46], whereas alleviation of anhedonia predicts improvement of functioning in depressed patients [47,48]. In rodents, assessing anhedonia by means of sucrose consumption preference has been the most robust and well-established readout of depressive-like behaviour induced by CUMS exposure [6]. Typically, either a 2% or 1% sucrose solution is used in this paradigm. Our CUMS animals displayed an acutely decreased preference for a 1%, but not a 2%, sucrose solution immediately after stress cessation. However, when assessed several weeks later using a 1% sucrose solution, no effects of CUMS were found whatsoever. While reductions in preference can generally already be observed after two weeks of CUMS exposure [32], data on the persistence of these effects are inconclusive. Some studies have reported on preference deficits continuing up to four to six weeks after stress cessation [32,49]. However, these studies also applied CUMS exposure for a prolonged duration of four to nine weeks, suggesting that longer CUMS exposure is potentially necessary to produce persistent anhedonic effects. Additionally, repeated testing did not seem to affect sucrose consumption in the second test series. Our data indicate that two weeks of CUMS exposure is sufficient to acutely impair sucrose preference, yet these effects are not persistent.

Several large studies have shown that depressive and anxious disorders often occur simultaneously, which is associated with lower chances of remission [50], and with anxiety preceding depression in most cases [51]. Accordingly, we also assessed symptoms of anxiety following CUMS. We observed no indications of heightened anxiety after CUMS exposure, either closely after its cessation or the recovery period.

While increases in anxiety-like behaviour following CUMS exposure have been reported before, this often involved stress durations of 5 weeks or more [52,53]. It is possible that longer exposure to CUMS is necessary to induce anxiety-like behaviour. It is also possible that the age of our animals played a role here. In adult Wistar rats, the absence of chronic mild stress effects in the elevated plus maze has been reported more often [54], even when tested acutely after 56 days of chronic mild stress exposure [55]. The double-tested CUMS animals did display more anxious behaviour than the single late-tested CUMS group; possibly the combination of CUMS and subjection to the first series of behavioural tests, especially the learned helplessness task, made the animals more anxious. Strikingly, the single late-tested CUMS group was less anxious than their control group 4 weeks post-stress cessation, but this was not observed in the groups tested immediately after CUMS exposure as well. The fact that our single-late tested CUMS animals present less anxiety-like behaviour compared to controls might suggest that the previous stress exposure has rendered them stress-resilient to a certain degree. In previous studies, anxiolytic effects have been observed more often after exposure to chronic mild stress [56,57], which have been interpreted as a blunting of the emotional response to the environment. Exposure to mild stress has previously been shown to induce resilience to stressful events in humans and animals alike [58–60]; a phenomenon referred to as stress inoculation. It was previously thought that stress exposure needed to take place in early life for these protective effects to occur, but more recently it was found that stress inoculation is not necessarily linked to the most critical or sensitive periods in development [61,62], and can also occur in adulthood. Our data suggest that the 4-week post-CUMS recovery period provided the rats the opportunity to recover and adapt; this could imply that a recovery period after stress exposure might be a determinative factor for successful stress inoculation.

Overall, our data suggest that two weeks of CUMS exposure induces neither acute nor long-term anxiogenic effects. Moreover, when followed up by a sufficiently long, undisturbed recovery period, anxiolytic effects might even be observed.

Many psychiatric disorders, including depression, display impaired social behaviours [63,64]. Studies have shown that social anhedonia could be present in up to one third of patients with depression [65], and correlates with poor treatment response and symptom severity [66,67]. Social anhedonia has even been suggested as endophenotype of depression [68].

Reduced social behaviour has also been observed in rodent models for depression [69,70]. However, in our study, social behaviour and memory in the social Y maze appeared unaffected by CUMS exposure. Prolonged social isolation as often implemented in prior literature [71] likely facilitates the gradual stress-induced impairment of social behaviour [72]. Therefore, it is possible that our paired housing interfered in the development of these effects in our animals. Additionally, contrary to findings in literature, our CUMS animals did not display preference for the familiar over unfamiliar animals in either test series. Importantly, the CUMS animals were stressed while housed with their cage mates that served as the familiar animals in the social Y maze, which might have reduced their preference for the familiar animal. Moreover, incorporating cage mates as familiar animals obviated the need for separate sampling and testing phases (as are often implemented), which makes it more difficult to compare our results to other studies. Overall, it seems that our CUMS model does not induce social impairment, at least not in the aspect of social behaviour that we assessed in our paradigm. It is possible that other types of social behaviour (such as social interaction) might be affected. Additional inclusion of individual housing could possibly promote the development of impaired social behaviour.

Lastly, we assessed learned helplessness behaviour; an individual's belief that one cannot cope with adverse circumstances [28,73], a feeling that typically develops after repeated exposure to uncontrollable adverse events. In rodents, the learned helplessness paradigm is often used to induce a depressive-like phenotype by means of exposure to inescapable foot shocks [31,74]. However, the assessment of escape behaviour after exposure to unescapable shocks can also reveal differences in susceptibility to the display of learned helplessness. We adapted a protocol which has been applied successfully to this end in our lab [19]. We observed neither acute nor long-term effects of CUMS on escape latencies. Moreover, both CUMS and control groups did not improve their escape behaviour across test trials. Potentially, the animals learned that shuttling between chambers did not prevent shocks during training. In line with this idea, we did observe alternative active escape behaviours (e.g. attempting to exit through the lid through which they entered, balancing on one paw against the wall to avoid shocks). These behaviours were however rather variable, and difficult to quantify, refraining us from drawing conclusions based on these observations. Future work should improve reliability of the assessment potentially by excluding the shuttling possibility during training, and instead implement more clearly different contexts for training and test sessions.

By including assessments of circadian rhythm, anhedonia, anxiety-like behaviour, sociability, and learned helplessness, and not simply one or two components of depressive-like behaviour, we set out to gain a broad, complete insight into the depressive-like phenotype resulting from CUMS exposure as well as its persistent nature. Moreover, we aimed at providing novel insights into the interactions between CUMS and repeated testing. Oftentimes when CUMS is applied in combination with another condition, e.g. a treatment, it is not verified whether stress effects are actually present before treatment initiation to prevent potential confounding effects of repeated testing. Here, we specifically investigated whether repeated testing could be considered in such cases. With regard to our prior expectations, indeed, we found that CUMS exposure caused our animals to display various components of depressive-like behaviour. However, the longevity of these effects varied per component; we observed circadian activity pattern disruptions both immediately after stress exposure and after a rest period, while anhedonia as indicated by a reduced sucrose preference was only present immediately after stress exposure. While we expected some degree of habituation of the double-tested animals, manifested as less anxious behaviour in the open field and elevated plus maze tests, we actually observed that the CUMS-exposed single late-tested animals were the least anxious. This is thought to be due to a phenomenon referred to as stress inoculation, a process in which a recovery period might seemingly be crucial.

Some limitations to the work should be mentioned. As the current study also piloted a larger project, test parameters needed to be slightly adjusted between the two time points of test series, and also across animals within the second series. Therefore, data obtained at the different time points cannot be compared directly, and some animals needed to be excluded from the analysis to ensure consistency in parameters within test series. Due to the original purpose of this study, another critical limitation consists of the fact that only male animals were used. As depression is known to be more prevalent in women than in men [75], it is highly important that it is also modeled in female animals so that sex differences can be investigated. While a sex-balanced investigation was beyond the scope of this validation study, we strongly emphasize the importance of this inclusion in future studies. As opposed to the majority of previous studies, we here opted for CUMS exposure of relatively short duration, which ceased during testing, as this facilitated compatibility with later planned treatment studies. Whereas this short period of CUMS exposure has been shown effective in inducing behavioural changes before [32,76], this means that our results cannot be readily extrapolated to CUMS paradigms of longer durations. Finally, the inclusion of 5 different behavioural tests, of which some quite stressful (e.g. the learned helplessness task), complicates the interpretation of the results slightly; it is difficult to know whether observed differences between single- and double-tested animals stem from learning effects or stress effects as a result from test exposure.

5. Conclusions

Taken together, our data indicate that behavioural effects of CUMS exposure are not only dependent on the timing of the assessment after stress cessation, but also on previous exposure to the behavioural paradigms. Moreover, different components of depressive-like behaviour seem to be affected differentially by these factors. Future work implementing CUMS in the investigation of treatments for depression should carefully consider these aspects when developing an experimental design.

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Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2022.113960.

References

[1] H. Li, S. Lin, T. Qin, H. Li, Z. Ma, S. Ma, Senegenin exerts anti-depression effect in mice induced by chronic un-predictable mild stress via inhibition of NF-kappaB regulating NLRP3 signal pathway, Int. Immunopharmacol. 53 (2017) 24–32, https://doi.org/10.1016/j.intimp.2017.10.001.

[2] G.S. Malhi, J.J. Mann, Depression, Lancet 392 (2018) 2299–2312, https://doi.org/10.1016/S0140-6736(18)31948-2.

[3] N.B. Bosaipo, M.P. Foss, A.H. Young, M.F. Juruena, Neuropsychological changes in melancholic and atypical depression: a systematic review, Neurosci. Biobehav. Rev. 73 (2017) 309–325, https://doi.org/10.1016/j.neubiorev.2016.12.014.

[4] S.M. Monroe, G.M. Slavich, I.H. Gotlib, Life stress and family history for depression: the moderating role of past depressive episodes, J. Psychiatr. Res. 49 (2014) 90–95, https://doi.org/10.1016/j.jpsychires.2013.11.005.

[5] V. Krishnan, E.J. Nestler, Animal models of depression: molecular perspectives, Curr. Top. Behav. Neurosci. 7 (2011) 121–147, https://doi.org/10.1007/7854_2010_108.

[6] P. Willner, The chronic mild stress (CMS) model of depression: history, evaluation and usage, Neurobiol. Stress 6 (2017) 78–93, https://doi.org/10.1016/j.ynstr.2016.08.002.

[7] Z. Gorka, E. Moryl, M. Papp, Effect of chronic mild stress on circadian rhythms in the locomotor activity in rats, Pharmacol. Biochem. Behav. 54 (1996) 229–234, https://doi.org/10.1016/0091-3057(95)02173-6.

[8] F. Calabrese, E. Savino, M. Papp, R. Molteni, M.A. Riva, Chronic mild stress-induced alterations of clock gene expression in rat prefrontal cortex: modulatory effects of prolonged lurasidone treatment, Pharmacol. Res. 104 (2016) 140–150, https://doi.org/10.1016/j.phrs.2015.12.023.

[9] F. Calabrese, P. Brivio, G. Sbrini, P. Gruca, M. Lason, E. Litwa, M. Niemczyk, M. Papp, M.A. Riva, Effect of lurasidone treatment on chronic mild stress-induced behavioural deficits in male rats: The potential role for glucocorticoid receptor signalling, J. Psychopharmacol. 34 (2020) 420–428, https://doi.org/10.1177/0269881119895547.

[10] Y.L. Wang, Q.Q. Han, W.Q. Gong, D.H. Pan, L.Z. Wang, W. Hu, M. Yang, B. Li, J. Yu, Q. Liu, Microglial activation mediates chronic mild stress-induced depressive- and anxiety-like behavior in adult rats, J. Neuroinflamm. 15 (2018) 21, https://doi.org/10.1186/s12974-018-1054-3.

[11] R.J. Katz, S. Hersh, Amitriptyline and scopolamine in an animal model of depression, Neurosci. Biobehav. Rev. 5 (1981) 265–271, https://doi.org/10.1016/0149-7634(81)90008-7.

[12] R.J. Katz, G. Baldrighi, A further parametric study of imipramine in an animal model of depression, Pharmacol. Biochem. Behav. 16 (1982) 969–972, https://doi.org/10.1016/0091-3057(82)90054-5.

[13] J. Sun, F. Wang, G. Hong, M. Pang, H. Xu, H. Li, F. Tian, R. Fang, Y. Yao, J. Liu, Antidepressant-like effects of sodium butyrate and its possible mechanisms of action in mice exposed to chronic unpredictable mild stress, Neurosci. Lett. 618 (2016) 159–166, https://doi.org/10.1016/j.neulet.2016.03.003.

[14] S. Antoniuk, M. Bijata, E. Ponimaskin, J. Wlodarczyk, Chronic unpredictable mild stress for modeling depression in rodents: meta-analysis of model reliability, Neurosci. Biobehav. Rev. 99 (2019) 101–116, https://doi.org/10.1016/j.neubiorev.2018.12.002.

[15] L. Prut, C. Belzung, The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review, Eur. J. Pharmacol. 463 (2003) 3–33, https://doi.org/10.1016/s0014-2999(03)01272-x.

[16] M.P. Leussis, V.J. Bolivar, Habituation in rodents: a review of behavior, neurobiology, and genetics, Neurosci. Biobehav Rev. 30 (2006) 1045–1064, https://doi.org/10.1016/j.neubiorev.2006.03.006.

[17] V. Voikar, E. Vasar, H. Rauvala, Behavioral alterations induced by repeated testing in C57BL/6J and 129S2/Sv mice: implications for phenotyping screens, Genes Brain Behav. 3 (2004) 27–38, https://doi.org/10.1046/j.1601-183x.2003.0044.x.

[18] J.D. Olivier, M.G. Van Der Hart, R.P. Van Swelm, P.J. Dederen, J.R. Homberg, T. Cremers, P.M. Deen, E. Cuppen, A.R. Cools, B.A. Ellenbroek, A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders, Neuroscience 152 (2008) 573–584, https://doi.org/10.1016/j.neuroscience.2007.12.032.

[19] R.H. van der Doelen, T. Kozicz, J.R. Homberg, Adaptive fitness; early life adversity improves adult stress coping in heterozygous serotonin transporter knockout rats, Mol. Psychiatry 18 (2013) 1244–1245, https://doi.org/10.1038/mp.2012.186.

[20] R.W. Logan, N. Edgar, A.G. Gillman, D. Hoffman, X. Zhu, C.A. McClung, Chronic stress induces brain region-specific alterations of molecular rhythms that correlate with depression-like behavior in mice, Biol. Psychiatry 78 (2015) 249–258, https://doi.org/10.1016/j.biopsych.2015.01.011.

[21] W.S. Lai, R.E. Johnston, Individual recognition after fighting by golden hamsters: a new method, Physiol. Behav. 76 (2002) 225–239, https://doi.org/10.1016/s0031-9384(02)00721-7.

[22] C.M. Hoyos, C. Gordon, Z. Terpening, L. Norrie, S.J.G. Lewis, I.B. Hickie, S. L. Naismith, Circadian rhythm and sleep alterations in older people with lifetime depression: a case-control study, BMC Psychiatry 20 (2020) 192, https://doi.org/10.1186/s12888-020-02606-z.

[23] M.E. Rumble, W.V. McCall, D.A. Dickson, A.D. Krystal, P.B. Rosenquist, R. M. Benca, An exploratory analysis of the association of circadian rhythm dysregulation and insomnia with suicidal ideation over the course of treatment in individuals with depression, insomnia, and suicidal ideation, J. Clin. Sleep. Med. 16 (2020) 1311–1319, https://doi.org/10.5664/jcsm.8508.

[24] A. Borsini, A.S.J. Wallis, P. Zunszain, C.M. Pariante, M.J. Kempton, Characterizing anhedonia: a systematic review of neuroimaging across the subtypes of reward processing deficits in depression, Cogn. Affect Behav. Neurosci. 20 (2020) 816–841, https://doi.org/10.3758/s13415-020-00804-6.

[25] G.K. Khazanov, C. Xu, B.D. Dunn, Z.D. Cohen, R.J. DeRubeis, S.D. Hollon, Distress and anhedonia as predictors of depression treatment outcome: a secondary analysis of a randomized clinical trial, Behav. Res. Ther. 125 (2020), 103507, https://doi.org/10.1016/j.brat.2019.103507.

[26] J.W. Tiller, Depression and anxiety, Med. J. Aust. 199 (2013) S28–31, https://doi.org/10.5694/mja12.10628.

[27] J. Bralten, N.R. Mota, C. Klemann, W. De Witte, E. Laing, D.A. Collier, H. de Kluiver, S. Bauduin, C. Arango, J.L. Ayuso-Mateos, et al., Genetic underpinnings of sociability in the general population, Neuropsychopharmacology 46 (2021) 1627–1634, https://doi.org/10.1038/s41386-021-01044-z.

[28] I.A. Trindade, A.L. Mendes, N.B. Ferreira, The moderating effect of psychological flexibility on the link between learned helplessness and depression symptomatology: a preliminary study, J. Context. Behav. Sci. 15 (2020) 68–72, https://doi.org/10.1016/j.jcbs.2019.12.001.

[29] K.D. Cairncross, B. Cox, C. Forster, A.F. Wren, A new model for the detection of antidepressant drugs: Olfactory bulbectomy in the rat compared with existing models, J. Pharmacol. Methods 1 (1978) 131–143, https://doi.org/10.1016/0160-5402(78)90018-9.

[30] R.D. Porsolt, M. Le Pichon, M. Jalfre, Depression: a new animal model sensitive to antidepressant treatments, Nature 266 (1977) 730–732, https://doi.org/10.1038/266730a0.

[31] J.B. Overmier, M.E. Seligman, Effects of inescapable shock upon subsequent escape and avoidance responding, J. Comp. Physiol. Psychol. 63 (1967) 28–33, https://doi.org/10.1037/h0024166.

[32] P. Willner, A. Towell, D. Sampson, S. Sophokleous, R. Muscat, Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant, Psychopharmacology 93 (1987) 358–364, https://doi.org/10.1007/BF00187257.

[33] R.J. Katz, K.A. Roth, B.J. Carroll, Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression, Neurosci. Biobehav. Rev. 5 (1981) 247–251, https://doi.org/10.1016/0149-7634(81)90005-1.

[34] W.N. Marsden, Synaptic plasticity in depression: molecular, cellular and functional correlates, Prog. Neuro Psychopharmacol. Biol. Psychiatry 43 (2013) 168–184, https://doi.org/10.1016/j.pnpbp.2012.12.012.

[35] A.C. Pereira, M.C. Carvalho, C.M. Padovan, Both serotonergic and noradrenergic systems modulate the development of tolerance to chronic stress in rats with lesions of the serotonergic neurons of the median raphe nucleus, Behav. Brain Res. 357–358 (2019) 39–47, https://doi.org/10.1016/j.bbr.2017.06.037.

[36] T.J. Schoenfeld, H.C. McCausland, H.D. Morris, V. Padmanaban, H.A. Cameron, Stress and loss of adult neurogenesis differentially reduce hippocampal volume, Biol. Psychiatry 82 (2017) 914–923, https://doi.org/10.1016/j.biopsych.2017.05.013.

[37] B. Czéh, E. Fuchs, O. Wiborg, M. Simon, Animal models of major depression and their clinical implications, Prog. Neuro Psychopharmacol. Biol. Psychiatry 64 (2016) 293–310, https://doi.org/10.1016/j.pnpbp.2015.04.004.
[38] R. Jankord, M.B. Solomon, J. Albertz, J.N. Flak, R. Zhang, J.P. Herman, Stress vulnerability during adolescent development in rats, Endocrinology 152 (2011) 629–638, https://doi.org/10.1210/en.2010-0658.
[39] T. Ricon, E. Toth, M. Leshem, K. Braun, G. Richter-Levin, Unpredictable chronic stress in juvenile or adult rats has opposite effects, respectively, promoting and impairing resilience, Stress 15 (2012) 11–20, https://doi.org/10.3109/10253890.2011.572207.
[40] H. Fang, S. Tu, J. Sheng, A. Shao, Depression in sleep disturbance: a review on a bidirectional relationship, mechanisms and treatment, J. Cell Mol. Med. 23 (2019) 2324–2332, https://doi.org/10.1111/jcmm.14170.
[41] B. Bei, L.D. Asarnow, A. Krystal, J.D. Edinger, D.J. Buysse, R. Manber, Treating insomnia in depression: Insomnia related factors predict long-term depression trajectories, J. Consult. Clin. Psychol. 86 (2018) 282–293, https://doi.org/10.1037/ccp0000282.
[42] E. Souetre, E. Salvati, J.L. Belugou, D. Pringuey, M. Candito, B. Krebs, J. L. Ardisson, G. Darcourt, Circadian rhythms in depression and recovery: evidence for blunted amplitude as the main chronobiological abnormality, Psychiatry Res. 28 (1989) 263–278, https://doi.org/10.1016/0165-1781(89)90207-2.
[43] J.Z. Li, B.G. Bunney, F. Meng, M.H. Hagenauer, D.M. Walsh, M.P. Vawter, S. J. Evans, P.V. Choudary, P. Cartagena, J.D. Barchas, et al., Circadian patterns of gene expression in the human brain and disruption in major depressive disorder, Proc. Natl. Acad. Sci. USA 110 (2013) 9950–9955, https://doi.org/10.1073/pnas.1305814110.
[44] D.L. McMakin, T.M. Olino, G. Porta, L.J. Dietz, G. Emslie, G. Clarke, K.D. Wagner, J.R. Asarnow, N.D. Ryan, B. Birmaher, et al., Anhedonia predicts poorer recovery among youth with selective serotonin reuptake inhibitor treatment-resistant depression, J. Am. Acad. Child Adolesc. Psychiatry 51 (2012) 404–411, https://doi.org/10.1016/j.jaac.2012.01.011.
[45] J. Spijker, R.V. Bijl, R. de Graaf, W.A. Nolen, Determinants of poor 1-year outcome of DSM-III-R major depression in the general population: results of the Netherlands Mental Health Survey and Incidence Study (NEMESIS), Acta Psychiatr. Scand. 103 (2001) 122–130, https://doi.org/10.1034/j.1600-0447.2001.103002122.x.
[46] E. Vrieze, K. Demyttenaere, R. Bruffaerts, D. Hermans, D.A. Pizzagalli, P. Sienaert, T. Hompes, P. de Boer, M. Schmidt, S. Claes, Dimensions in major depressive disorder and their relevance for treatment outcome, J. Affect. Disord. 155 (2014) 35–41, https://doi.org/10.1016/j.jad.2013.10.020.
[47] P. Llorca, D. Gourion, Management of anhedonia and depressive symptoms in depressed outpatients: benefit for functioning, Eur. Psychiatry 30 (2015), https://doi.org/10.1016/S0924-9338(15)31881-2.
[48] F. Vinckier, D. Gourion, S. Mouchabac, Anhedonia predicts poor psychosocial functioning: Results from a large cohort of patients treated for major depressive disorder by general practitioners, Eur. Psychiatry 44 (2017) 1–8, https://doi.org/10.1016/j.eurpsy.2017.02.485.
[49] E. Toth, R. Gersner, A. Wilf-Yarkoni, H. Raizel, D.E. Dar, G. Richter-Levin, O. Levit, A. Zangen, Age-dependent effects of chronic stress on brain plasticity and depressive behavior, J. Neurochem. 107 (2008) 522–532, https://doi.org/10.1111/j.1471-4159.2008.05642.x.
[50] M. Fava, A.J. Rush, J.E. Alpert, G.K. Balasubramani, S.R. Wisniewski, C.N. Carmin, M.M. Biggs, S. Zisook, A. Leuchter, R. Howland, et al., Difference in treatment outcome in outpatients with anxious versus nonanxious depression: a STAR*D report, Am. J. Psychiatry 165 (2008) 342–351, https://doi.org/10.1176/appi.ajp.2007.06111868.
[51] F. Lamers, P. van Oppen, H.C. Comijs, J.H. Smit, P. Spinhoven, A.J. van Balkom, W. A. Nolen, F.G. Zitman, A.T. Beekman, B.W. Penninx, Comorbidity patterns of anxiety and depressive disorders in a large cohort study: the Netherlands Study of Depression and Anxiety (NESDA), J. Clin. Psychiatry 72 (2011) 341–348, https://doi.org/10.4088/JCP.10m06176blu.
[52] X. Sun, T. Zhang, Y. Zhao, E. Cai, H. Zhu, S. Liu, Panaxynol attenuates CUMS-induced anxiety and depressive-like behaviors via regulating neurotransmitters, synapses and the HPA axis in mice, Food Funct. 11 (2020) 1235–1244, https://doi.org/10.1039/c9fo03104a.
[53] H.J. Huang, X.C. Zhu, Q.Q. Han, Y.L. Wang, N. Yue, J. Wang, R. Yu, B. Li, G.C. Wu, Q. Liu, et al., Ghrelin alleviates anxiety- and depression-like behaviors induced by chronic unpredictable mild stress in rodents, Behav. Brain Res. 326 (2017) 33–43, https://doi.org/10.1016/j.bbr.2017.02.040.
[54] H.P. Jose Jaime, B.C. Venus, J.R. Graciela, H.H. Tania, M.M. Lucia, Young-adult male rats' vulnerability to chronic mild stress is reflected by anxious-like instead of depressive-like behaviors, Neurosci. J. 2016 (2016), 5317242, https://doi.org/10.1155/2016/5317242.
[55] A. Karson, T. Demirtas, D. Bayramgurler, F. Balci, T. Utkan, Chronic administration of infliximab (TNF-alpha inhibitor) decreases depression and anxiety-like behaviour in rat model of chronic mild stress, Basic Clin. Pharmacol. Toxicol. 112 (2013) 335–340, https://doi.org/10.1111/bcpt.12037.
[56] H. Kompagne, G. Bardos, G. Szenasi, I. Gacsalyi, L.G. Harsing, G. Levay, Chronic mild stress generates clear depressive but ambiguous anxiety-like behaviour in rats, Behav. Brain Res. 193 (2008) 311–314, https://doi.org/10.1016/j.bbr.2008.06.008.
[57] P.S. D'Aquila, P. Brain, P. Willner, Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression, Physiol. Behav. 56 (1994) 861–867, https://doi.org/10.1016/0031-9384(94)90316-6.
[58] N. Garmezy, A.S. Masten, A. Tellegen, The study of stress and competence in children: a building block for developmental psychopathology, Child Dev. 55 (1984) 97–111.
[59] M. Katz, C. Liu, M. Schaer, K.J. Parker, M.C. Ottet, A. Epps, C.L. Buckmaster, R. Bammer, M.E. Moseley, A.F. Schatzberg, et al., Prefrontal plasticity and stress inoculation-induced resilience, Dev. Neurosci. 31 (2009) 293–299, https://doi.org/10.1159/000216540.
[60] D.M. Lyons, K.J. Parker, A.F. Schatzberg, Animal models of early life stress: Implications for understanding resilience, Dev. Psychobiol. 52 (2010) 402–410, https://doi.org/10.1002/dev.20429.
[61] A.G. Lee, C.L. Buckmaster, E. Yi, A.F. Schatzberg, D.M. Lyons, Coping and glucocorticoid receptor regulation by stress inoculation, Psychoneuroendocrinology 49 (2014) 272–279, https://doi.org/10.1016/j.psyneuen.2014.07.020.
[62] J. Brockhurst, C. Cheleuitte-Nieves, C.L. Buckmaster, A.F. Schatzberg, D.M. Lyons, Stress inoculation modeled in mice, Transl. Psychiatry 5 (2015), e537, https://doi.org/10.1038/tp.2015.34.
[63] A. Kupferberg, L. Bicks, G. Hasler, Social functioning in major depressive disorder, Neurosci. Biobehav. Rev. 69 (2016) 313–332, https://doi.org/10.1016/j.neubiorev.2016.07.002.
[64] E. Barkus, J.C. Badcock, A transdiagnostic perspective on social anhedonia, Front Psychiatry 10 (2019) 216, https://doi.org/10.3389/fpsyt.2019.00216.
[65] L. Pelizza, A. Ferrari, Anhedonia in schizophrenia and major depression: state or trait? Ann. Gen. Psychiatry 8 (2009) 22, https://doi.org/10.1186/1744-859X-8-22.
[66] B.D. Atherton, R.M. Nevels, M.T. Moore, Predicting symptoms of depression from social anhedonia and emotion regulation, J. Nerv. Ment. Dis. 203 (2015) 170–174, https://doi.org/10.1097/NMD.0000000000000262.
[67] G. Rey, R. Jouvent, S. Dubal, Schizotypy, depression, and anxiety in physical and social anhedonia, J. Clin. Psychol. 65 (2009) 695–708, https://doi.org/10.1002/jclp.20577.
[68] V. Enneking, P. Krussel, D. Zaremba, K. Dohm, D. Grotegerd, K. Forster, S. Meinert, C. Burger, F. Dzvonyar, E.J. Leehr, et al., Social anhedonia in major depressive disorder: a symptom-specific neuroimaging approach, Neuropsychopharmacology 44 (2019) 883–889, https://doi.org/10.1038/s41386-018-0283-6.
[69] M. van Boxelaere, J. Clements, P. Callaerts, R. D'Hooge, Z. Callaerts-Vegh, Unpredictable chronic mild stress differentially impairs social and contextual discrimination learning in two inbred mouse strains, PLoS One 12 (2017), e0188537, https://doi.org/10.1371/journal.pone.0188537.
[70] M. Rincon-Cortes, R.M. Sullivan, Emergence of social behavior deficit, blunted corticolimbic activity and adult depression-like behavior in a rodent model of maternal maltreatment, Transl. Psychiatry 6 (2016), e930, https://doi.org/10.1038/tp.2016.205.
[71] A. Du Preez, D. Onorato, I. Eiben, K. Musaelyan, M. Egeland, P.A. Zunszain, C. Fernandes, S. Thuret, C.M. Pariante, Chronic stress followed by social isolation promotes depressive-like behaviour, alters microglial and astrocyte biology and reduces hippocampal neurogenesis in male mice, Brain Behav. Immun. 91 (2021) 24–47, https://doi.org/10.1016/j.bbi.2020.07.015.
[72] J.G. de Jong, B.J. van der Vegt, B. Buwalda, J.M. Koolhaas, Social environment determines the long-term effects of social defeat, Physiol. Behav. 84 (2005) 87–95, https://doi.org/10.1016/j.physbeh.2004.10.013.
[73] M.E. Seligman, Learned helplessness, Annu Rev. Med. 23 (1972) 407–412, https://doi.org/10.1146/annurev.me.23.020172.002203.
[74] H. Anisman, Z. Merali, Rodent models of depression: learned helplessness induced in mice, Curr. Protoc. Neurosci. (2001), https://doi.org/10.1002/0471142301.ns0810cs14.
[75] C. Kuehner, Why is depression more common among women than among men? Lancet Psychiatry 4 (2017) 146–158, https://doi.org/10.1016/S2215-0366(16)30263-2.
[76] C. Hu, Y. Luo, H. Wang, S. Kuang, G. Liang, Y. Yang, S. Mai, J. Yang, Re-evaluation of the interrelationships among the behavioral tests in rats exposed to chronic unpredictable mild stress, PLoS One 12 (2017), e0185129, https://doi.org/10.1371/journal.pone.0185129.