Socioemotional deficit and HPA axis time response in high compulsive rats selected by schedule-induced polydipsia

Elena Martín-González, Manuela Olmedo-Córdoba, Ángeles Prados-Pardo, Daniel J. Cruz-Garzón, Pilar Flores, Santiago Mora, Margarita Moreno

Department of Psychology and Health Research Center (CEINSA), University of Almería, Spain

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ABSTRACT

Compulsivity is a failure to stop an ongoing behavior that has become inappropriate to the situation and is recognized as a transdiagnostic trait present in different neuropsychiatric disorders. The implication of motivation and emotion, as well as the stress response in compulsive population has not been fully understood. We assessed the motivation to reward and cues, the emotional response in different contexts and the hypothalamic-pituitary-adrenal (HPA) axis response in rats selected by a preclinical model of compulsive behavior. Firstly, high (HD) or low (LD) drinkers were selected according to their drinking behavior on schedule-induced polydipsia (SIP). Then, we assessed motivation by the propensity to attribute incentive salience to rewards on Pavlovian Conditioned Approach (PavCA) and motivation to gain reward on Progressive Ratio Schedule of Reinforcement (PRSR). Emotion was measured by Social Dominance on the Tube Test (SDTT) and emotional memory on Passive Avoidance (PA). Plasma corticosterone (CORT) levels in response to SIP were assessed. HD rats showed a socioemotional deficit by fewer victories on the SDTT, and an increased latency to enter the dark compartment on the PA. No differences were found between groups regarding to motivational assessment. Moreover, HD rats revealed a blunted time response in the increase of CORT levels at 45 min after SIP compared to LD rats. The findings show that the compulsive phenotype of HD rats exhibit less social dominance, more resistance to extinction and a differential CORT time response to SIP. These findings may contribute to highlight the relevance of assessing socioemotional behaviors and stress response for a better characterization of the vulnerability to compulsive spectrum disorders.

1. Introduction

Compulsions are stereotyped behaviors, conducted following to rigid rules and performed to decrease or avoid unpleasant consequences (Chamberlain et al., 2009). The presence of compulsions characterize obsessive-compulsive and related disorders (OCRDs), which include obsessive-compulsive disorder (OCD), hoarding disorder, body dysmorphic disorder, skin-picking disorder, and hair-pulling disorder (American Psychiatric Association, 2013). Thus, compulsivity could be considered a transdiagnostic trait, which may be a problem for the traditional diagnostic systems, prevention, and treatment (Den Ouden et al., 2020). The new strategies proposed, the Roadmap for Mental Health Research in Europe ROAMER (Haro et al., 2014) and U.S. National Institute of Mental Health (NIMH) Research Domain Criteria RDoC (Insel et al., 2010), are based on the dimension of altered behavior (Fineberg et al., 2016), highlighting the importance of evaluating behavioral and cognitive patterns associated with certain transdiagnostic traits, such as compulsivity.

There is some evidence that shows how motivation and emotion might be altered in compulsive spectrum disorders, and we have tried to collate some of the main findings. In this sense, altered motivation has been observed in OCD patients by impairment in goal-directed behavior and maladaptive habit learning (Gillan and Robbins, 2014), as well as the processing of motivational incentive stimuli and motivation to gain a reward (Jung et al., 2011). Moreover, altered emotion has been observed by disrupted affective processing of feedback from both social and environmental circumstances is linked to OCD symptoms (O’Kearney, 2001), and the social deficit is highly linked to compulsive
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2. Materials and methods

2.1. Animals

48 male Wistar rats from Envigo (Barcelona, Spain), weighing between 225 and 250 g at the beginning of the experiment, were used in the present study. Animals were housed four rats per cage (50 × 35 × 20 cm), kept in a temperature-controlled environment at 22 °C, with a 12:12 h light-dark cycle (lights off at 08:00 h). Water and food were freely available and environmental enrichment consisting of wooden blocks provided throughout the experiment. After 10 days for habituation and before behavioral tasks, animals were gradually reduced to 85% of their free-feeding body weight relative to a standard growth curve through controlled feeding (data not shown). 30 min after each daily experimental session, food was provided. All testing was carried out between 9:00 and 15:00 h. All the procedures were conducted in accordance with the Spanish Royal Decree 53/2013 and the European Community Directive (2010/63/EU) for animal research. The present study was also approved by the Animal Research Committee of the University of Almeria and complied with the ARRIVE guidelines. The authors declare that the research shows commitment to the 3Rs principle (replacement, reduction, refinement).

2.2. General procedure

2.2.1. Schedule-induced polydipsia (SIP)

2.2.1.1. Description of the apparatus

Rats were tested in 8 standard operant chambers (32 × 25 × 34 cm) (MED Associates, St. Albans, VT, USA) equipped with a pellet dispenser, bottle of water, and ambient light. The programing and recording of experimental events were automatically controlled using Med PC IV computer and commercial software (Cibertec SA, Spain).

2.2.1.2. Behavioral procedure

Before carrying out SIP, for two consecutive days, the amount of water ingested was evaluated for 60 min, to obtain a baseline. There was unlimited access to a bottle of fresh water and a reward of 60 pellets (Noyes 45-mg dustless reward pellets; TSE Systems, Germany) deposited together in each feeder in each baseline session. After one day of habituation to the operant boxes, rats were exposed during 60 min sessions to a food pellet presentation using a fixed time 60s (FT-60s) schedule. There was a bottle containing fresh tap water in the wall opposite the pellet dispenser. After 20 daily sessions and following the protocol described by Moreno et al. (2012), animals were classified into low drinkers (LD) and high drinkers (HD), depending on whether the water intake (average of the last 5 sessions) was above or below the median of the group. The following measures were recorded for each rat: the total amount of water (milliliters) removed from the bottle, the total number of licks to the bottle, and the total number of entries to the food magazine (Mora et al., 2018).

2.2.2. Experimental design

The experimental design is depicted in Fig. 1. The order of testing was as follows: SIP, motivational measures (Pavlovian Conditioned Approach, PavGA and Progressive Ratio Schedule of Reinforcement, PRSR), and emotional measures (Social Dominance Tube Test, SDTT and Passive Avoidance, PA). The order of the tasks was chosen based on their presumably increasing stressful effect, since they could not be randomized due to their different duration in days. Each task commenced at least 20 days after the previous one. Twenty days after the end of the motivational and emotional assessment, we assessed the HPA axis response to SIP in HD and LD rats, with a control of each group that were neither re-exposed. Therefore, half of each group of rats, HD and LD, were re-exposed to SIP procedure (RE); while the other half of each group of rats were not re-exposed to SIP (NRE), and rested in their home-cage but under the same food deprivation conditions. Consequently, HD and LD rats were divided into two different conditions: no re-exposure (NRE) and re-exposure (RE), with four experimental groups: HD-NRE (n = 12), HD-RE (n = 12), LD-NRE (n = 12), and LD-RE (n = 12). After SIP re-exposure (session 20), three blood samples were obtained in LD-RE and HD-RE groups: immediately (time zero, t0), at 45 (145), and 90 min (190) after SIP. Blood samples were collected after 20 sessions to ensure a stable level of SIP acquisition and the timing was chosen.

spectroscopy such as OCD, autism spectrum disorder (ASD) or attention deficit hyperactivity disorder (ADHD) (Baribeau et al., 2019). Indeed, social defeat and subordination might be an important contributor factor to developing emotional disorders such as depression (Gardner and Wilson, 2004). Finally, harm avoidance is an important motivational factor underlying compulsive behavior in OCD (Bejerot et al., 1998). Avoidance behavior is a characteristic pattern that may be also associated with disorders with compulsive symptomatology, such as post-traumatic stress disorder (PTSD), avoidant personality disorder, anxiety disorders, alcohol use disorder, and avoidant/restRICTive food intake disorder (American Psychiatric Association, 2013). Moreover, people with a behavioral compulsive pattern in OCD and addiction disorders exhibit higher experiential avoidance as an effect of the distress generated by the situation (Gillan et al., 2020; Den Ouden et al., 2020).

Compulsivity might be also related to an aberrant response to stress. Clinical studies have indicated that stressors such as significant loss, increased responsibility, and exposure to traumatic situations may precede the vulnerability of the development of OCD (Brandriff et al., 2016). According to that, the hypothalamic-pituitary-adrenal (HPA) axis, one of the most important endogenous adrenal steroid system in mammals, which modulates cortisol secretion (corticosterone in rodents), has been associated with psychopathological symptoms (Randelow et al., 2017). However, different studies have found some contradictory results. In baseline conditions, some studies did not find any differences (Kawano et al., 2013), whereas Kluge et al. (2007) found an increase in cortisol and adrenocorticotropic hormone (ACTH) in OCD patients compared to healthy subjects. In contrast, hair cortisol levels were lower in OCD patients compared to healthy subjects, showing a possible down-regulation of the HPA axis (Koumantarou Malisiova et al., 2020). Moreover, cortisol levels were increased in healthy subjects and reduced in OCD after a stressor (Gustafsson et al., 2008).

Preclinical research could help to understand the relationship between compulsivity and their behavioral and neuroendocrine markers. Using an animal model of compulsivity (Moreno and Flores, 2012) called schedule-induced polydipsia (SIP), food-deprived animals exposed to an intermittent reinforcement schedule develop a persistent and excessive addictive behavior (Falk, 1961, 1966). This animal model is effective not only for the study of the compulsive phenotype but also for common compulsive spectrum disorders due to their transdiagnostic characteristic (Moreno and Flores, 2012; Belin-Rauscent et al., 2016). According to SIP acquisition, two populations can be selected: high drinker (HD) rats, considered as compulsive, versus low drinker (LD) rats, considered as non-compulsive (Moreno and Flores, 2012). HD animals exhibit reinforcer devaluation insensitivity and persistent behavior (Merchán et al., 2019). In addition, corticosterone (CORT) had been measured after SIP exposure in classical studies (Brett and Levine, 1979, 1981; Wallace et al., 1983; Tazi et al., 1986; Dantzer et al., 1988; Mittleman et al., 1988; L´opez-Grancha et al., 2018), and following the protocol described by Moreno et al. (2012), animals were classified into low drinkers (LD) and high drinkers (HD), depending on whether the water intake (average of the last 5 sessions) was above or below the median of the group. The following measures were recorded for each rat: the total amount of water (milliliters) removed from the bottle, the total number of licks to the bottle, and the total number of entries to the food magazine (Mora et al., 2018).
according to the literature (Gagliano et al., 2014; Nadal et al., 2021). In the no re-exposure condition (NRE), only one blood sample was collected in LD-NRE and HD-NRE groups. The rationale of this experimental design was to expand the knowledge on CORT time-response after SIP and also without its exposure in previously selected HD and LD rats. In our group, Merchan et al., 2019 measured CORT in rats before SIP exposure, finding no difference between HD and LD rats. Food deprivation levels were maintained in all experimental conditions.

2.2.3. Motivational assessment

2.2.3.1. Pavlovian Conditioned Approach (PavCA): propensity to attribute incentive salience to rewards and their associated stimuli

2.2.3.1.1. Description of the apparatus. Rats were tested in 6 standard operant chambers identical to those described in Section 2.2.1 but equipped with one retractable lever located on the right or on the left (counterbalanced) of the pellet dispenser.

2.2.3.1.2. Behavioral procedure. Five daily sessions of Pavlovian training were carried out. During training, the lever was extended into the operant chamber for 8 s, while the light behind the lever was illuminated. After 8 s the lever was retracted, the light extinguished, and a food pellet delivered. In each training session, 25 lever-pellet were pairings using a variable time VT-90 s schedule (i.e., presentation of the conditioned stimulus CS and unconditioned stimulus US varied pairings using a variable time VT-90 s schedule (i.e., presentation of the operant chamber for 8 s, while the light behind the lever was illuminated. After 8 s the lever was retracted, the light extinguished, and a food pellet delivered. In each training session, 25 lever-pellet were pairings using a variable time VT-90 s schedule (i.e., presentation of the conditioned stimulus CS and unconditioned stimulus US varied randomly between 30 and 150 s, with an average of 90 s). Measured recorded during the 5 sessions were lever presses, magazine entries during the presence of the CS, the probability of lever press or magazine entry during the presence of the CS (number of trials with a lever press or magazine entry/total number of trials), and the latency to the first lever press or magazine entry (Meyer et al., 2012).

2.2.3.2. Progressive Ratio Schedule of Reinforcement (PRSR): motivation to gain reward

2.2.3.2.1. Description of the apparatus. Rats were tested in 6 standard operant chambers identical to those described in Section 2.2.1 but equipped with two retractable levers located on each side of the pellet dispenser.

2.2.3.2.2. Behavioral procedure. PRSR session began with both levers extended and response on one of them (onwards the “active” lever) was rewarded with a food pellet. During the session, responses on the “active” lever were rewarded, but the number of lever presses required augmented progressively with each successive food reward obtained. The steps of the exponential progression used were (56×0.2×reward number) – 5, rounded to the nearest integer (i.e. 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc.). Both levers were retracted after each pellet delivery for an inter trial interval (ITI) of 10 s. Test session lasted 30 min. Responding on the “inactive” lever had no consequences. PRSR lasted one session. The following variables were measured: total number of lever presses in the “active” lever, the total number of lever presses in the “inactive” lever, and the breakpoint (ratio at which an animal stops responding in a session). The behavioral procedure was modified from a previously described task (Rygula et al., 2015).

2.2.4. Emotional assessment

2.2.4.1. Social Dominance Tube Test (SDTT): social dominance

2.2.4.1.1. Description of the apparatus. SDTT was conducted using opaque PVC tubes (100 × 7 cm), with a small longitudinal aperture at the top to check the animal localization. Three gates were placed at the tube external segments and the center.

2.2.4.1.2. Behavioral procedure. After one day of habituation to the experimental room, animals were trained for 5 consecutive days. During training, after some gentle pressure to encourage rats to move through the tube, animals started to move back and forth into the apparatus to get reinforcement at the end of the tube. All rats were rewarded for traveling through the tube in both directions. Training sessions last about 10 min per rat. After training, we assessed social dominance. Each animal was faced 3 consecutive times with 3 different unknown animals (9 approaches for each animal) with similar weight. Experimental group (LD or HD) belonging was randomized. Following each approach, animals were kept resting for 45 s until the next approach. The apparatus was cleaned with a disinfectant between animals. Secondly, 48 h after the dominance test, we assessed hierarchical status in the same apparatus, where each rat faced with the rest of the co-habitants 3 times each (9 approaches for each animal). The criteria for victories was defined when the opponent placed the four paws out of the tube in its initial external site. The percentage of victories of each animal was measured (for more details see Perez-Fernandez et al., 2020). No other behavior was measured during the approaches.

2.2.4.2. Passive Avoidance (PA): emotional memory and extinction

2.2.4.2.1. Description of the apparatus. For the Passive Avoidance (PA) test, a hand-made apparatus (60 × 30 × 30 cm) was used. It consisted of a lighted compartment, a dark compartment, a guillotine door that connected both compartments, and a grid floor connected to the scrambler shocker (Med Associated, Inc.).

2.2.4.2.2. Behavioral procedure. During the exploration sessions, the animal was placed in the lighted compartment and allowed to move freely through both compartments for 300 s for two consecutive days. In the conditioning session, 24 h after the last exploration session, the animal was placed in the lighted compartment and the door was closed and the US is presented when entering the dark compartment. The presentation of US (an inescapable foot shock, 2 mA, 2 s) leads to a learned emotional state and the subsequent conditioned responses, the avoidance response (Ogren and Stiedl, 2015). Timing and strength for the electric shock was carefully considered and based on published protocols (Anaeigoudari et al., 2015; Jahangiri et al., 2019). Finally,
during the test sessions, the animal was placed again into the lighted compartment and their latency to enter the dark compartment was measured at different time points after the conditioning session. Thus, the latency to enter the dark chamber (in seconds) was assessed at each test session (Jahangiri et al., 2019). During the test sessions, the animal was placed again into the lighted compartment and their latency to enter the dark compartment was measured (mean of two consecutive sessions), at 2 h (acquisition test), and at 24 h, 48 h, and 10 days (extinction tests) after the conditioning session (Jahangiri et al., 2019).

2.2.5. Plasma corticosterone levels: dynamic of HPA axis time response

Blood samples (300 μl) were collected in all animals (RE and NRE groups) in eppendorfs containing EDTA. For studying the dynamic of HPA axis time response, samples were obtained from the lateral tail vein in HD-RE and LD-RE groups, immediately after SIP re-exposure (time zero, t0) and at 45 min (t45). After each blood sample collection, rats recovered from anesthesia and were kept undisturbed in their home cage until the next extraction. Finally, at 90 min (t90) blood collection was obtained from the jugular vein after decapitation in all groups (including HD-NRE and LD-NRE). All procedures were done after being briefly anesthetized with isoflurane (approximately 30s), in order to reduce the stress of the tail-nick and decapitation. Isoflurane inhalation has been previously used in our lab and others for HPA studies (Merchan et al., 2019; Bach et al., 2019; Wegman-Points et al., 2020; Markey et al., 2020). Therefore, it is an ethical and optimal choice widely used for studies on HPA axis response (Altholtz et al., 2006; Wu et al., 2015).

Plasma was separated by centrifugation (Sigma 3-18KS, Germany) of the blood samples at 3000 rpm (RCF = 800 × g) for 20 min at 4 °C. The plasma was then stored at −20 °C until analysis. CORT levels were analyzed using DetectX® enzyme immunoassay kit (K014-H1, DetectX®, Arbor Assays™, Ann Arbor, USA). The inter- and intra-assay coefficients of variation were 2.5% and 6.3%, respectively. The sensitivity of the assay was 14.35 pg/ml.

2.3. Statistical analysis

SIP acquisition was analyzed using a two-way repeated-measures analysis of variance (ANOVA), with “group” (LD and HD) as between-subject factor and “sessions” (20 sessions) as the within-subject factor. Regarding PavCA, lever presses, magazine entries during the presence of the CS, magazine entries during the absence of the CS, and the latency to the first lever press or magazine entry were analyzed using a two-way repeated-measures ANOVA, with “group” (LD and HD) as between-subject factor and “sessions” (5 sessions) as the within-subject factor. The differences in the variables of PRSR (i.e. total number of lever presses in the “inactive” lever, the total number of lever presses in the “active” lever and the breakpoint) were analyzed using Student’s t-test (t-test). Moreover, the differences in hierarchy and dominance in the SDTT measured by the percentage of victories, were analyzed using t-test.

As the data is expressed in percentages, the variable of percentage of victories was arcsine transformed before analyses to limit the effect of an artificially-imposed ceiling (McDonald, 2009). For PA, data of the exploration sessions (mean of two days) and the test sessions were analyzed using a two-way repeated-measures ANOVA, with “group” (LD and HD) as the between-subject factor and “sessions” as the within-subject factor. SIP re-exposure for RE groups data was analyzed using either two-way repeated-measures analysis of variance (ANOVA) with a between-subject factor (group: HD and LD) and a within-subject factor (re-exposure: acquisition and re-exposure), for comparison acquisition vs re-exposure, or using one-way analysis of variance (ANOVA), with between-subject factor (group: HD and LD), for comparison HD vs LD within re-exposure. Differences in plasma CORT levels between LD-NRE and HD-NRE were analyzed by Student’s t-tests (t-test). For assessing the CORT time response to SIP in re-exposure groups, plasma CORT levels were analyzed using a two-way repeated-measures ANOVA, with “group” (LD and HD) as the between-subject factor and “times” (t0, t45, and t90) as the within-subject factor. Post hoc analyses were performed using Bonferroni correction when appropriate. Statistical significance was established at p < 0.05. Effect size is reported when appropriate; Partial eta-squared values of 0.01, 0.06, and 0.14 and Cohen’s d values of 0.2, 0.5, and 0.8 are considered to reflect small, medium, and large effects, respectively (Cohen, 1988). Data in graphs are expressed as mean ± SEM. All analyses were performed with Statistica® software (version 8.0) and all figures were made using GraphPad Prism 8.

3. Results

3.1. Screening compulsivity by schedule-induced polydipsia (SIP)

The mean total licks, water intake, and total magazine entries for LD and HD through 20 SIP sessions are shown in Fig. 2. The mean ± SEM total number of licks during the last 5 days of SIP was 874.02 ± 70.77 for LD and 2517.99 ± 235.69 for HD. SIP acquisition was also evident in the water intake. The mean ± SEM water intake during the last 5 days of SIP was 4.13 ± 0.27 ml for LD and 7.13 ± 1.06 ml for HD. Concerning the total number of licks, repeated measures ANOVA revealed significant differences according to the interaction between the SIP acquisition sessions and LD vs HD (interaction SIP session × group effect: F(19, 874) = 11.33, p < 0.001; η2p = 0.2). Repeated measures ANOVA also showed a significant interaction in water intake (interaction SIP session × group effect: F(19, 874) = 47.81, p < 0.001; η2p = 0.51). Post hoc analysis indicated that SIP induced different rates in drinking behavior across the 20 sessions in both groups. In the total number of licks, the LD and HD group differed in session 3 (p = 0.02; d = 1.47) and the HD group increased their number of licks in session 4 (p = 0.02; d = 1.38) compared to session 1. Similar differences between LD and HD were found in water intake: the LD and HD group differed in session 4 (p = 0.03; d = 1.17) and the HD group increased their number of licks in session 5 (p = 0.03; d = 1.1) compared to session 1. There were no significant differences between LD and HD animals in the total magazine entries on SIP (SIP session interaction × group effect: F(19, 874) = 0.58, p = 0.92).

3.2. Motivational assessment

3.2.1. Pavlovian Conditioned Approach (PavCA)

Lever-directed (sign-tracking) and magazine-directed (goal-tracking) behaviors were assessed across five consecutive PavCA sessions for LD and HD animals and data are shown in Fig. 3. Repeated measures ANOVA revealed no significant differences in the interaction between the PavCA sessions and LD vs HD in the probability to lever press (interaction PavCA session × group effect: F(4, 184) = 2.29, p = 0.06) or magazine entries (interaction PavCA session × group effect: F(4, 184) = 0.45, p = 0.77); totallever presses (interaction PavCA session × group effect: F(4, 184) = 0.34, p = 0.85) or total magazine entries during the presence of the CS (interaction PavCA session × group effect: F(4, 184) = 1.85, p = 0.12); latency to the first lever press (interaction PavCA session × group effect: F(4, 184) = 1.96, p = 0.1), or latency to the first magazine entry (interaction PavCA session × group effect: F(4, 184) = 1.36, p = 0.25).

3.2.2. Progressive Ratio Schedule of Reinforcement (PRSR)

The total number of lever presses in the active or the inactive lever and the breakpoint in the PRSR are shown in Fig. 4. t-Test analysis revealed that the total number of lever presses on the inactive lever was lower in HD animals compared to LD (df = 46; t-test = 2.24; p = 0.03; d = 0.66). However, no significant differences between phenotypes were observed in the total number of lever presses on the active lever (df = 46; t-test = −0.42; p = 0.68), or the breakpoint (df = 46; t-test = −0.6; p = 0.55).
3.3. Emotional assessment

3.3.1. Social Dominance Tube Test (SDTT)

Before SDTT analysis, transitivity (when animal A beats B, and B beats C, A must beat C) was evaluated to validate the paradigm and showed high rates of transitivity in both dominance (83%) and hierarchy (83%).

The percentage of victories against an unknown competitor (dominance) and the percentage of victories against a known competitor (hierarchy) are shown in Fig. 5. t-Test revealed that HD rats had a significantly reduced percentage of victories against unknown competitors compared to LD rats (df = 46; t-test = 2.23; p = 0.03; d = 0.65). However, no significant difference was found between phenotypes in the percentage of victories against known competitors (df = 46; t-test = 0.02; p = 0.98).

3.3.2. Passive Avoidance (PA)

Fig. 6 shows the latency to enter the dark compartment on PA for LD and HD rats. Repeated measures ANOVA showed a significant interaction in latency to enter the dark compartment between groups and test sessions on PA (session x group interaction effect: F(4,184) = 3.44; p = 0.01; η²p = 0.07). Post hoc analysis revealed that both groups showed acquisition of PA 2 h after receiving the electric shock, by the significant increase in latency to enter the dark compartment in LD (p < 0.001; d = 3.55) and HD (p < 0.001; d = 6.64), compared to the exploration session. This significant increase was maintained under the extinction tests in both groups, 24 h (LD p < 0.001, d = 7.07; HD p < 0.001, d = 16.38), 48 h (LD p < 0.001, d = 3.69; HD p < 0.001, d = 15.09) and 10 days (LD p < 0.001, d = 2.92; HD p < 0.001, d = 5.52) after receiving the electric shock, compared to the exploration session. However, on the last day of the extinction tests (day 10), the LD group showed a significant reduction in their latency to enter the dark compartment compared to 2 h (p = 0.04; d = 0.54), 24 h (p < 0.001; d = 1.03) and 48 h tests (p = 0.02; d = 0.6). In contrast, HD animals remained showing an increase in their latency to enter the dark compartment 10 days under extinction, showing no significant differences compared to the previous extinction tests: 2 h (p = 1), 24 h (p = 1) and 48 h (p = 1). Therefore, HD rats showed a significantly higher increased latency to enter the dark compartment 10 days on extinction test compared to LD rats (p = 0.01; d = 0.83).

3.4. SIP re-exposure

In SIP acquisition, HD and LD animals selected for no re-exposure (NRE) and re-exposure (RE) sub-groups, showed significant differences in the mean of total number of licks (in NRE: F(1,22) = 104.78; p < 0.001; η²p = 0.83; and in RE: F(1,22) = 13.24; p = 0.001; η²p = 0.38) and water intake (in NRE: F(1,22) = 26.3; p < 0.001; η²p = 0.54; and in RE: F(1,22) = 62.96; p < 0.001; η²p = 0.74) in the last 5 sessions. RE groups carried out a second SIP procedure, until they recovered their previous drinking levels. On the 20th session of re-exposure to SIP, there were no differences compared to their last 5 SIP sessions exposure in total number of licks (SIP re-exposure effect: F(1,22) = 3.86; p = 0.25) or water intake (SIP re-exposure effect: F(1,22) = 3.86; p = 0.06); however, group differences between HD and LD were still significant both in total number of licks (group effect: F(1,22) = 5.11; p = 0.03; η²p = 0.19) and water intake (group effect: F(1,22) = 7.64; p = 0.01; η²p = 0.26) after SIP re-exposure. Data not shown.

3.5. Corticosterone assessment

Fig. 7 shows the mean of plasma CORT levels (ng/ml) for the non-re-exposure (LD-NRE and HD-NRE) and re-exposure groups to SIP (LD-RE and HD-RE). In NRE SIP condition (Fig. 7A), no significant differences were identified between HD and LD rats in plasma CORT levels (t22 = 1.49; p = 0.15). Fig. 7B shows the mean plasma CORT levels (ng/ml) for
the time response after SIP in the re-exposed groups. Repeated measures ANOVA revealed significant differences in plasma CORT levels according to the interaction between time and groups (interaction time point x group effect: $F_{(2,44)} = 7.39; p < 0.002; \eta^2p = 0.25$). Post hoc analysis showed significant differences in the CORT time response to SIP in both groups. Specifically, at t45 and at t90 there were an increase of plasma CORT levels in the HD-RE group ($p = 0.02; d = 1.81$; $p = 0.03; d = 1.32$) and in the LD-RE group ($p < 0.001; d = 2.57; p < 0.001; d = 1.99$) compared to the measure t0. However, HD-RE rats showed significantly lower plasma CORT level compared to LD-RE rats at t45 after SIP re-exposure ($p = 0.04; d = 2.98$) and this difference is not significant at t90 ($p = 0.28$).

4. Discussion

The present study explored the possible alteration in motivational and emotional mechanisms, assessing the dynamic of HPA axis time response, in a compulsive phenotype of rats selected by SIP. There were no differences in motivational behaviors between compulsive HD and non-compulsive LD animals. However, in the assessment of emotional behaviors, HD rats, characterized by persistent and excessive compulsive drinking on SIP, were prone to be submissive during a social
encounter with an unknown competitor on SDTT, and also were more resistant to extinction on PA test, shown by a sustained latency to enter the dark compartment at the last extinction session compared to LD rats.

Moreover, both groups increased plasma CORT levels after SIP re-exposure, but HD animals had a significant blunted response compared to LD animals. These results are discussed in terms of the implication of motivational and emotional factors on compulsivity, and its relation to the HPA axis time response.

4.1. Preserved motivation in compulsive HD rats selected by SIP

We did not observe differences between LD and HD rats in their propensity to attribute incentive-motivational salience to rewards and their associated stimuli on PavCA. According to our data, the transgenic SAPAP3−/− (animals with genetic deletion of synapse-associated protein 90/postsynaptic density protein 95 associated protein 3) a model of compulsive behavior, showed a similar acquisition rate in the Pavlovian conditioning task compared to wild type animals (van den Boom et al., 2019). Nevertheless, there could be a relationship between the individual variation in the attribution of incentive salience to cues and certain compulsive behaviors such as compulsive checking. Some studies revealed that animals characterized as sign-trackers on PavCA develop more dysfunctional extra observing lever presses on observing response tasks, a model of OCD (Eagle et al., 2020). In clinical studies, OCD and generalized anxiety disorder (GAD) patients, a compulsivity dimension was negatively associated with goal-directed performance. However, other symptom dimensions such as obsessionality or general distress were related to goal-directed behavior (Gillan et al., 2020). These results indicate that the propensity to attribute incentive-motivational salience to cues might be associated with a compulsivity
dimension that the HD compulsive phenotype of rats selected by SIP might not resemble, which is in accordance with the multifaceted nature of compulsive spectrum disorders.

LD and HD rats selected by SIP did not exhibit any differences in motivation to gain reward on PRSR. Our data suggest that motivation measured by PRSR is not related to compulsivity on SIP. The reason might rely on the fact that PRSR is testing the cognitive component of motivation (Rygula et al., 2015) but not the incentive value of the food reinforcer (Cordony et al., 2019) that could underlie the development of compulsive behavior. Interestingly, HD animals showed a reduced number of lever presses to the inactive lever compared to LD animals. This result may be related to cognitive inflexibility in HD animals demonstrated by an increased number of perseverative responses on Reversal Learning (Navarro et al., 2017; Merchán et al., 2019). Therefore, the progressive increase in the requirement of lever presses on the PRSR task might cause an extinction condition; where the LD rats performed a flexible response and change to check the inactive lever, while the HD rats were more resistant to change and persevered in the same lever. Thus, pointing towards an alteration in the neuropsychological mechanisms that underlie the learning of habits in the behavioral compulsive pattern (Everitt and Robbins, 2016).

4.2. Altered emotion in compulsive HD rats selected by SIP: impaired social dominance and resistance to extinction

Previous results in our group using classical emotional tests, have demonstrated that HD animals presented an increased fear memory by a higher percentage of freezing in the retrieval day in the Fear Conditioning test compared to LD animals (Prados-Pardo et al., 2019). However, this emotional response of HD rats might not be associated with an anxiety trait, because we did not find any differences between HD and LD groups in the time and number of entries in the open arms in EPM (López-Grancha et al., 2008; Prados-Pardo et al., 2019). According to these findings, we further explored the emotional behavior by procedures that assess the responses to potential aversive events, such as the SDTT and the PA task. Both tests have stressful events, such as a social competitor or an electric shock, respectively. The study of the emotional behavioral responses to negative events extends the knowledge about the compulsive phenotype of rats selected by SIP.

The assessment of social dominance by SDTT revealed that HD rats selected by SIP showed less significant victories against unknown opponents compared to LD rats. Many authors have linked social dominance to emotional domains (Kroes et al., 2006; Jones and Monfils, 2016; Kondrakiewicz et al., 2019). In fact, social dominance has been proposed as a basic animal and human emotion (for a review see van der Westhuizen and Solms, 2015). Our results are in accordance with the literature on preclinical models of compulsive-like disorders. In a rat model of autism-like behavior, experimental animals showed impaired social dominance in SDTT compared to control animals (Win-Shwe et al., 2021). Clinical studies linked inhibitory control deficit with some socialization disorders (Ma et al., 2016). Thus, OCD patients had impairment in areas of work, social life, and family life (Huppert et al., 2009).

The assessment of emotional memory by PA test revealed no differences in conditioned fear acquisition between HD and LD rats. This result confirms previous studies in which HD rats did not exhibit differences in conditioned fear acquisition on FC test (Prados-Pardo et al., 2019) or Latent Inhibition (LI) test (Navarro et al., 2017) compared to LD rats. However, HD animals were more resistant to extinction on the PA test, shown by a sustained higher latency to enter the dark compartment at the last extinction session, 10 days after receiving an electric shock, compared to LD rats. Similar data were found in previous experiments where HD rats showed an augmented time of freezing compared to LD rats at the retrieval day during cued-fear memory in the FC test (Prados-Pardo et al., 2019). Interestingly, rats selected as Roman high avoidance (RHA) by their avoidance performance in the active avoidance (AA) test showed more compulsive drinking than Roman low avoidance (RLA) (Moreno et al., 2010). RHA rats also have a longer time to lead the extinction in the cocaine self-administration procedure (Fattore et al., 2009) and the partial reinforcement extinction effect was larger and longer-lasting in RHA (Fuentes-Verdugo et al., 2020) compared to RLA. Resistance to extinction is also observed using non-emotional tasks in HD rats: in a devaluation test (Merchán et al., 2019), on the Reversal Learning task (Navarro et al., 2017; Merchán et al., 2019), and under extinction on 5-CSRTT (Moreno et al., 2012) compared to LD rats. Extinction does not involve the destruction of the original learning but implies a new inhibitory association that is context-dependent (Bouton, 2004). In this sense, HD animals could have a deficit in extinction learning and/or processing of contextual cues.

In clinical studies using FC paradigms, OCD patients also exhibited a deficit in fear renewal and extinction recall (Fyer et al., 2020).

4.3. CORT time response in HD compulsive rats selected by SIP

LD and HD rats selected by SIP showed no differences in CORT plasma levels in basal conditions (non-re-exposure condition). This is in accordance with previous data in our laboratory, in which LD and HD animals showed no differences in plasma CORT levels before SIP exposure (Merchán et al., 2019). Moreover, our data resemble the first demonstrations carried out by Dantzer et al. (1988), where at baseline levels in the colony room, there were no significant differences in CORT levels between SIP-positive (animals with excessive drinking) and SIP-negative (animals with low drinking rates). Regarding the CORT time response, both groups, LD and HD increased CORT levels at 45 and 90 min after SIP re-exposure. This result is in accordance with previous studies on SIP with subtle differences. Animals exposed to an inter-food interval of 30s (F30s) increased CORT plasma levels at 40th SIP session compared to the 3rd SIP session (López-Grancha et al., 2006). Animals after the 10th SIP sessions had an increase in CORT levels compared to the pretraining session (Mittelman et al., 1988). Rats exposed to a timed food delivery condition for 10 days increased CORT levels compared to rats that received no timed food (Wallace et al., 1983). Indeed, in a recent study, HD rats exhibited increased plasma CORT levels 24 h after the last SIP session compared to their basal level before SIP acquisition (Merchán et al., 2019). However, HD animals showed a blunted CORT time response compared to LD rats at 45 after the last SIP session. Our results are in accordance with several classic studies in which SIP exposure led to a reduction in CORT levels after 21 SIP sessions (Brett and Levine, 1979) and at 10, 20, and 30 min during the 22nd SIP session (Brett and Levine, 1981). Therefore, a decrease in CORT levels was shown during SIP in SIP-positive animals compared to SIP-negative animals, at 10 and 30 min during the 11th SIP session (Dantzer et al., 1988). Moreover, there was a negative significant correlation between the amount of water consumed and CORT levels after the 10th SIP session (Mittelman et al., 1988).

A classic explanation of this phenomenon is that excessive drinking may be a coping response to stress caused by intermittent food delivery (Brett and Levine, 1979; Brett and Levine, 1981; Wallace et al., 1983; Tazi et al., 1986; Dantzer et al., 1988; Mittelman et al., 1988; López-Grancha et al., 2006). In accordance with this hypothesis, animals with a proactive coping style exhibit low CORT production compared to animals with a reactive coping style that reacts passively to stress (Bowen et al., 2014). Rats exposed to early life stress showed compulsive behavior on the 5-CSRTT and a reduction in the reactivity of the HPA axis pointing towards an increase in the active coping strategy (Fuentes et al., 2014). Internal mechanisms lead to proactive coping responses; making animals with proactive styles insensitive to environmental changes in contrast to reactive coping animals (see review by Coppens et al., 2019). The insensitivity to environmental changes could be related to the deficit in extinction. Proactive animals show behavioral inflexibility compared to reactive animals (see review by Cockrem, 2013). In this sense, HD rats exhibited behavioral inflexibility in the
Reversal Learning task (Navarro et al., 2017; Merchán et al., 2019) and resistance to extinction in 5-CSRTT (Moreno et al., 2012) compared to LD rats.

4.4. Can CORT modulate the different facets of compulsivity?

The manipulation of the HPA axis might modulate compulsive drinking behavior on SIP. The intracerebroventricular administration of corticotropin-releasing hormone (CRH) reduced water intake on SIP (Cole and Koob, 1994). Moreover, adrenalectomized female rats failed to develop SIP but CORT administration restored the SIP expression in adrenalectomized rats, suggesting that corticosterone plays a main role in the development of excessive drinking (Levine and Levine, 1989). Moreover, the pharmacological manipulation of the HPA axis might moderate the ability to extinction (Lesuis et al., 2015). The relationship between social dominance and CORT levels remains unclear. In a model of social stress, subordinate stressed animals had higher levels of CORT at baseline but, in van der Straten according to our data, these animals showed decreased levels of CORT after EPM paradigm compared to control animals (Löfgren et al., 2012).

Both domains, resistance to extinction and social impairment are observed in OCD (Brock and Hany, 2021). Moreover, high cortisol levels have been observed in OCD in aversive situations (Fluitman et al., 2010). Indeed, different studies have found a blunt cortisol response to distress in OCD patients compared to healthy controls (van der Straten et al., 2020; Gustafsson et al., 2008). Thus, chronic low cortisol in OCD patients has been suggested to be linked to a down-regulation of the HPA axis, as an adaptive response to chronic stress exposure (Koumantarou Malisova et al., 2020). Cortisol facilitates the consolidation of extinction learning in differential fear conditioning in healthy participants (Brueckner et al., 2019). Moreover, high levels of cortisol improve the effects of exposure treatment in patients with anxiety disorders (Meuret et al., 2016). In fact, PTSD patients who positively respond to therapy, increase their cortisol levels after exposure therapy (van Gelderen et al., 2020).

5. Conclusions

The present study suggests that HD rats selected by SIP exhibit alterations in socioemotional but not motivational mechanisms. The lack of differences in both motivational paradigms might be explained by the transition between goal-directed and habitual actions: compulsive behavior might emerge from excessive habit formation, not due to a goal-directed learning per se. Moreover, differences in both emotional, but not motivational, measures might be explained by the hypothesis that compulsive animals develop an aberrant behavior when faced with negative consequences, such as an unknown competitor on the SDTT or an electric shock on the PA, but they do not show any alteration when they faced with positive consequences, such as a food reward; suggesting that the HD vulnerability is related to the processing of negative rather than positive reinforcement. Finally, our data provided evidence of the possible implication of the HPA axis for the development and maintenance of compulsivity. Further studies should aim to disentangle the role of CORT and socioemotional dysregulation in the vulnerability mechanisms that might lead to compulsive behavior on SIP, a suitable preclinical model for evaluating the complex neuropsychopathology of OCRRs.

Ethics

All the procedures were conducted in accordance with the Spanish Royal Decree 53/2013 and the European Community Directive (2010/63/EU) for animal research. The present study was also approved by the Animal Research Committee from the University of Almería and complied with the ARRIVE guidelines. The authors declare that the research shows commitment to the 3Rs principle (replacement, reduction, refinement).

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Data availability

Data will be made available on request.

CRediT authorship contribution statement

EM-G: Methodology, Investigation, Data analysis, Writing-Original draft preparation.
MO-C: Investigation, Data analysis, Writing-Original draft preparation.
AP-P: Investigation, Writing—Review & editing.
DJC-G: Methodology.
PF: Conceptualization, Writing—Review & editing.
SM: Investigation, Writing—Review & editing, Supervision.
MM: Conceptualization, Methodology, Writing—Review & editing, Resources, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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