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Lena-Sophie Martis[a,b], Claudia Brision[a], Megan C. Holmes[b,c], Ove Wiborg[a,d,*]

[a] *Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, Denmark*

[b] *Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Scotland, United Kingdom*

[c] *Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Scotland, United Kingdom*

[d] *Department of Health Science and Technology, Aalborg University, Denmark*

** ow@hst.aau.dk, Aalborg University, Fredrik Bajers Vej 7, E4-119, 9220 Aalborg, Denmark*

Abstract

Depression-associated cognitive impairments persist after remission from affective symptoms of major depressive disorder (MDD), decreasing quality of life and increasing risk of relapse in patients. Conventional antidepressants are ineffective in restoring cognitive functions. Therefore, novel antidepressants with improved efficacy for ameliorating cognitive symptoms are required. For tailoring such antidepressants, translational animal models are in demand. The chronic mild stress (CMS) model is a well-validated preclinical model of depression and known for eliciting the MDD core symptom "anhedonia" in stress-susceptible rats. Thus, cognitive performance was assessed in rats susceptible (depressive-like) or resilient to CMS and in unchallenged controls. The rodent analogue of the human touchscreen Paired-Associates Learning (PAL) task was used for cognitive assessment. Both stress groups exhibited a lack of response inhibition compared to controls while only the depressive-like group was impaired in task acquisition. The results indicate that cognitive deficits specifically associate with the anhedonic-like state rather than being a general consequence of stress exposure. Hence, we propose that the application of a translational touchscreen task on the etiologically valid CMS model, displaying depression-associated cognitive impairments, provides a novel platform for pro-cognitive and clinically pertinent antidepressant drug screening.

Keywords:

Depression, Cognitive impairments, Chronic mild stress (CMS), Resilience, Preclinical touchscreen task, Paired-associates learning (PAL)

1. Introduction

Major depressive disorder (MDD) is the leading cause of disability worldwide affecting 300 million people and constituting a major socio-economic burden to society (World health organisation, 2017). The core symptoms of MDD are lack of energy, depressed mood and anhedonia, which refers to a decreased sensitivity or anticipation to reward (American Psychiatric Association, 2013; Rizvi, Pizzagalli, Sproule, & Kennedy, 2016). Additionally, depressed patients can exhibit a plethora of other manifestations including feelings of guilt and worthlessness, altered sleep architecture, change in body weight, suicidal thoughts, or impairments in cognition, primarily in attention, executive function and memory (Reppermund, Ising, Lucae, & Zihl, 2009; Rock, Roiser, Riedel, & Blackwell, 2014). After remission from the affective symptoms of MDD, these cognitive impairments still persist in 30–60% of patients (Darcet, Gardier, Gaillard, David, & Guilloux, 2016; Jaeger, Berns, Uzelac, & Davis-Conway, 2006; Reppermund et al., 2009; Rock et al., 2014) and were found to be the longest present residual symptom (Conradi, Ormel, & de Jonge, 2011). Cognitive impairments are a major contributor to the disabling impact of MDD (Naismith, Longley, Scott, & Hickie, 2007) and, thus, in patients with persistent cognitive impairments quality of life is decreased and risk of relapse elevated (Gonda et al., 2015; Reppermund et al., 2009). Accordingly, treatment of depression associated cognitive impairments in addition to the affective symptoms is considered crucial for complete remission (Gonda et al., 2015; Jaeger et al., 2006; Reppermund et al., 2009; Rock et al., 2014).

Although many resources have been directed towards depression research, the causal mechanisms of MDD remain unknown due to the complex gene x environment interaction emerging in a variety of symptoms. A major environmental risk factor for developing MDD is the exposure to stress (de Kloet, Joëls, & Holsboer, 2005). Stress can cause neuropsychological changes which can lead, in predisposed individuals, to an excessive or prolonged stress response and increased risk for mental diseases, such as depression (Cattaneo & Riva, 2016; de Kloet et al., 2005; Risch et al., 2009). Indeed, a hyperactive hypothalamic-pituitary-adrenal (HPA) axis is found in the majority of MDD patients (Barden, 2004; Pariante & Lightman, 2008). The consequently high circulating glucocorticoids will have deleterious effects, on both structure and function, in a key glucocorticoid sensitive region, namely the hippocampus that is central in memory formation and retrieval (Czéh & Lucassen, 2007; de Kloet et al., 2005). MDD

patients show memory impairments and a decreased hippocampal volume, which is associated with the duration and number of depressive episodes (MacQueen et al., 2003; Sheline, Gado, & Kraemer, 2003; Sheline, Sanghavi, Mintun, & Gado, 1999; Sheline, Wang, Gado, Csernansky, & Vannier, 1996; Videbech & Ravnkilde, 2004). Both, hippocampal atrophy and memory impairments might be a direct consequence of stress in MDD patients. Moreover, chronically elevated cortisol levels, as a consequence of prolonged stress exposure, can impair cognition in non-depressed individuals (Lupien et al., 1998). This highlights the possibility that stress is a causal factor in the development of depression-associated cognitive impairments. To gain further insight into the relationship of stress and cognitive impairments in depression, a preclinical stress model exhibiting depression associated cognitive impairments is indispensable (Darcet et al., 2016).

A number of preclinical models of depression apply stressors (etiological validity) to provoke a depressive-like phenotype. Some milder paradigms, such as the chronic mild stress (CMS) model, also enable the segregation of a stress-resilient subgroup, which allows investigation of distinct stress- and depression-related effects as well as the study of potential resilience mechanisms. Comparable studies are impossible in humans since stress intensity, nature and duration, as well as time point in life of stress experience, differ greatly between subjects. Depressed patients are often medicated and a "resilient" group with comparable stress experience is difficult to identify. These confounding factors are controlled for in preclinical MDD models applying defined stress paradigms. The CMS model, mimicking daily stress experience in humans, is a highly validated preclinical model of depression, well known for the manifestation of the MDD core symptom of anhedonia (face validity). Additionally, CMS exposed rats exhibit other depressive-like symptoms such as changes in sleep architecture, changes in body weight, decreased sexual activity and altered aggression behaviour (Wiborg, 2013; Willner, 2005). Impaired CMS-induced working memory, spatial memory and object recognition memory were shown with classical rodent behavioural tests (Elizalde et al., 2008; Henningsen et al., 2009; Li et al., 2008; Li, Wang, Wang, Yukihisa, & Kinzo, 2006; Papp et al., 2017). However, these cognitive behavioural findings were reported for all rats that underwent CMS rather than for anhedonic-like rats only, i.e. CMS rats were not segregated into resilient and anhedonic-like phenotypes. Furthermore, as classical tests are designed for the rodent, translation of the results to the clinic is limited (Bussey et al., 2012). In contrast, the touchscreen operant platform is considered to be more translational because it uses a similar test setup and readouts to those in cognitive testing in humans and is more standardized in its testing setup (Bussey et al., 2008; Morton, Skillings, Bussey, & Saksida, 2006; Nithianantharajah et al., 2015). These touchscreen tasks were developed based on the Cambridge Neuropsychological Test Automated Battery (CANTAB), the most frequently applied cognitive test battery in MDD patients (Darcet et al., 2016). Further advantages of the rodent touchscreen platform include standardized experimental equipment and tasks,

objective readouts, minimization of experimenter's bias, a cognitive test battery and high throughput (Bussey et al., 2012; Horner et al., 2013). In the present study, we applied the different Paired-Associates Learning (dPAL) task which has been used in preclinical models of schizophrenia and Alzheimer's disease, and is known for being a hippocampus-dependent task (Hvoslef-Eide et al., 2015; Talpos, Aerts, Fellini, & Steckler, 2014). Hence, we investigated if the translational touchscreen platform is sensitive for detecting cognitive impairments in stress exposed rats. Furthermore, we determined if the impairments observed are the consequence of general stress exposure or specially associated with the depressive-like phenotype by including stress-susceptible and stress resilient rats in the study. This will provide insight in the relationship of stress, mood (anhedonia) and cognitive symptoms. The aim of this study was to establish a clinically relevant platform for developing and tailoring pro-cognitive antidepressant treatments.

We hypothesized that cognitive impairments would be observed in both stress exposed groups in the dPAL task but that the stress-susceptible, depressive-like, rats may be impaired in a different cognitive area or more severely than the CMS resilient rats. These cognitive impairments might possibly be observed in attention, executive function or memory.

2. Materials and Methods

2.1. Animals

Male Long Evans rats (LE; Janvier Labs, France) were 5–6 weeks and 100–120 g at arrival to our facility. Animals were housed four per cage for one week and afterwards they were single-housed. Rats were kept on a 12-h light/dark cycle (lights on at 6:00 am) with free access to food and water (otherwise stated). All experiments were conducted according to EU Directive 2010/63/EU and approved by the Danish National Committee for Ethics in Animal Experimentation (2013-15-2934-00814).

A timeline of the experiment is shown in Fig. 1.

[Figure 1; colour for online, grey for print; 2 columns]

Fig. 1. Experimental timeline. Depiction of the different experimental stages and their duration. Touchscreen pre-training included 8 days of gradual food restriction to 80% of *ad libitum* intake during the 10th week of CMS. Here, the original CMS protocol (dark green) was continued without the stressor "food deprivation". Food restriction was followed by operant conditioning in the touchscreen setup during which a modified CMS protocol (light green) was initiated. The acquisition of the touchscreen dPAL task was conducted until passing criterion was reached and retention was determined in two additional dPAL sessions after a 10-day hiatus without touchscreen testing. (SCT–sucrose consumption test, CMS–chronic mild stress, dPAL–different paired-associates learning, Ø–average time for rats to learn the relevant stage).

2.2. Chronic mild stress paradigm

2.2.1. Baseline sucrose consumption test

The SCT was carried out to assess the rats' hedonic state during stress exposure. Animals were acclimatized to the facility for one week. In the next two weeks, rats were habituated to SCTs by drinking a palatable sucrose solution (1.5%) semi-weekly for 1 h following 14 h of food and water deprivation. Thereafter, weekly SCTs were carried out twice and averaged to a baseline sucrose consumption for each rat individually (Fig. 1). Animals were split in two matched groups with equal group mean and standard deviation (SD) of their baseline sucrose consumption. CMS exposure was initiated for one of the groups (n = 148) and the other group was housed in a separate room and left unchallenged (n = 24). Weekly SCTs were conducted throughout the original CMS paradigm including stressed and control animals**.** After 9 weeks of CMS, the stress exposed group was divided into subgroups depending on their sucrose index (mean of last two SCTs during CMS / baseline SCT). Rats were categorized as stress-susceptible, thus anhedonic-like, with a SCT index ≤ 0.7 and as stress resilient with a SCT index ≥ 0.9 based on an *a priori* criteria used in previous studies (Wiborg, 2013). Mean sucrose intake of the last two weeks of the original CMS protocol, i.e. week eight and nine, indicated 61 rats (41%) to be anhedonic-like and 29 (20%) to be resilient.

2.2.2. CMS paradigm and hedonic state

Rats entering the CMS paradigm were exposed to a series of stressors lasting between 5–14 h (Jayatissa, Bisgaard, Tingström, Papp, & Wiborg, 2006). Stress duration and type of stressors were varied across a two-week protocol (Table 1) to increase unpredictability of stressors and avoid habituation. During the stressor "grouping", a CMS rat was transferred to the home cage of another CMS rat (resident-intruder). Grouping partners were exchanged weekly and individual rats were alternated in being resident or intruder.

Following 9 weeks of CMS, 11 of the resilient, 10 of the anhedonic-like and 11 of the non-stressed control rats were subjected to gradual food restriction (see 2.3.2). During gradual food restriction, the stressor "food deprivation" was excluded from the CMS protocol. Thereafter, touchscreen pre-training was initiated and the original CMS protocol was modified (Table A.1) to avoid interference with touchscreen performance: First, stressors were only applied during night-time because touchscreen training took place during daytime. Furthermore, "grouping" overnight, which is a harsh stressor, was replaced in this protocol to prevent poor touchscreen performance due to fatigue rather than poor cognition in the CMS group. Finally, the stressor "water deprivation" was abandoned complementary to

the already excluded "food deprivation" as these stressors likely affect the rat's motivation for consuming the sugar pellet rewards used for touchscreen operant conditioning.

Table 1

The original chronic mild stress protocol. Time of stress exposure is presented in brackets. Stressors are imposed on the CMS group only, whereas '*' indicates that both, CMS and control rats, undergo the procedure.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Morning	New cage*, body weight measurement*, light off (11.00–13.00, 15.00–17.00)	Water deprivation (08.00–17.00)	Stroboscopic light (10.00–16.00)	New cage	SCT* (08.00–09.00), alternating weekly: food or water deprivation (09.30–18.00)	Alternating weekly: water or food deprivation (08.30–17.30)	Cage tilt 45° (08.00–17.00)
Evening	–	Cage tilt 45° (18.00–08.00)	Wet bedding (18.00–08.00)	Food and water deprivation* (18.00–09.00)	Grouping (18.00–08.00)	Cage tilt 45° (18.00–08.00)	Wet bedding (18.00–08.00)

SCT – sucrose consumption test

2.3. Touchscreen operant platform

Learning and memory were assessed with the translational touchscreen platform dPAL task.

2.3.1. Apparatus

A detailed description and visualization of the equipment can be found in Horner et al. (2013). In brief, the Bussey-Saksida operant chambers (Campden Instruments Ltd., Loughborough, UK) are sound- and light-attenuated boxes with a trapezoid shaped interior (height 300 mm, length 332 mm, width screen 240 mm, width magazine 126 mm). Opposite to a reward delivery system (magazine), a touch-sensitive screen was located and covered by a mask with three windows (height 100 mm, width 60 mm). A spring-hinged shelf (90°) was installed below the windows to slow the rat down before touching the screen and avoiding hasty choices. The chambers were further equipped with a house and magazine light, a metal grid floor, a tone generator and a fan, which ensured sufficient ventilation and masked external noise. The touchscreen program was controlled by Whisker Server Abett II (Campden Instruments Ltd.).

2.3.2. Touchscreen pre-training

Before undergoing the dPAL task, rats were pre-trained to use the touchscreen setup following 9 weeks of CMS. First, rats were food restricted to reinforce operant conditioning. Food was gradually

decreased by 5% every second day to 80% of free feeding consumption. On the last two days, rats were additionally habituated to consume five touchscreen reward pellets (sugar coated, 45 mg dustless precision pellets, Bio Serv, Flemington, NJ, USA) in their home cage. Body weight was monitored daily. Touchscreen training was carried out every day in a session of 45 min or 75 trials maximum (except for "habituation" step) during the light phase. All rats were moved to the testing room 30 min prior to touchscreen training. Rats of each group were tested simultaneously and balanced across touchscreen chambers to prevent possible chamber effects. Touchscreen testing was conducted every day and individual testing time point was varied (Martis et al., 2018). Pre-training consisted of five steps (Horner et al., 2013). First, in the "habituation" step, rats were left in the touchscreen box with house light off and had to consume five reward pellets from the food magazine within 30 min. Second, "initial touch", rats automatically received one reward pellet every 30 s or three reward pellets if the rat touched the stimulus (randomly, one of the three touchscreen windows was illuminated). Reward collection was followed by a 20 s inter-trial-interval (ITI) after which the next trial would automatically start. Rats were moved on to "must touch" if they touched the stimulus ≥ 30 times in one session (passing) or, alternatively, if they touched the stimulus ≤ 5 times per session on two consecutive days (failing). In "must touch", the rat had to touch the stimulus in order to receive a reward pellet. If the rat was new to "must touch" or had ≤ 40 touches the day before, peanut butter was introduced to each screen window prior to session start to draw attention to the screen. If the rat touched the stimulus ≤ 5 times per session on two consecutive days (failing), it was moved back to "initial touch" (only if it had failed "initial touch") or passed on to "must initiate" if it touched the stimulus 75 times within a session. "Must initiate" was similar to "must touch", additionally the rat had to initiate each trial by nose poking in the food magazine. Finally during "punish incorrect", a touch on the two non-illuminated windows on the screen resulted in a 5 s time-out period with house light on, followed by the ITI. To pass "punish incorrect" and thus pre-training, the rat had to accomplish 75 trials within 45 min with at least 60 correct touches to the illuminated window (≥ 80% accuracy) for two consecutive days. The rat was stressed again for 3 h ("grouping") the day following pre-training as a reminder of the original stress protocol and hence received one day without touchscreen testing.

2.3.3. Different paired-associates learning task

dPAL training (Horner et al., 2013) began the day after grouping. In this task, three symbols (white on black background) should be associated with one of the touchscreen windows, respectively (Fig. 2A). A session followed the same rules as in "punish incorrect", but instead of one illuminated and two blank windows in each trial, two windows displayed two of the three symbols. One of the symbols would be in its correct window, whereas the other one in an incorrect window. The remaining window

was left blank (Fig. 2B). This resulted in six different experimental trials, which were randomly balanced within a session. A correct response was registered if the rat touched the symbol that was displayed in the correct location. An incorrect response was followed by a 5 s time-out with house light on. After the ITI, a correction trial was initiated, i.e. the previous incorrectly responded trial was displayed again. If the rat responded incorrectly to the correction trial, another one would be displayed until the rat managed a correct choice. Criterion to pass was accomplished by completing 75 trials (not counting correction trials) within 45 min, with 80% accuracy, on two consecutive days.

[Figure 2; 1 column]

Fig. 2. Different paired-associates learning task scheme. (A) Each symbol is shown in its correct location (L): spider-L1, plane-L2, flower-L3. (B) An example trial is displayed with one symbol (spider) in its correct location, and one symbol (plane) in an incorrect location.

2.3.4. Retention

Passing dPAL was followed by 10 days without touchscreen testing and an increase in food accounting for the lack of reward pellets during this period. After the 10-day hiatus, rats were returned to 80% baseline food restriction and retested on the dPAL task for two days.

2.4. Statistical Analysis

SCT data were analysed without the baseline SCT, applying mixed effects model for repeated measurements with post-hoc Bonferroni-corrected pairwise group comparisons. dPAL summary statistics was evaluated with Shapiro-Wilk test for residual normality and Levene's test for homogeneity of variance with non-significant results allowing for statistical analysis by ANOVA and LSD post-hoc analysis. dPAL repeated measurements data were analysed using univariate repeated measurements ANOVA and Greenhouse-Geisser correction if sphericity was violated. Retention was analysed applying multivariate repeated measures ANOVA. Moreover, memory and relearning performance were analysed by one-way ANOVA, and assumption of normality and homogeneity were reviewed. Rats that did not acquire the dPAL task could evidently not be included in summary statistics (3.2.1.) and retention (3.3.) as they never reached criterion (two CMS resilient and one control rat), but were included in data analyses over time (3.2.2. and 3.2.3.). Data of summary statistic and retention were reviewed for outliers with Grubb's test ($\alpha = 0.05$) and ROUT test (Q = 1%; GraphPad Prism 6, GraphPad Software Inc., California, USA) and revealed no outliers. For response latency, the median for a given session was included in the data analysis instead of the mean value to avoid distorted values by rats taking a break rather than responding extremely slow (Kim, Heath, Kent, Bussey, & Saksida, 2015). The parameter "redundant screen touches" describes the number of touches to the blank screen additionally to the one for making a choice and is expressed as number of redundant touches divided by the total number of trials (trials plus

correction trials). The "maximum number of consecutive correct touches" refers to the highest number of trials that a rat carried out in row within a session. Statistical analysis was conducted with RStudio (RStudio Inc., Massachusetts, USA) and rdata.online (Montreal, Canada). Data were displayed with GraphPad Prism 5.

3. Results

3.1. Hedonic state changes in response to stress

Rats exposed to CMS were segregated into anhedonic-like and resilient phenotypes based on their sucrose consumption test (SCT) index. The CMS groups responded differently to stress in respect to their sucrose consumption (interaction effect of group x weeks of CMS: $\chi^2(16) = 41.84$, $p = 0.0004$; Fig. 3). The CMS anhedonic-like group significantly decreased their sucrose intake over the course of stress exposure compared to non-stressed controls (Bonferroni-corrected group-wise comparisons $p < 0.0001$) and to CMS resilient rats (Bonferroni-corrected group-wise comparisons $p < 0.0001$). There was no significant difference between the non-stressed control and CMS resilient group. The SCT results show that stress clearly provoked distinct phenotypes in regard to the hedonic state with only a fraction of rats becoming anhedonic-like, thus depressive-like, and with another subgroup of rats being stress resilient.

[Figure 3; 1.5 columns]

Fig. 3. Sucrose consumption during CMS. The weekly sucrose consumption, normalised to baseline, is shown as group mean (± SEM). Statistically significant group-wise Bonferroni-corrected comparisons over all time points are indicted by ****$p < 0.0001$ (Control: $n = 11$, Resilient: $n = 11$, Anhedonic: $n = 10$).

3.2. Paired-associates learning touchscreen task

3.2.1. Learning of the dPAL task

Learning behaviour until attaining dPAL acquisition criterion was evaluated with summary statistics comparing non-stressed controls, CMS anhedonic-like and resilient rats.

The number of sessions required to learn the dPAL task was not significantly different between groups ($F(2,26) = 2.40$, $p = 0.111$), even though the controls needed the least number of sessions (Group mean ± SD = 25.00 ± 9.20 sessions), followed by the resilient group (26.11 ± 4.70 sessions) and, eventually, by the anhedonic-like group (31.70 ± 7.07 sessions). The number of sessions does not reveal the exact number of repetitions, thus practice, completed by individual rats to reach criterion, since the number of trials can vary from zero to 75 trials within a session. This means that rats with the same number of sessions may potentially have performed a variable number of trials to acquire the dPAL task.

Thus, number of trials to reach dPAL criterion was also analysed. Anhedonic-like rats needed substantially more trials (1821.40 ± 153.56 trials) to acquire the dPAL task compared to controls (1305.80 ± 176.11 trials; LSD post-hoc test $p = 0.021$). Resilient rats (1426.56 ± 108.36 trials) displayed a trend to require less trials than anhedonic-like rats (LSD post-hoc test $p = 0.079$), but their performance was not significantly different from controls (LSD post-hoc test $p = 0.581$; marginally significant main effect of group: $F(2,26) = 3.26$, $p = 0.054$; Fig. 4A).

The total number of correction trials to acquire the dPAL task was higher in the anhedonic-like group than in controls or resilient rats but did not attain significance (Fig. 4B).

The time to collect the touchscreen reward pellet (collection latency) did not differ significantly between groups, nor did the median time to respond to the stimuli on the screen (response latency; Fig. 4C) or the number of screen touches additionally to the one for making a choice (redundant screen touches per trial).

We examined the highest number of correct trials that the rats were able to carry out in a row within a session. This parameter was used to assess sustained attention and is referred to as "maximum consecutive correct trials per session". CMS anhedonic-like rats carried out significantly more maximum consecutive correct trials per session than controls (LSD post-hoc $p = 0.005$; main effect of group: $F(2,26) = 4.65$, $p = 0.019$; Fig. 4D). This result suggests that anhedonic-like rats have a different strategy for learning the touchscreen task compared to non-stressed controls and possibly CMS resilient rats. Overall, CMS anhedonic-like, but not resilient rats, exhibited impaired learning behaviour.

[Figure 4; 2 columns]

Fig. 4. Summarized touchscreen parameters of dPAL task acquisition. (A) Absolute number of trials needed to pass the dPAL task. (B) Absolute number of correction trials needed for learning the dPAL task. (C) Median response latency to touchscreen stimuli. (D) Average number of maximum consecutive correct trials per session. Group means (± SEM) and individual results are shown. LSD post-hoc comparisons are indicated with $*p < 0.05$, $**p < 0.01$.

3.2.2. dPAL task acquisition over time

Blocks of equal numbers of trials were used for analysing the touchscreen data over time, since the number of trials within a session varies across time and, consequently, also the learning process (Kim et al., 2015). More precisely, the total number of trials (trials plus correction trials) required to learn the dPAL task was split into ten equal bins. Thus, the variable number of sessions, and consequently the total number of trials, between individual rats was normalised to ten time points (bins) for each rat. This permitted a more direct comparison of individual rats as well as statistical analysis with repeated measurements ANOVA.

The accuracy of learning ($F(5.72,165.88) = 63.04$, $p < 0.0001$; Fig. 5A) and the number of trials completed ($F(2.76,80.10) = 46.91$, $p < 0.0001$; Fig. 5B) significantly increased over time with increasing bin number. No differences for these parameters were observed between groups. The number of correction trials ($F(2.75,79.68) = 47.44$, $p < 0.0001$) significantly decreased over time. Therefore, there is no apparent difference in the learning performance over time of this task between groups.

The CMS resilient rats executed more redundant screen touches than controls or CMS anhedonic-like rats (interaction effect of group x bin: $F(5.04,73.12) = 3.35$, $p = 0.009$) in the initial phase of dPAL learning (Fig. 5C). Furthermore, the number of redundant screen touches per trial significantly decreased over time for all groups ($F(2.52,73.12) = 10.92$, $p < 0.0001$).

Interestingly, the CMS anhedonic-like animals executed more consecutive correct trials (12.42 ± 5.99 trials) than non-stressed controls (10.58 ± 5.18 trials; LSD post-hoc $p = 0.016$) or CMS resilient rats (10.92 ± 5.25 trials; LSD post-hoc $p = 0.048$; main effect of group: $F(2,29) = 5.64$, $p = 0.009$; Fig. 5D).

A trend in group x bin interaction was observed for collection latency ($F(5.56,80.59) = 2.18$, $p = 0.058$). Post-hoc comparisons showed that CMS resilient took longer to collect their reward than controls ($p < 0.05$) in block 5–7 and 9. Collection latency decreased significantly with increasing bin number ($F(2.78, 80.59) = 9.07$, $p < 0.0001$).

Over time, i.e. with increasing bin number, median response latency decreased significantly ($F(3.13,90.64) = 12.99$, $p < 0.0001$), whereas maximum number of consecutive correct trials increased ($F(4.78,138.75) = 17.16$, $p < 0.0001$; Fig. 5D). Both parameters indicate task improvement over the course of dPAL task acquisition.

These results show that all groups were able to learn the task over time, but differences in learning strategies between groups were evident.

[Figure 5; 2 columns]

Fig. 5. Learning of the dPAL task over time. Total number of trials (trials plus correction trials) are split into bins of ten accounting for increasing number of trials per session over task acquisition (Kim et al., 2015). (A) Accuracy over time. (B) Number of trials (black) and total number of trials (trials plus correction trials; grey). (C) Number of redundant screen touches per trial. Significant post-hoc comparisons are indicated by ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ compared to the CMS resilient group respectively, and the control versus the anhedonic-like group by $^{\#}p < 0.06$. (D) The maximum number of consecutive correct trials. LSD post-hoc comparisons between groups are indicted by *$p < 0.05$. Group means are shown (± SEM).

3.2.3. Learning behaviour within the course of an average dPAL session

All sessions of one animal were averaged to a single session. This session was then split into six equal blocks by the total number of trials (trials plus correction trials). This allowed for the analysis of learning behaviour within the course of a session.

Accuracy (Fig. 6A) and number of trials were not significantly altered over the course of a session or between groups. However, the number of correction trials decreased significantly with increasing session block ($F(5,145) = 3.18$, $p = 0.009$).

Non-stressed controls executed less redundant touches per trial than CMS resilient and anhedonic-like rats in the first third of a session (interaction effect of group x session block: $F(4.78,69.34) = 3.40$, $p = 0.009$). The number of redundant touches per trial decreased within the course of a session ($F(2.39,69.34) = 7.22$, $p = 0.0007$; Fig. 6B).

During the progression of a session, thus with increasing block number, the maximum number of consecutive trials increased significantly ($F(5,145) = 14.61$, $p < 0.0001$; Fig. 6C) as well as median response latency ($F(1.59,46.16) = 10.19$, $p < 0.0001$; Fig. 6D). Collection latency varied with block number ($F(3.22,93.38) = 2.25$, $p < 0.0001$).

Thus within a session, primary readout parameters, like accuracy and number of trials, seemed not to change, but secondary parameters did, such as decreased number of correction trials and redundant touches, increased number of consecutive correct trials and median response latency.

[Figure 6; 2 columns]

Fig. 6. Learning parameters within the course of a session. (A) Percent of correct choices. (B) Number of redundant screen touches per trial. Post-hoc group-wise comparisons are indicated by $^{**}p < 0.01$, $^{*}p < 0.05$ comparing to the control group, respectively. (C) Maximum number of consecutive correct trials and (D) average median response latency significantly increased within a session. Group means (± SEM) over the course of session blocks are displayed.

3.3. Retention of the dPAL task assessing long-term memory

Following dPAL acquisition and a 10-day hiatus, animals were retested on the dPAL task over two days to assess long-term memory performance. The final session of dPAL acquisition as well as the two retention sessions were included in the analysis (mixed model repeated measurements ANOVA).

Accuracy of performance was significantly decreased in the first retention session after the hiatus (74.30 ± 6.42%) compared to accuracy at time of acquisition (80.27 ± 6.21%; post-hoc $p = 0.002$). However, accuracy increased from the first retention session to the second retention session (80.47 ± 5.83%; post-hoc $p = 0.0001$; main effect of session: $\chi^2(9) = 16.17$, $p = 0.0003$; Fig. 7A).

Next, memory (difference in accuracy between the last session passing dPAL criterion and the first retention session) and relearning (difference in accuracy between the first and second retention session) were analysed with one-way ANOVA. Neither memory nor relearning performance differed statistically between groups. Individual changes in accuracy are shown in Fig. 7B.

Hence, results show changes in performance due to the 10-day hiatus, but long-term memory differences were not observed between groups.

[Figure 7; 1.5 columns]

Fig. 7. Long-term memory and relearning performance in the dPAL task. (A) Accuracy is shown for the last session before the 10-day hiatus and the two retention sessions afterwards. Changes in group accuracy are displayed for memory (····) and relearning (—). Passing criterion is indicated at 80% accuracy. (B) The rats' individual changes in accuracy from last dPAL criterion session to the first retention session (memory) and first to second retention session (relearning). Group means (± SEM) and individual results are displayed.

4. Discussion

In the present study, translational testing applying the touchscreen operant platform revealed that anhedonic-like, but not the resilient subgroup of CMS exposed rats are impaired in task acquisition. This was mainly apparent from the finding that anhedonic-like rats are slower at acquiring the dPAL task compared to controls, whereas resilient rats required a comparable number of trials to learn the dPAL touchscreen task as controls. However, CMS resilient rats increased impulsive behaviour, as suggested from a higher number of redundant screen touches, than non-stressed controls and anhedonic-like rats. This suggests a differential but still efficient learning ability in the resilient group compared to controls. The results show that the cognitive impairments are specific to the depressive-like phenotype making it an excellent model for testing antidepressant drugs aiming to target both depressive and cognitive symptoms of MDD.

As shown previously (Bergström, Jayatissa, Mørk, & Wiborg, 2008; Christensen, Bisgaard, & Wiborg, 2011; Martis et al., 2018), CMS induces reduced reward sensitivity, which is demonstrated by reduced sucrose consumption in a subgroup of stress exposed rats, whereas another subgroup is resilient and remains hedonic. Reduced reward sensitivity is believed to be the biological underpinning of the MDD core symptom anhedonia (Sibille & French, 2013).

This study aimed to determine whether cognitive ability is altered in response to stress generally or specifically in association with the anhedonic-like phenotype, which appears more susceptible to the detrimental stress effects. To our knowledge, the use of standardized touchscreen testing in depression and anxiety models is not established (Darcet et al., 2016) and, hence, the different touchscreen parameters are discussed in detail in the following.

The anhedonic-like, thus depressive-like rats, needed more trials to acquire the dPAL task than non-stressed control rats, and hence appear impaired in their cognitive performance. Performance of CMS resilient rats was not different to controls nor anhedonic-like rats. However, post-hoc analysis of number of trials needed to learn the dPAL task suggests that resilient rats' performance mirrors more closely that of control rats than anhedonic-like rats. Consequently, impaired learning is specific to the depressive-like phenotype and not a consequence of stress exposure in general.

It could be argued that prolonged dPAL acquisition in the anhedonic-like group is due to reduced motivation. However, reward collection latency did not differ between groups indicating similar motivation to consume the reward and perform the touchscreen task. This is likely explained by rats being food deprived, and therefore as hunger is a strong motivator, differences in reward sensitivity are masked. Likewise in MDD patients, cognitive impairments are ascribed to deficits in cognition and not to a lack of motivation (Jaeger et al., 2006; Richards & Ruff, 1989).

The total number of correction trials needed to acquire the dPAL task was not significantly different between groups. However, anhedonic-like rats needed on average more correction trials than controls or resilient rats (Fig. 4B), which might indicate learning deficits and is concordant with faster task acquisition in controls and resilient rats. Regarding the learning curves, the number of correction trials decreased over time, as well as within a session, indicating improved task comprehension over time (Fig. 5B).

Deficits in attention are observed in depressed patients (Rock et al., 2014). Therefore, we introduced the parameter "maximum consecutive correct trials". It assesses the highest number of trials a rat is able to perform correctly in a row within a session and, thus, provides a readout for sustained performance. All three groups increased the number of consecutive correct trials in the course of dPAL acquisition, therefore indicating that learning improves sustained performance (Fig. 5D). Surprisingly, anhedonic-like rats were able to perform a higher number of maximum consecutive correct trials than non-stressed controls (Fig. 4D) and, to a smaller extent, to resilient rats as well (Fig. 5D). This finding appears counterintuitive since anhedonic-like rats showed overall inferior performance in the dPAL task acquisition. However, "maximum consecutive correct trials" measures only the highest score within a session and therefore resembles the best performance at a single time point, but does not capture performance over the whole course of the session. This suggests that anhedonic-like rats are generally able to perform well, however, they are not capable of maintaining their performance for successfully acquiring the dPAL task faster.

Elevated numbers of redundant screen touches may suggest increased impulsive or habit-like behaviour and decreased response inhibition as part of executive functions, a feature of the prefrontal cortex (PFC) (Koechlin & Summerfield, 2007; Miller & Cohen, 2001). Both CMS groups (Fig. 6B), particularly resilient rats (Fig. 5C), showed an increased number of redundant screen touches per trial. Ideally, we would expect only one touch per trial. Contrary to Talpos et al. (2014) suggesting that the dPAL task may not be sufficiently sensitive for detecting failures in response inhibition as an effect of LSD treatment, we suggest from our present findings that an increased number of redundant touches display a failure in response inhibition in CMS exposed rats. This finding is unlikely attributed to altered locomotion since, in a previous study, LE controls and LE CMS rats were indifferent in their activity in

the open field test (Martis et al., 2018). In a classical operant learning study, stress exposed rats shifted from effortful decision-making to increased habit-like behaviour, which was accompanied by atrophy in the medial PFC and associative striatum and hypertrophy in the sensorimotor striatum (Dias-Ferreira et al., 2009). Dias-Ferreira et al. (2009) explained this behavioural shift as a coping strategy to avoid effortful goal-directed behaviour during stress exposure. Thus, the present findings might suggest redundant screen touches as an indicator of utilization of different coping strategies and PFC functioning. Both CMS groups, especially the resilient group, seem to abandon effortful control in favour of habitual behaviours whereas unchallenged controls rely to a greater extent on appraisal.

All groups showed a decrease in accuracy in the first retention session compared to their performance in the final dPAL session before the 10-day hiatus. Although the anhedonic-like group showed a greater decrease in accuracy (-13.6%) than CMS resilient (-10.2%) or controls (-8.9%; Fig. 7B), no effect of group on memory performance was observed, indicating intact long-term memory in the CMS groups or failure to reach significance due to the high variance in performance, particularly in the control group.

Formation of long-term memory and object-in-place tasks are hippocampus-dependent (Eichenbaum, Sauvage, Fortin, Komorowski, & Lipton, 2012; Swainson et al., 2001) and both are main components of the rodent dPAL task (Hvoslef-Eide et al., 2015). It was shown that post-acquisition hippocampal lesions severely impair dPAL retrieval, whereas pre-acquisition hippocampal lesions only moderately affected dPAL learning in mice (Kim et al., 2015). Furthermore, dPAL performance was impaired in mild cognitive impairment patients displaying altered hippocampal function in an fMRI version of the PAL task (De Rover et al., 2011). Functional and structural alterations of the HPC are found in MDD patients (Chan et al., 2016; McEwen, 2005; Sheline et al., 2003, 1996) and in the CMS model, i.e. in anhedonic-like as well as in resilient animals (Delgado Y Palacios et al., 2011; Delgado Y Palacios, Verhoye, Henningsen, Wiborg, & Van der Linden, 2014; Jayatissa et al., 2006). Here, we found that CMS anhedonic-like rats require longer for learning the dPAL task, although their memory appears intact. The incremental learning of the rodent dPAL task might result in the task becoming hippocampus-independent (McClelland, McNaughton, & O'Reilly, 1995) or, alternatively, Kim et al. (2015) suggests that pre-acquisition lesions obligate other brain regions to compensate. Thus, longer task acquisition specifically in the anhedonic-like, but not resilient CMS group compared to non-stressed controls might be explained by alteration of the HPC.

Another brain region that was shown to be involved in dPAL acquisition (McAllister, Mar, Theobald, Saksida, & Bussey, 2015) and altered in MDD patients (Coffey et al., 1993; Landrø, Stiles, & Sletvold, 2001; Mayberg, Lewis, Regenold, & Wagner, 1994; Potter, Kittinger, Ryan Wagner, Steffens,

& Ranga Rama Krishnan, 2004) is the PFC. In the present study, the increased number of redundant touches in the CMS groups indicate a lack of response inhibition, a function of the PFC (Graybeal, Kiselycznyk, & Holmes, 2012). Hence, this suggests impaired executive functions by CMS exposure observed in the dPAL touchscreen task and also found in MDD patients (Rock et al., 2014; Swainson et al., 2001).

The behavioural changes observed in the present study were salient in visuo-spatial learning (acquisition of the dPAL task) and sustained attention (maximum number of consecutive trials). These processes appear to be a major contributor to disability in life functioning in humans even after half a year of remission from depression (Jaeger et al., 2006). Moreover, we applied chronic stress, which is a major risk factor in MDD, to provoke a depressive-like phenotype. Thus, clinical relevance and translational value of the present study is further supported.

A limitation of the present study is that the SCT test was abandoned during the touchscreen testing due to the sugary touchscreen pellets desensitising the rats for consumption of a dilute 1.5% sucrose solution. Hence, anhedonic-like rats could potentially have recovered from their depressive-like state. However, it is unlikely as rats have been shown to recover spontaneously only after 4–5 weeks following cessation of CMS (Wiborg, 2013). Furthermore, the continuation with a modified CMS protocol during touchscreen testing may have delayed spontaneous recovery. Muscat & Willner (1992) have shown that a two-week over-night stress protocol, applying similar stressors as in our modified CMS protocol, elicited comparable hedonic phenotypes as their original CMS protocol. Moreover, food restriction accompanying touchscreen testing may have added to the delaying effect of the modified CMS protocol (Mallien et al., 2016). Hence, it appears likely that spontaneous recovery after cessation of the original CMS protocol was prevented by the modified version in the present study.

4.1. Conclusion

In summary, the present study demonstrated that prolonged task acquisition was specifically associated with the depressive-like phenotype. These impairments were not a result of lacking motivation but can be attributed to cognitive deficits in anhedonic-like rats. Surprisingly, anhedonic-like rats showed superior sustained attention, which was, however, not reflected in their overall performance. In both CMS groups, response inhibition was impaired indicating deficits in executive functions as result of stress exposure. This increased habitual behaviour was especially prominent in the resilient group, which performed as well as non-stressed controls in the dPAL task, and, thus, this mechanism might be part of the stress-coping strategy of this group.

To our knowledge, this is the first study to show that the touchscreen dPAL task can be applied to detect depression-associated cognitive impairments in a preclinical MDD stress rat model. Accordingly,

the present study suggests CMS anhedonic-like rats, assessed with touchscreen tasks, as a translational and standardized platform for developing and screening novel pro-cognitive antidepressant treatment regimens, which are deemed necessary for obtaining higher remission rates of MDD and reducing risk of relapse.

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Declaration of interest

None.

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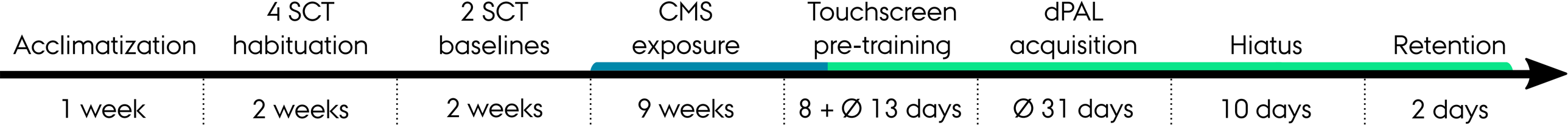
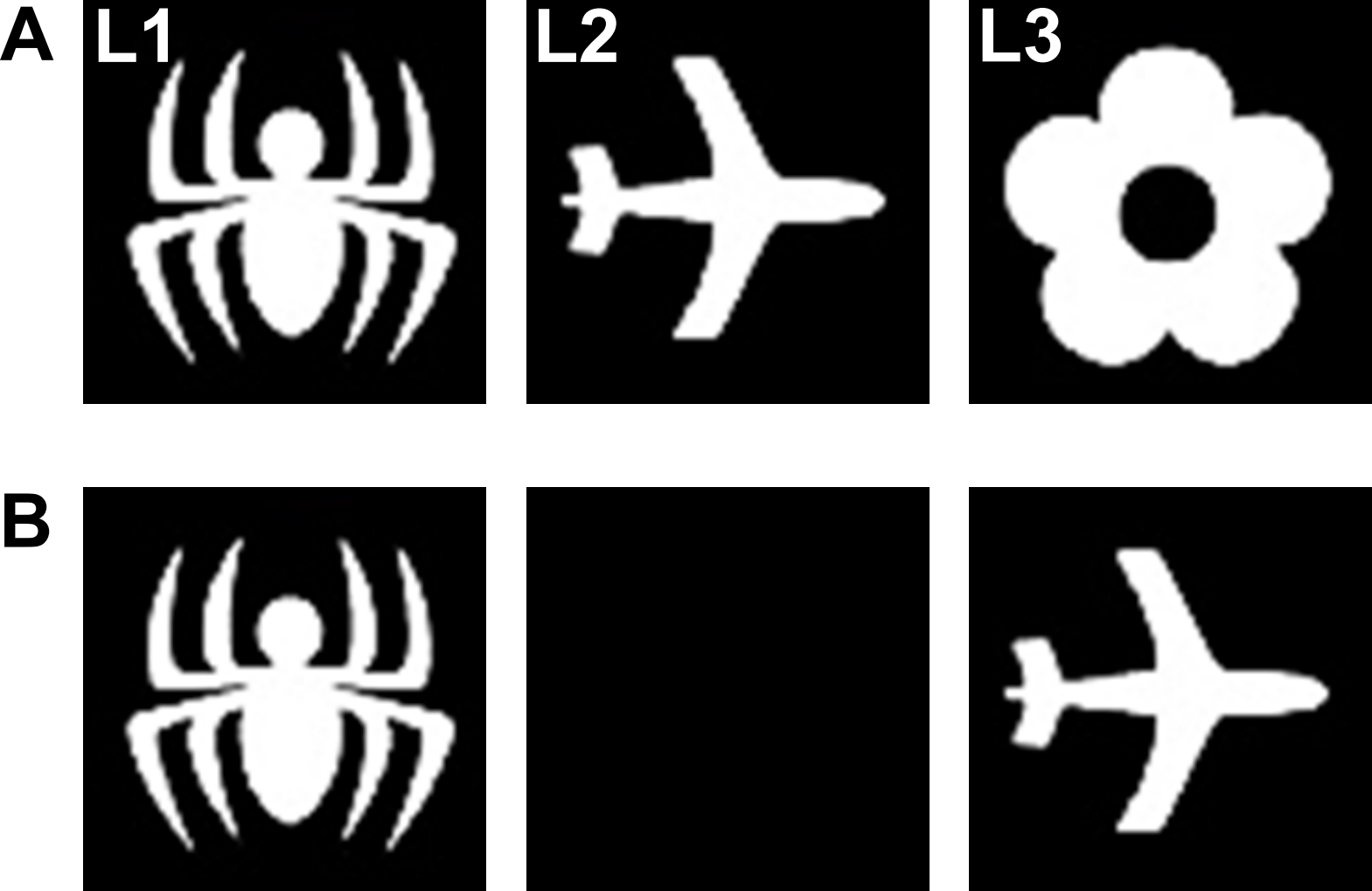
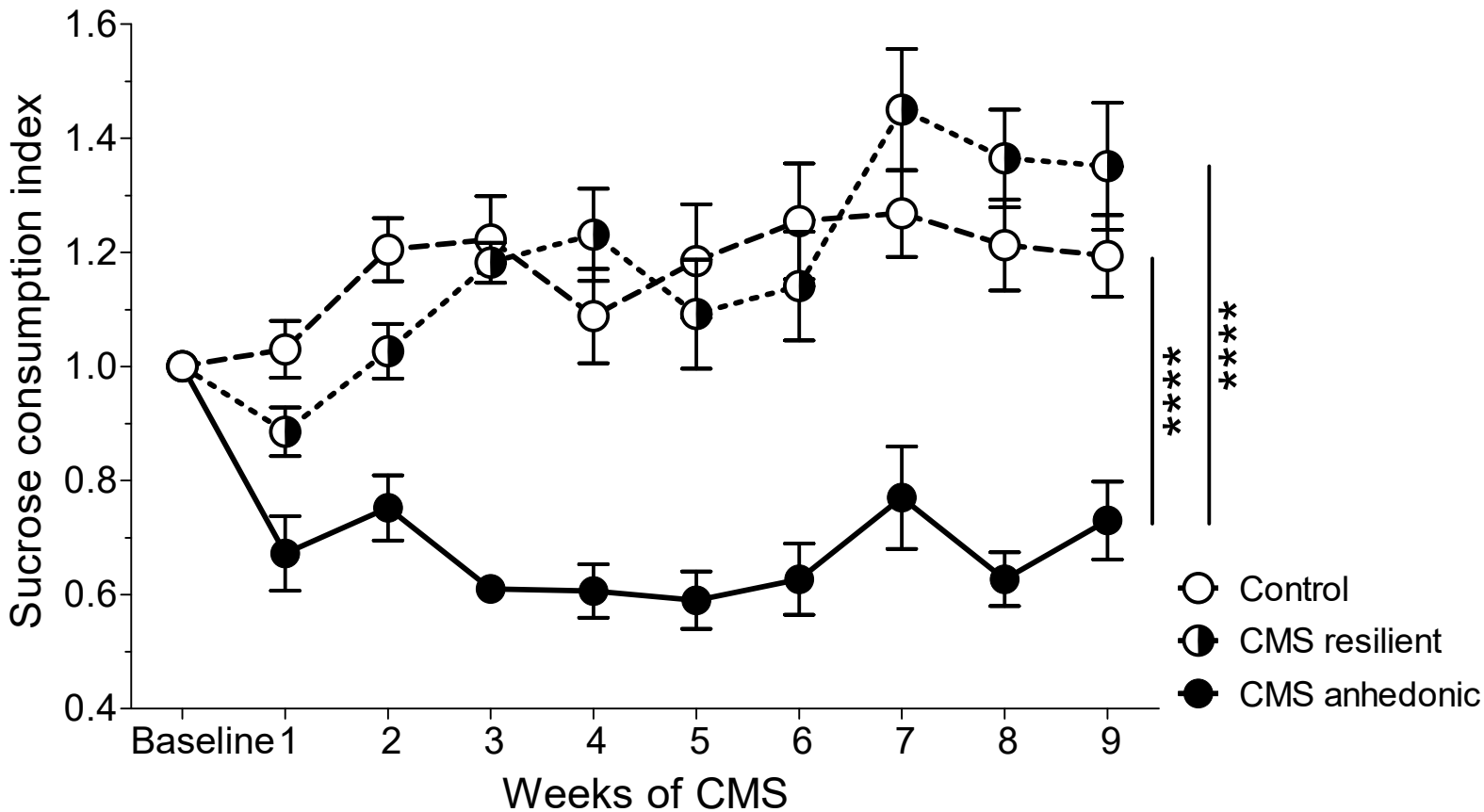
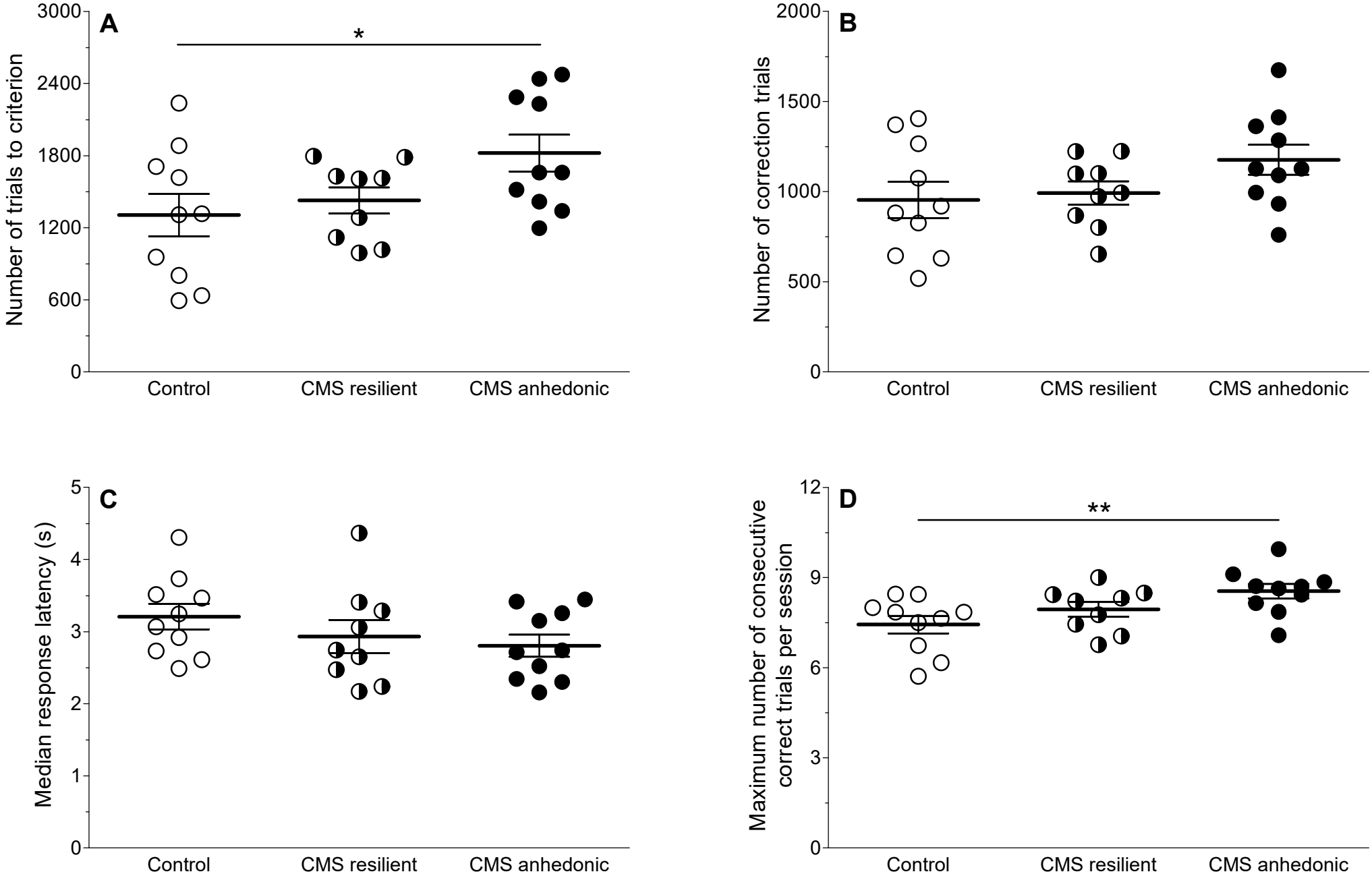
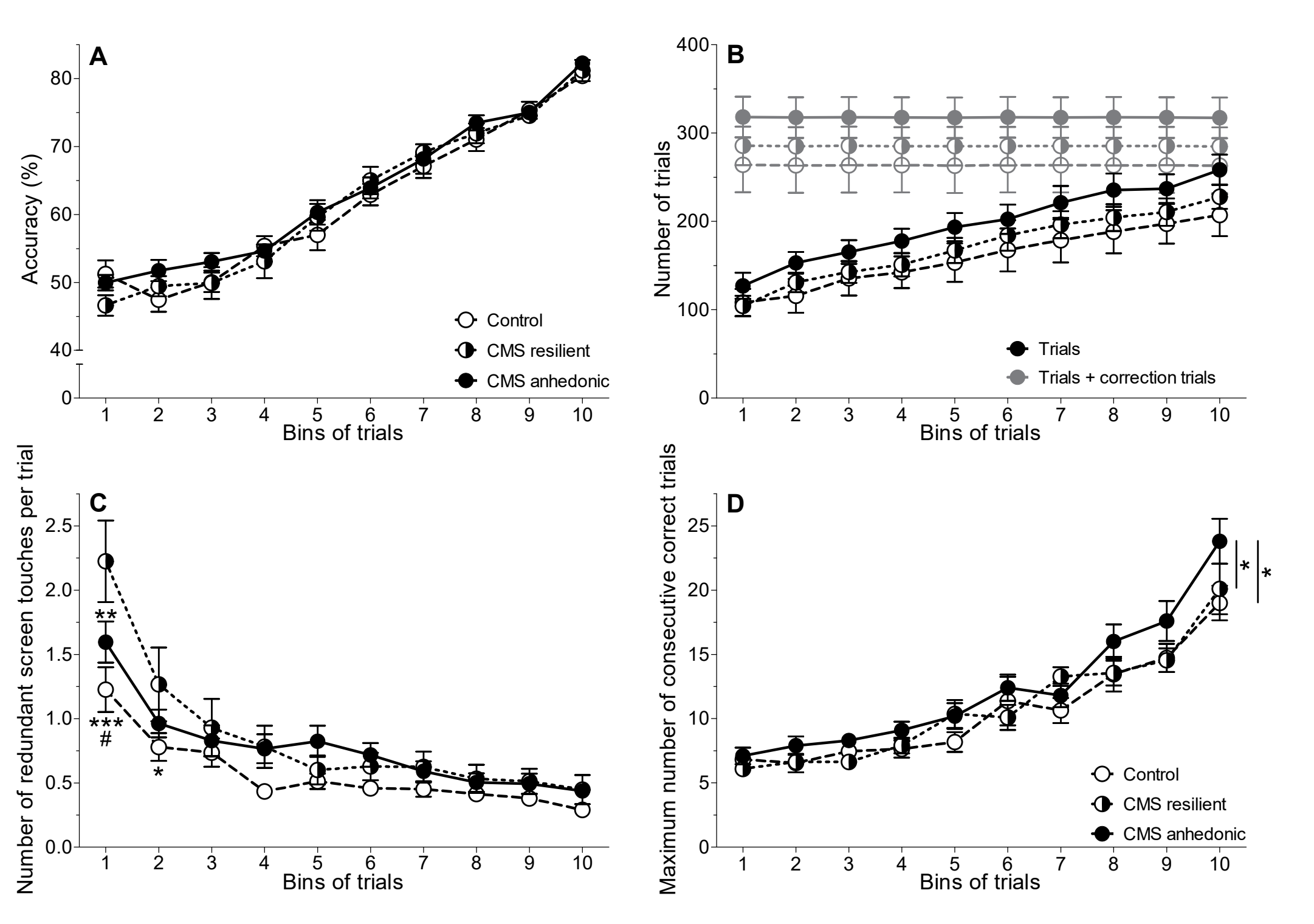
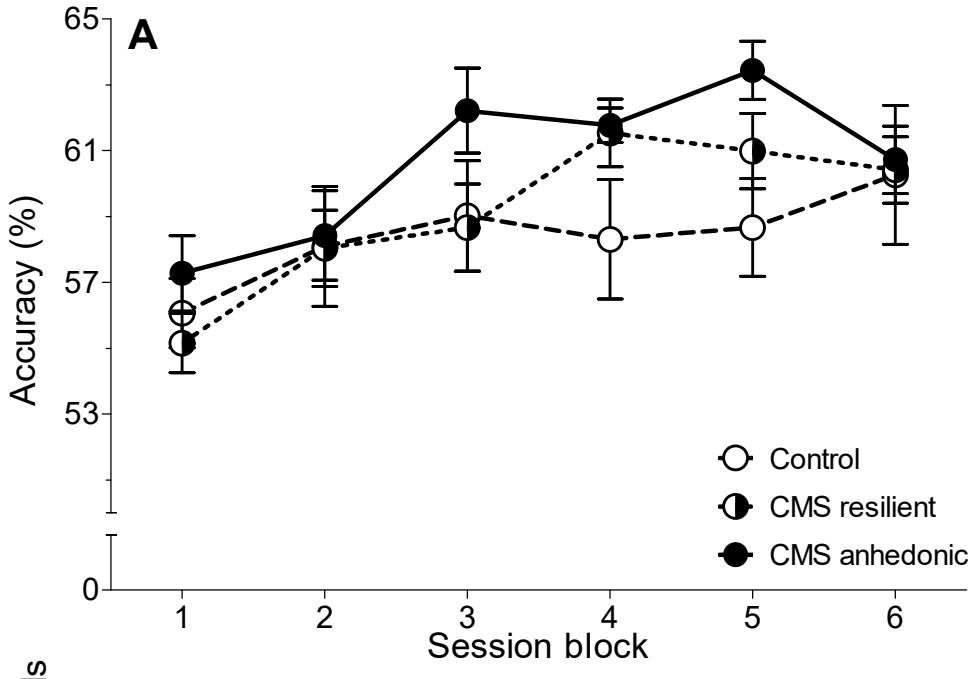

Acclimatization
4 SCT habituation
2 SCT baselines
CMS exposure
Touchscreen pre-training
dPAL acquisition
Hiatus
Retention
1 week
2 weeks
2 weeks
9 weeks
8 + Ø 13 days
Ø 31 days
10 days
2 days

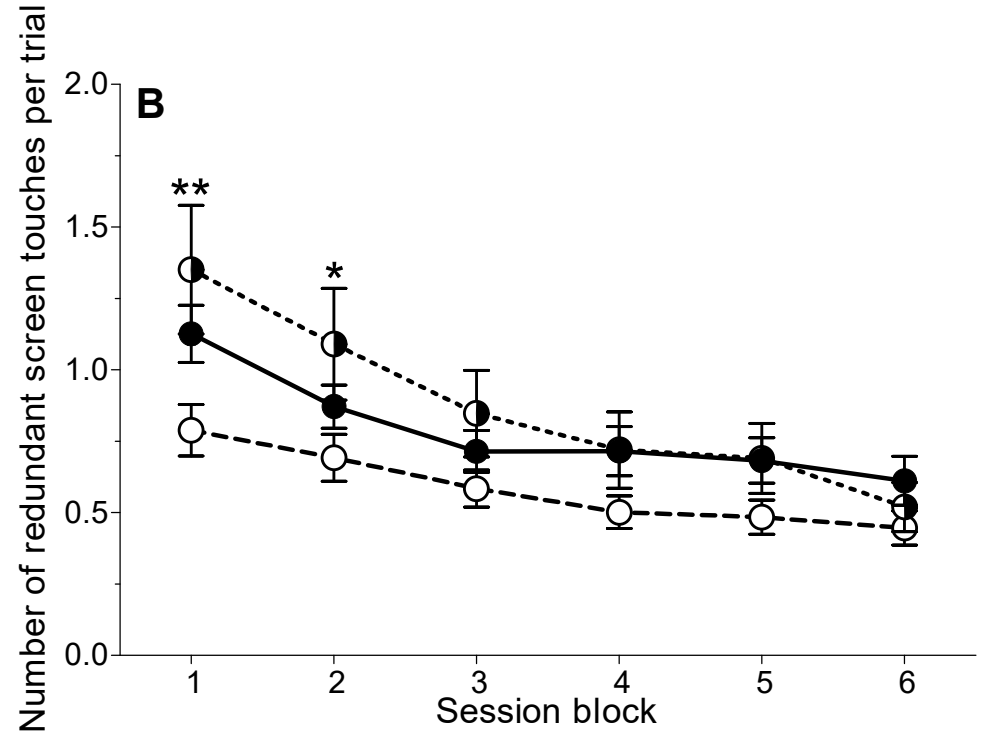

A
L1
L2
L3
B

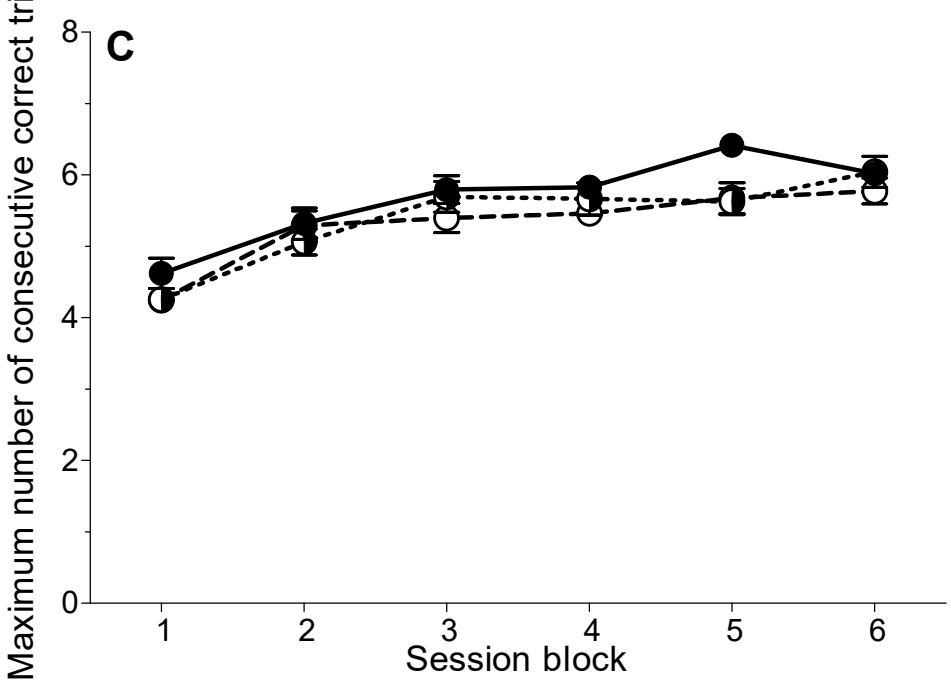

Sucrose consumption index
1.6
1.4
1.2
1.0
0.8
0.6
0.4
Baseline
1
2
3
4
5
6
7
8
9
Weeks of CMS
****
****
Control
CMS resilient
CMS anhedonic

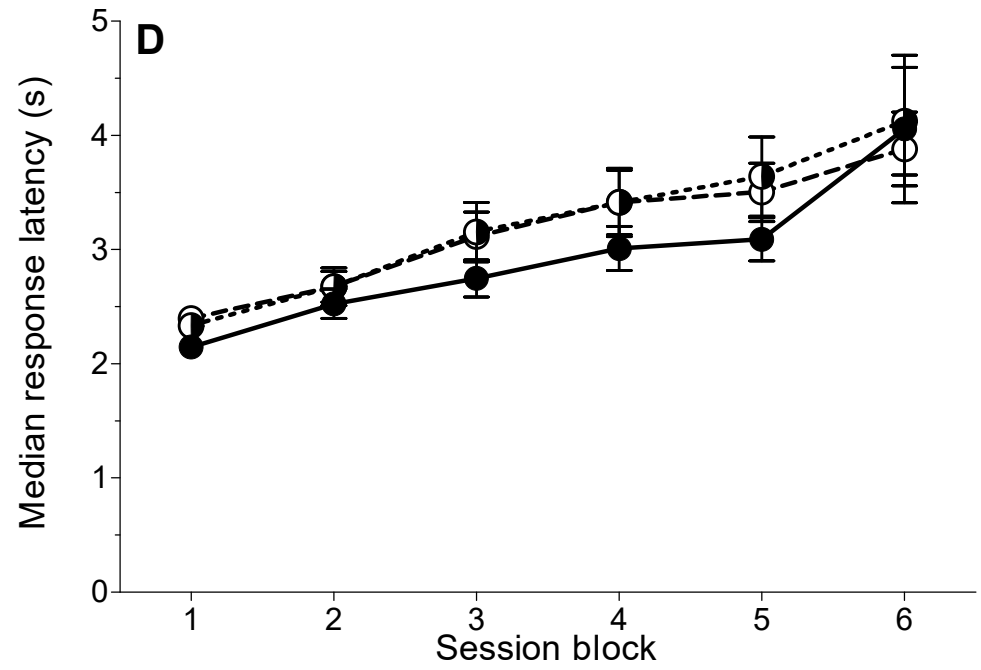

A
*
Number of trials to criterion
3000
2400
1800
1200
600
0
Control
CMS resilient
CMS anhedonic
B
Number of correction trials
2000
1500
1000
500
0
Control
CMS resilient
CMS anhedonic
C
Median response latency (s)
5
4
3
2
1
0
Control
CMS resilient
CMS anhedonic
D
**
Maximum number of consecutive correct trials per session
12
9
6
3
0
Control
CMS resilient
CMS anhedonic

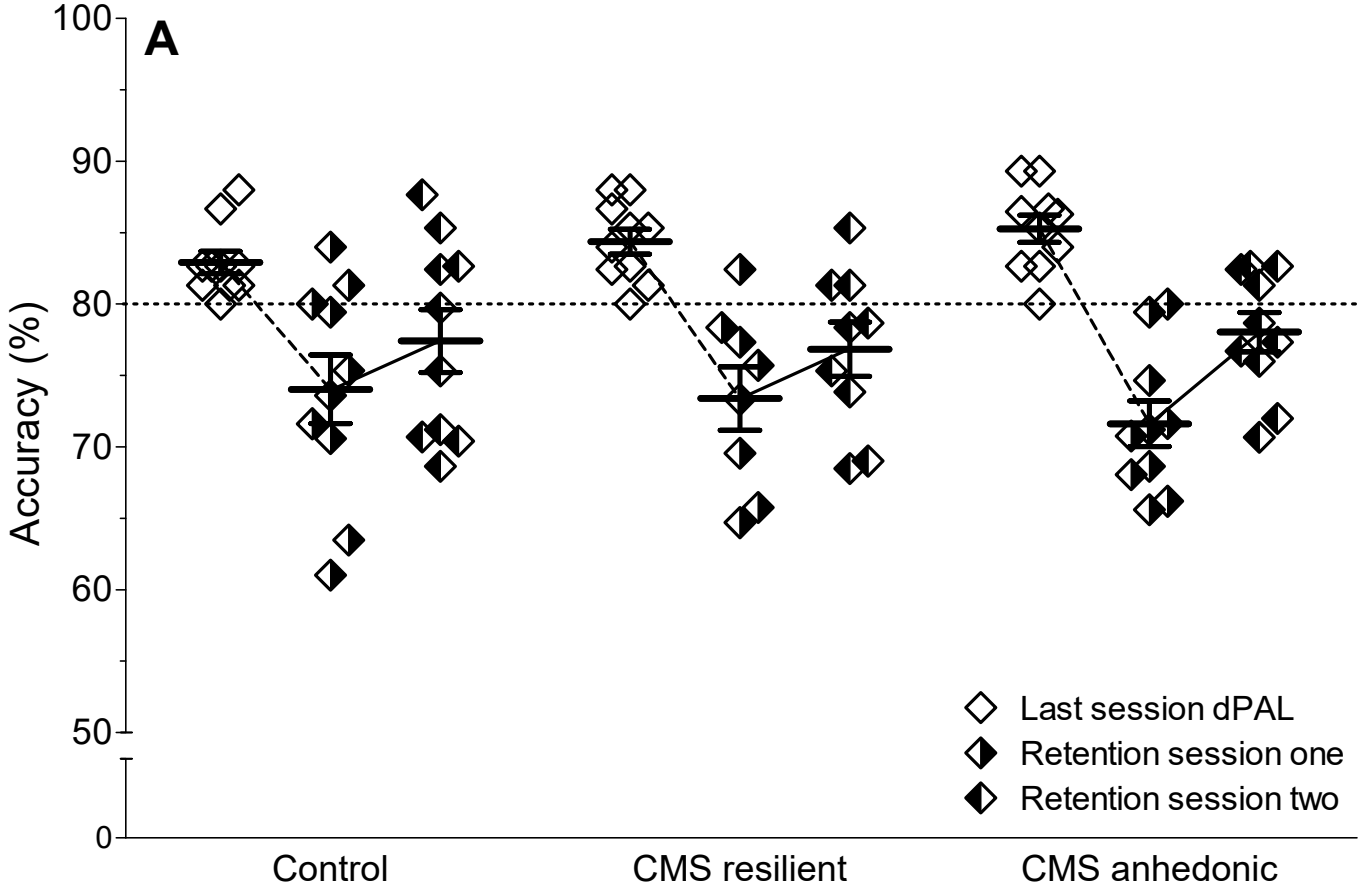

A
Accuracy (%)
Bins of trials
Control
CMS resilient
CMS anhedonic
B
Number of trials
Bins of trials
Trials
Trials + correction trials
C
Number of redundant screen touches per trial
Bins of trials
**
***
#
*
D
Maximum number of consecutive correct trials
Bins of trials
Control
CMS resilient
CMS anhedonic
*
*


A
Accuracy (%)
65
61
57
53
0
Control
CMS resilient
CMS anhedonic
1
2
3
4
5
6
Session block


B
Number of redundant screen touches per trial
2.0
1.5
1.0
0.5
0.0
**
*
1
2
3
4
5
6
Session block


C
Maximum number of consecutive correct trials
8
6
4
2
0
1
2
3
4
5
6
Session block


D
Median response latency (s)
5
4
3
2
1
0
1
2
3
4
5
6
Session block


A
100
90
80
70
60
50
0
Accuracy (%)
Last session dPAL
Retention session one
Retention session two
Control
CMS resilient
CMS anhedonic

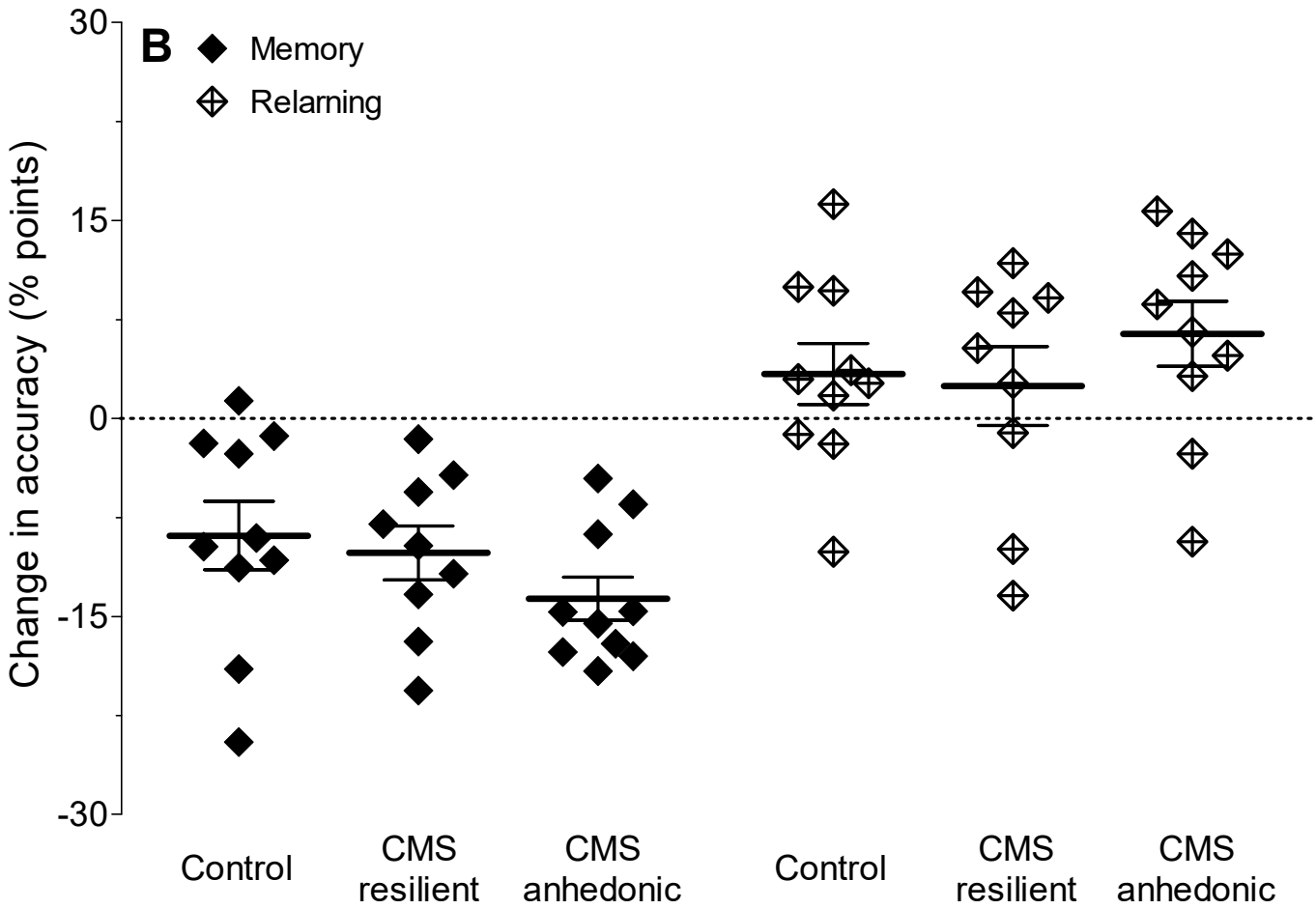

B
Memory
Relarning
30
15
0
-15
-30
Change in accuracy (% points)
Control
CMS resilient
CMS anhedonic
Control
CMS resilient
CMS anhedonic

Table A.1

The modified chronic mild stress (CMS) protocol. Time of stress exposure is given in brackets. Stressors are imposed on the CMS group only; whereas '*' indicates that both, CMS and control rats, undergo the procedure.

	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
Morning	New cage*, body weight measurement*			New cage			
Evening	Stroboscopic light (20.00–02.00)	Cage tilt 45° (17.15–07.15)	Wet bedding (17.15–07.15)	Light on (20.00–22.00, 00.00–02.00, 02.00–04.00)	Cage tilt 45° (17.15–07.15)	Stroboscopic light (20.00–22.00, 03.00–04.00) Light on (00.00–02.00)	Wet bedding (17.15–07.15)