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Impulsivity Characterization in the Roman High- and Low-Avoidance Rat Strains:
Behavioral and Neurochemical Differences

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Running title: Impulsivity in RHA and RLA rats

Abstract

The selective breeding of Roman high- (RHA) and low-avoidance (RLA) rats for rapid vs extremely poor acquisition of active avoidance behavior in a shuttle-box has generated two phenotypes with different emotional and motivational profiles. The phenotypic traits of the Roman rat lines/strains (outbred or inbred, respectively) include differences in sensation/novelty seeking, anxiety/fearfulness, stress responsivity, and susceptibility to addictive substances. We designed the present study to characterize differences between the inbred RHA-I and RLA-I strains in the impulsivity trait by evaluating different aspects of the multifaceted nature of impulsive behaviors using two different models of impulsivity, the delay-discounting task and five-choice serial reaction time (5-CSRT) task. Previously, rats were evaluated on a schedule-induced polydipsia (SIP) task that has been proposed as a model of obsessive-compulsive disorder. RHA-I rats showed an increased acquisition of the SIP task, higher choice impulsivity in the delay-discounting task, and poor inhibitory control as shown by increased premature responses in the 5-CSRT task. Therefore, RHA-I rats manifested an increased impulsivity phenotype compared to RLA-I rats. Moreover, these differences in impulsivity were associated with basal neurochemical differences in striatum and nucleus accumbens monoamines found between the two strains. These findings characterize the Roman rat strains as a valid model for studying the different aspects of impulsive behavior and for investigating the mechanisms involved in individual predisposition to impulsivity and its related psychopathologies.

Keywords: impulsivity; Roman high- and low-avoidance rats; schedule-induced polydipsia; delay-discounting task; 5-choice serial reaction time task; monoamines

INTRODUCTION

Impulsivity encompasses a range of actions that are poorly conceived, prematurely expressed, unduly risky, or inappropriate to the situation, often leading to undesirable consequences (Daruna and Barnes, 1993). High levels of impulsivity are associated with disorders including obsessive-compulsive disorder (OCD), attention-deficit hyperactivity disorder, schizophrenia and antisocial behavior (American Psychiatric Association, 2000). Moreover, accumulating evidence suggests that the comorbidity between impulsivity and other behavioral traits, as sensation/novelty seeking or risk taking and physiological stress responsivity (Chakroun *et al*, 2004; Sher *et al*, 2000), may share genetic factors that contribute to the vulnerability to addictive disorders (Kreek *et al*, 2005).

In recent years, important efforts have been made to characterize valid animal models for studying the impact of genetic background on the neural substrates of psychiatric (Annetrude *et al*, 2008; Gould and Gottesman, 2006; Machado-Vieira *et al*, 2004) and addiction disorders (Crabbe, 2002; Laakso *et al*, 2002). Two phenotypes with different emotional and motivational profiles have been developed through bidirectional selection and breeding of the Roman high- (RHA) and low-avoidance (RLA) rat lines/strains (outbred or inbred, respectively), selected for rapid (RHA) vs extremely poor (RLA) acquisition of two-way active avoidance in the shuttle-box (Broadhurst and Bignami, 1965; Driscoll and Bättig, 1982) (for reviews, see Driscoll *et al*, 1990; Driscoll *et al*, 1998; Driscoll *et al*, 2009; Escorihuela *et al*, 1995; Escorihuela *et al*, 1999; Fernández-Teruel *et al*, 1997; Giorgi *et al*, 2007; Steimer and Driscoll, 2005). These lines/strains show consistent differences in sensation/novelty seeking and in stress/anxiety endocrine and

behavioral responses when confronted with novel environments (ie, intended to measure anxiety, emotionality, fearfulness, or novelty seeking), such as the open-field test (Aubry *et al*, 1995; Carrasco *et al*, 2008; Escorihuela *et al*, 1999; Gentsch *et al*, 1991), elevated plus maze (Escorihuela *et al*, 1999; Steimer and Driscoll, 2003), elevated zero-maze (López-Aumatell *et al*, 2009b), black-white box test, dark/light open-field test, dark-light hexagonal tunnel maze (Fernández-Teruel *et al*, 2002b; Steimer and Driscoll, 2005), and hole-board test (Escorihuela *et al*, 1999; Fernández-Teruel *et al*, 1992). In all of these situations and in fear-conditioning procedures (eg, fear-potentiated startle; López-Aumatell *et al*, 2009a), RLA rats display increased anxiety or fear, a passive coping style, and increased stress responses by hypothalamus-pituitary-adrenal (HPA) axis activation (Carrasco *et al*, 2008; Steimer and Driscoll, 2003). RHA rats tend to be novelty/sensation seekers with relatively low HPA axis activation (Driscoll *et al*, 1998, 2009). Moreover, these differences occur with individual differences in vulnerability to drug addiction, manifested as behavioral and neurochemical responses to different drugs of abuse such as ethanol, morphine, and cocaine (Fattore *et al*, 2009; Giorgi *et al*, 2007). Abundant evidence implicates dopaminergic mechanisms in these differences. Compared to RLA rats, RHA rats exhibit different dopaminergic responses: activation in the mesocortical dopaminergic pathway by stressors and anxiogenic drugs (Giorgi *et al*, 2003); more rapid baseline turnover rate of dopamine (DA) in the caudate nucleus (Driscoll *et al*, 1990); and more intense stereotypy in response to acute challenge with the DA receptor agonist apomorphine (Giménez-Llort *et al*, 2005).

Despite the role of impulsivity as a principal symptom of most of the above mentioned psychiatric disorders (Evenden and Ryan, 1996), only one study has investigated impulsivity

differences in these two rat lines (Zeier *et al*, 1978). During the acquisition of a DRL-20 task, RHA rats displayed more irrelevant lever-pressing behavior than their RLA counterparts, suggesting reduced inhibition of impulsive behavior. However, there have been no other studies that have systemically investigated differences in impulse control between RHA-I and RLA-I rat strains.

Here we investigated possible differences in impulsivity between the RHA-I and RLA-I inbred strains to assess their potential as a valid model for studying the basis of impulsivity, dividing this multifaceted trait (Evenden, 1999) into two testable categories: (1) impulsive choice, measured by intolerance of gratification delay in the delay-discounting task (Cardona *et al*, 2006; Winstanley *et al*, 2004, 2006), and (2) impulsive behavior, measured by poor inhibitory control of inappropriate responses in a five-choice serial reaction time (5-CSRT) task (Robbins, 2002; Winstanley *et al*, 2006). We also explored possible between-strain differences on schedule-induced polydipsia (SIP) task that has been proposed as a model of OCD (Platt *et al*, 2008; Rosenzweig-Lipson *et al*, 2007; Woods *et al*, 1993; Woods-Kettelberger *et al*, 1996). Since impulsive behaviors have been related to monoaminergic dysfunctions (Winstanley *et al*, 2006), we assessed basal neurochemical function in the striatum and nucleus accumbens of RHA-I and RLA-I rats.

MATERIAL AND METHODS

Animals

Fourteen RHA-I and 14 RLA-I inbred female rats, between 90 and 120 days of age at the start of the experiments, were used. They were bred at the Autonomous University of Barcelona (Spain) from colonies established in 1997 (Driscoll *et al*, 1998; Escorihuela *et al*, 1997; Escorihuela *et al*, 1999). Although the literature refers to gender differences in the expression of impulsive behavior, we used female rats in the present experiments because the behavioral traits that distinguish the Roman lines have been consistently confirmed in both sexes across several laboratories and along different generations (Aguilar *et al*, 2003; Corda *et al*, 2005; Ferre *et al*, 1995; Rosas *et al*, 2007).

Animals were housed 3/cage (50 cm × 15 cm × 25 cm) at 22°C with 8:00/20:00 light/dark cycle, water available, and deprived to 85% of *ad libitum* feeding weight via daily feedings of lab chow approximately 30 min after each experimental session. All testing was done between 08:00 and 14:00 h. All procedures were performed in accordance with Spanish Royal Decree 1201/2005 on the protection of experimental animals and with the European Communities Council Directive (86/609/EEC).

The order of training and screening was as follows: SIP, delay-discounting, and 5-CSRT task. Each task commenced at least 2 weeks after the previous one, and interference among tasks was unlikely because each involved a different type of operant response for food reward.

Apparatus: Operant Chambers

We conducted the tests in seven standard operant conditioning chambers (MED Associates, USA) 32 cm long × 25 cm wide × 34 cm high, with stainless-steel grid floors. A detailed description of the apparatus has been provided previously for SIP and delay-discounting task (Cardona *et al*, 2006; López-Grancha *et al*, 2008). The same chambers were also used in the 5-CSRT task, with an array of five contiguous square holes (2.5 cm), 2.2 cm deep and 2 cm above floor level at the back panel. These apertures had photocell beams at the entrance and a yellow stimulus light for the nose-poke response. The scheduling and recording of experimental events was controlled by a Med PC computer and commercial software (Cibertec SA, Spain).

Experimental Procedures

SIP: Over two successive days, a water-ingestion test was given (baseline). Sixty pellets were placed together, and the amount of water each rat consumed in 60 min was measured. After one adaptation day, the animals were exposed to a FT-60s (Fixed Time 60 s) schedule of food pellet presentation during 60-min sessions. Water bottles containing 100 ml fresh water were provided immediately before each session. The following measures were recorded for each rat: (a) total amount of water (ml) removed from the bottle, (b) total number of licks, and (c) total number of nose-pokes (see López-Grancha *et al*, 2008, for more details).

Delay-discounting task: In this task, response on one lever (A) produced a one-pellet reward; response on the other lever (B) produced a four-pellet reward. Each session consisted of 60 trials,

30 forced choice and 30 free choice in which both levers were presented. Illumination of the house light and tray light signaled the start of each trial. A nose-poke response was required in the food pellet receptacle to initiate lever presentation. Failure to make the response or to depress the lever within 10 s terminated the trial. When either lever was pressed, the house light turned off, the “clicker” sounded, and both levers were retracted. Food reward was then delivered, either almost immediately or after a delay. The experiment was divided into five sets of five sessions. During the first set, both levers had a delay of 0 s. The delay to reward A remained constant at 0 s throughout the experiment, whereas the delay to reward B increased to 10 s in set 2, 20 s in set 3, and 40 s in set 4. Set 5 was identical to set 1: levers A and B were both 0 s. Only data from the last two sessions per set were analyzed (see Cardona *et al*, 2006, for more details).

5-CSRT task: Animals were required to respond to brief flashes of light presented randomly in one of five spatial locations (Carli *et al*, 1983). Pretraining and training were based on previous procedures with subtle modifications (Robbins, 2002; van Gaalen *et al*, 2006). Rats were habituated to the apparatus over 2 days for 20 min/session. In these sessions, the house light was switched on and 20 food pellets were placed in the magazine and two in each open aperture.

Pre-training, phase 1: All cue lights and house light were switched on throughout the session. A nose-poke in one of the five holes elicited a food pellet delivery. A session ended after delivery of 100 pellets or after 30 min. Animals were trained until all rats reliably earned 100 pellets within 30 min.

Pre-training phase 2: Rats were trained to detect brief flashes of light (stimulus duration, SD 30 s) presented in any one of the five spatial locations (in pseudorandom order) with the house light switched on during the entire session. A response into the illuminated (correct) hole elicited a

food pellet delivery in the food magazine (concurrently with its illumination), and the stimulus light was extinguished. The collection of the pellet from the food magazine started a new trial. Responses into the other holes were counted but had no further consequences, with the opportunity to make a response in the correct aperture within the 30 s of SD. Animals were tested until all animals earned 100 pellets within 30 min. After these pre-training phases the 5-CSRT task training commenced.

Training: Animals were on the 5-CSRT task to criterion performance (accuracy > 80%, omissions < 20%), with stimuli duration (SD) of 1 s. Each test session commenced with the illumination of the chamber by the house light and of the food magazine where a food pellet was delivered. The collection of this pellet from the feeder started the first trial, the next trials were self-initiated by a nose-poke response in the food magazine; then the light of the food magazine was extinguished and a fixed inter-trial interval (ITI) of 5 s started. At the end of the ITI, a visual stimulus with 1 s duration (SD) was presented in a random location of the apertures at the rear. Responses in this aperture within 5 s, the limited hold (LH), were recorded as *correct responses* and were rewarded by a food pellet delivery in the magazine feeder with its illumination. Response errors were *omissions* (failure to respond to the stimulus within the LH), *errors of commission* (incorrect responses made to the wrong location) and *premature responses* (responses made before the presentation of the visual stimulus in any of the five apertures during ITI). These response errors were all punished with a 5 s period of darkness (time-out), during which no food was delivered. An additional response in an aperture after a correct response and before the food collection was recorded as a *perseverative response* and had no punishment of time-out period; the collection of the pellet from the feeder started the next trial. A response in the food magazine after the delivery of food or after a time-out period initiated the next trial; a

response in the food magazine after a premature response restarted the same trial. Each session terminated after 100 trials or after 30 min. To facilitate acquisition of the 5-CSRT task, SD of target stimuli was progressively shortened from 6 s to 1 s over 8 training stages. Thus, in stage 1, SD = 6 s with LH = 6 s and ITI = 5 s, while SDs of stages 2 through 8 were 5, 2.5, 2, 1.75, 1.5, 1.25, and 1 s, respectively (LH and ITI = 5 s). Animals advanced through stages by completing at least 50 correct trials with 80% accuracy and less than 20% omissions. Due to problems of RLA-I to reach the criterion of less than 20% omissions, rats were allowed to progress with less than 30% omissions and stable 80% accuracy for at least three consecutive sessions.

The recorded measures were as follows:

Accuracy: percentage of correct trials $(\text{correct trials} / (\text{correct trials} + \text{incorrect trials}) \times 100$.

Correct responses: nose-poke responses during the LH period in the same aperture as the stimulus light presentation.

Incorrect responses: nose-poke responses during the LH period in a different aperture from the one where the stimulus light was presented.

Omissions: percentage of omissions among total number of trials $(\text{number of omissions} / (\text{correct responses} + \text{incorrect responses} + \text{omissions}) \times 100$

Premature responses: number of responses before the visual target presents in the apertures during the ITI periods.

Perseverative responses: number of repetitive responses at the response apertures after a correct response and before collection of the food reward.

Latencies (in seconds) to: *correct response* from stimulus presentation to a correct nose-poke response; *incorrect response* from stimulus presentation to an incorrect nose-poke response; and to *reward* to collect a food pellet in the magazine following a correct response.

Brain Monoamine Analyses

One day after the last behavioral task, eight animals of each strain were rapidly decapitated using a guillotine. Brains were quickly removed, frozen, and stored at -80°C until preparation (Moreno *et al*, 2008). For brain tissue preparation, the samples were thawed enough to allow dissection of the striatum and nucleus accumbens. These were weighed and homogenized in 0.4 N perchloric acid with 0.1% metabisulfite, 0.01% EDTA, and 1 mg/ml cysteine. The homogenates were centrifuged at 15,000 rpm for 20 min at 4°C , and supernatants were collected and frozen at -80°C until biochemical analyses for determining the levels of norepinephrine (NE), DA, 5-HT, and their metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxy-3-indolacetic acid (5-HIAA). These were measured using reverse-phase high performance liquid chromatography with electrochemical detection. The mobile phase, containing (in mM) KCO_2H_4 , 0.1; $\text{Na}_2\text{-EDTA}$, 0.1; PICB_8 , 2.06; plus 18% methanol, adjusted to pH 2.63 with orthophosphoric acid, was delivered at 1 ml/min. Monoamines were separated on a 3- μm particle size column (Phenomenex C18 10×0.46 cm, Micron Analítica SA, Spain).

Data Analysis

Behavioral data were analyzed using repeated measures analysis of variance (ANOVA), with Strain (RHA-I and RLA-I) as the between-subject factor and Sessions or Delay as the within-subject factor. We used one-way ANOVA to evaluate behavioral parameters for the 5-CSRT task and the monoamine data, with Strain (RLA and RHA) as the between-subject factor. Where appropriate, post-hoc comparisons were made using the Newman-Keuls test. To assess the

integration between impulsive measures and neurochemistry, Spearman rank correlations were calculated in both groups. All analyses were computed using the Statistica software package. Statistical significance was set at $P < 0.05$.

RESULTS

Schedule-induced polydipsia

Figure 1 shows the mean water intake (A) and the mean total licks (B) for each strain during the acquisition and maintenance of SIP. Rats developed schedule-induced polydipsia after being exposed to the FT 60-s schedule of food presentation. During the baseline period, RHA-I and RLA-I rats drank a mean of 7.8 ± 0.9 and 8.7 ± 1.3 ml, respectively. The mean intake of water for these groups during the last 5 days of the experiment was 20.9 ± 1.6 and 12.3 ± 0.7 ml for RHA-I and RLA-I, respectively (Figure 1A). This SIP acquisition was also observed by the increase in the number of licks (B). During the first three days, RHA-I and RLA-I rats licked a mean of 1588.3 ± 160.3 and 1018 ± 123.3 , respectively. The mean total licks for these groups averaged across the last 5 days of the experiment were 3827.9 ± 435.1 and 1514.2 ± 148.1 for RHA-I and RLA-I, respectively (Figure 1B). Regarding the HD and LD assessment, we found a high correspondence between RHA-I and HD, as well as between RLA-I and LD (data not shown).

ANOVA revealed significant differences between RHA-I and RLA-I in water intake (A) ($F_{1,26} = 14.69$; $P < 0.001$) and total licks (B) ($F_{1,26} = 25.01$; $P < 0.001$). Session effects were significant

in both measures: water intake (A) ($F_{19,492} = 32.00$ $P < 0.001$) and in total licks (B) ($F_{19,492} = 18.98$; $P < 0.001$). Interaction between Sessions and Strain was also significant ($F_{19,492} = 6.72$; $P < 0.001$) in water intake (A) and in total licks (B) ($F_{19,492} = 7.64$; $P < 0.001$). Post-hoc analysis indicated that the FT-60s schedule of food delivery induced different drinking rates across the 20 test sessions in both groups. Differences in water intake between RHA-I and RLA-I animals were evident from session 5, and from session 3 in total licks. A main effect of Session ($F_{1,26} = 4.77$; $P < 0.001$) emerged for the nose-poke measure, but not for Strain ($F_{19,492} = 0.63$; $P > 0.05$). The RHA-I rats reached an asymptotic level on SIP sooner, evidenced by the increased water intake and number of total licks in the first sessions and a higher rate of licks at the end of training.

Insert figure 1

Delay-Discounting Task

Figure 2 shows the mean number of choices of the large reward during the delay-discounting task. All rats chose the large reward in almost every trial when the delay to the large reward was 0 s. ANOVA showed a Delay effect ($F_{3,78} = 81.80$; $P < 0.001$): as the delay to the large reward increased, the preference of all the rats shifted towards the smaller but more immediate reward. A Strain effect was also observed ($F_{1,26} = 4.51$; $P < 0.001$), and the interaction between Strain and Delay was significant ($F_{3,78} = 5.87$; $P < 0.001$). Post-hoc analyses indicated significant differences for 20-s and 40-s delays ($P < 0.05$). Finally, when the delay to the large reward was removed in the final set of sessions, all animals returned to choosing the large reward in almost all trials. Taken together, these results show that the RHA-I rats exhibited a significantly greater

preference for small immediate rewards versus large delayed rewards compared with RLA-I rats (i.e., they showed increased choice impulsivity).

Insert figure 2

Five-Choice Serial Reaction Time Task

Figure 3 shows the effect of rat strain on the acquisition of the 5-CSRT task. RLA-I rats required an average of 24.9 ± 1.3 sessions to achieve criterion performance ($SD = 1$) on the 5-CSRT task, compared to 21.3 ± 1.6 sessions for RHA-I rats. The ANOVA revealed no significant differences between RHA and RLA in number of sessions ($F_{1,21} = 3.75$; $P > 0.05$). Interaction between Sessions and Strain was significant ($F_{19,147} = 3.11$; $P < 0.05$). Post-hoc analysis indicated significant differences between Strain \times Sessions from training stage 4 until the last training stage, 8. This difference in number of sessions to reach the training stage was the result of problems the RLA-I animals had in meeting the criterion of less than 20% of omissions required to progress to the next stage.

Insert figure 3

Figure 4 shows the performance in different parameters of the 5-CSRT task ($SD = 1$) by RLA-I and RHA-I rats. One-way ANOVA revealed no differences in accuracy between RLA-I and RHA-I animals (Figure 4A). In contrast, compared to RHA-I rats, RLA-I rats exhibited a significantly increased percentage of omission errors out of the total number of trials ($F_{1,21} = 36.50$; $P < 0.001$) (Figure 4B). The increased omissions among RLA-I animals were observed across the training stages. On the other hand, compared to the RLA-I group, the RHA-I animals had a significantly higher number of premature responses, defined as the number of responses

before the visual target occurs ($F_{1,21} = 8.80$; $P < 0.05$) (Figure 4C). No between-strain differences emerged in perseverative responses ($F_{1,21} = 0.0002$; $P > 0.05$) (Figure 4D). Compared to the RHA-I group, the latencies were slower in RLA-I rats for all parameters (Figure 5), ie, from stimulus presentation to a correct response ($F_{1,21} = 11.53$; $P < 0.05$); to an incorrect response ($F_{1,21} = 14.31$; $P < 0.05$); and to collect the reward in the magazine following a correct response ($F_{1,21} = 10.45$; $P < 0.05$).

Insert figure 4

Insert figure 5

Monoamine Analysis

RLA-I and RHA-I rats showed differences in monoamine systems in the striatum and nucleus accumbens under basal conditions. In the striatum and nucleus accumbens, levels of 5-HT ($F_{1,14} = 28.18$; $P < 0.001$; $F_{1,14} = 5.8$; $P < 0.05$, respectively), 5-HIAA ($F_{1,14} = 6.7$; $P < 0.001$; $F_{1,14} = 10.76$; $P < 0.05$), and NE ($F_{1,14} = 12.67$; $P < 0.05$; $F_{1,14} = 14.83$; $P < 0.05$) were significantly higher in the RHA-I compared to RLA-I rats (Table 1). An increased 5-HIAA/5-HT turnover ratio in nucleus accumbens ($F_{1,14} = 6.10$; $P < 0.05$) was also observed in RHA-I rats. No significant changes were found in the striatum (Figure 6A).

Although no significant between-strain differences emerged in the levels of DA and its metabolites, DOPAC and HVA, we did identify a significant decrease in (DOPAC+ HVA)/DA turnover ratio in the RHA-I in both the striatum and nucleus accumbens ($F_{1,14} = 5.24$; $P < 0.05$; $F_{1,14} = 12.44$; $P < 0.05$, respectively) (Figure 6B).

Insert figure 6

Insert table 1

When comparing individual data of impulsivity measures with neurochemical findings, we found significant correlations for RHA-I, but not for the RLA-I group (Figure 7). In RHA-I rats, there was a negative correlation between premature responding in the 5-CSRT task with 5-HIAA metabolite ($r=-0.90$, $P<0.05$) in nucleus accumbens. On the other hand, there was positive correlation between choice of large reward in delay discounting task and 5-HIAA/5-HT turnover ratio (delay 40, $r=0.72$, $P<0.05$), and DA (delay 20, $r=0.90$; delay 40, $r=0.78$, $P<0.05$) and DOPAC (delay 20, $r=0.93$, $P<0.001$; delay 40, $r=0.67$, $P=0.06$) in striatum.

Insert figure 7

DISCUSSION

We have examined the impulsive profile of psychogenetically selected RHA-I and RLA-I female rats in different experimental paradigms frequently used for studying impulsive behavior. We also explored between-strain differences in SIP (a proposed model for OCD). RHA-I rats showed a higher choice impulsivity in the delay-discounting task, poor inhibitory control as shown by increased premature responses in the 5-CSRT task, and increased acquisition of the SIP task compared to RLA-I rats. In addition, RHA-I and RLA-I rats showed significant differences in monoamine measures in the striatum and nucleus accumbens under basal conditions, with significant relations for RHA-I rats between impulsivity assessment and neurochemical findings.

Increased Adjunctive Drinking Acquisition in RHA-I Rats

In the SIP task, despite the development of SIP in both rat strains, the RHA-I rats showed stronger acquisition of the adjunctive behavior. The RHA-I rats acquired the asymptotic level sooner than the RLA-I rats, exhibiting an increased level of water intake and number of total licks from the first sessions until the end of training.

Because of its characteristics of “excessiveness” and “persistence”, SIP (Falk, 1961) has been proposed as a model of OCD (Platt *et al*, 2008; Woods *et al*, 1993). Moreover, the SIP model seems to be sensitive to detect susceptibilities in individuals to addictive behavior (Gilpin *et al*, 2008; Mittleman *et al*, 2003; Toscano *et al*, 2008) and binge-eating behavior (Moreno *et al*, 2009). Curiously and relevant to the current work, these disorders also share impulsivity as one of the main symptoms (American Psychiatric Association, 2000). In fact, the development of SIP has revealed marked individual differences among animals (Cardona *et al*, 2006; López-Grancha *et al*, 2008; Mittleman and Valenstein, 1984, 1985; Piazza *et al*, 1993), allowing their separation into high (HD) and low drinkers (LD) populations, according to whether their rates of drinking are above or below the median. Our findings show a high correspondence of RHA-I and HD and between RLA-I and LD. Therefore, our present results point to SIP as a sensitive task for separating specific populations, which could be of potential interest as an animal model for investigating the neurobehavioral basis of vulnerability to several psychopathologies related to impulsivity.

Higher Choice Impulsivity and Poor Inhibitory Control in RHA Rats

RHA-I rats showed a high impulsivity profile in two distinct forms of measures related to the impulsivity trait: the delay-discounting and the 5-CSRT task. The delay-discounting paradigm is a widely used task to measure impulsive choice. This task evaluates whether animals prefer a smaller but immediate reward or a larger but delayed reward (Evenden and Ryan, 1996; Winstanley *et al*, 2004), with impulsive choice defined as the selection of the smaller immediate reward. We found that RHA-I rats made fewer choices of a large reward when the delay was increased from 0 to 20 and 40 s. Thus, RHA-I rats prefer an immediate reward and show marked choice impulsivity compared to RLA-I rats. Other studies have found strain differences in impulsivity with the same approach, such as greater impulsivity in Lewis rats compared to Fischer 344 rats (Anderson and Woolverton, 2005). This finding appears to agree with the increased susceptibility to addiction (and effects of addictive drugs) of Lewis vs Fischer 344 rats (Kosten and Ambrosio, 2002, for review; Sanchez-Cardoso *et al*, 2009) and is also in line with similar findings in RHA vs RLA rats (eg, Fattore *et al*, 2009; Fernández-Teruel *et al*, 2002a) and the current results.

The 5-CSRT task measures parameters related to attention and inhibitory control of impulsive actions, such as premature responses (Robbins, 2002; Robinson *et al*, 2009; Winstanley *et al*, 2006). Despite the absence of between-strain differences in accuracy, RHA-I rats displayed an increased number of premature responses compared to RLA-I rats. This measure is an index of impulsive behavior because it occurs when inhibitory control of highly prepotent responses has

been maladaptively lost (Robbins, 2002). Consequently, RHA-I animals showed higher impulsive behavior than RLA-I rats in the 5-CSRT task.

Moreover, the increased error omissions observed in RLA-I rats might hypothetically reflect sensory, motor, or motivational factors (Robbins, 2002). However, the concordant lack of changes in accuracy and the increased response latency in RLA-I rats point to motivational/emotional factors (rather than impairments in attentional processes) as a possible mechanism underlying strain differences for this parameter. Compared to RHA-I rats, RLA-I rats are characterized by increased anxiety/fear levels, enhanced (hormonal and behavioral) sensitivity to stressful situations, and displays of characteristic passive coping strategies (eg, freezing behavior) when faced with conflict or stressful situations (Carrasco *et al*, 2008; Driscoll *et al*, 2009; López-Aumatell *et al*, 2009a,b; Steimer and Driscoll, 2003, 2005). In this sense, it appears likely that the demanding (ie, stressful) conditions of the 5-CSRT task could have influenced motivational/emotional aspects in RLA-I rats, possibly leading to their longer response latencies and increased errors of omissions.

The differences in premature responding reported here are not the result of artefacts unrelated to impulsivity; recent work on Lister-Hooded rats selected for differences in impulsive behavior, showed similar baselines values of premature responding on the 5-CSRT task to those exhibited by RHA and RLA animals in the present experiment (Robinson *et al*, 2009). In fact, premature responses in both groups were above and below, respectively, the normal average for Wistar and Sprague-Dawley rats (Moreno *et al*, unpublished data from our laboratory; Semenova and Markou, 2007; Semenova *et al*, 2007; van Gaalen *et al*, 2006).

Neurochemical Differences in RHA vs RLA: Association with Impulsivity Phenotype

Impulsive behaviors have been related to monoaminergic dysfunction in limbic brain areas (Cardinal *et al*, 2001). Moreover, the relationship between monoamine levels and impulsivity depends on the particular impulsive behavior evaluated (Winstanley *et al*, 2006, for review). Convergent evidence suggests that monoaminergic (at least DA and 5-HT) functional differences in the mesolimbic-mesocortical systems and related areas mediate the impulsivity-related behavioral traits that distinguish the Roman rat lines/strains, including differential sensitivity to the effects of (and preference for) drugs of abuse (for reviews, see Charnay *et al*, 1995; Driscoll *et al*, 1980; Fattore *et al*, 2009; Fernández-Teruel *et al*, 2002a; Giorgi *et al*, 1994; Giorgi *et al*, 2003; Giorgi *et al*, 2007; Guitart-Masip *et al*, 2006a,b; Guitart-Masip *et al*, 2008a,b; Kulikov *et al*, 1995; Lecca *et al*, 2004). Collectively, the neurochemical findings reported in these studies suggest a relatively increased function (or tone) of DA and 5-HT neurotransmission in the RHA rat line/strain relative to RLA animals. However, regarding the DA system, these between-strain differences have been clearly identified after different challenges/treatments or stressors as being far from consistent in their direction when evaluated under baseline conditions. In fact, the common finding has been the absence of RHA vs RLA differences in DA function (outflow, as measured by microdialysis) under baseline conditions (Giorgi *et al*, 2007, for review).

The neurochemical results of the present study revealed between-strain differences in monoamine systems in the striatum and nucleus accumbens under basal conditions. Compared to the RLA-I rats, RHA-I animals showed 5-HT hyperactivity based on increased levels of 5-HT

and the metabolite 5-HIAA in both brain areas, and an increased 5-HIAA/5-HT turnover ratio in the nucleus accumbens. These results would appear to agree with the neurochemical evidence described above regarding 5-HT differences between RHA and RLA (outbred) rats. In addition, this is the first time that between-strain differences in NE levels has been reported in Roman rats, RHA-I showed increased NE levels in both brain areas compared to RLA-I rats. Conversely, although there were no between-strain differences in DA or its metabolites (DOPAC and HVA), RHA-I rats showed a relative (but significant) basal hypoactivity in the dopaminergic system as suggested by a decreased (DOPAC+HVA)/DA turnover ratio in the striatum and nucleus accumbens. This finding appears to be partially at variance with previous results (Driscoll *et al*, 1990) obtained with the RHA/Verh and RLA/Verh outbred rat lines (from which the present RHA-I/RLA-I inbred strains were derived in 1993); however, that previous study is not completely comparable to the present work because the authors measured DA turnover by determining DOPAC disappearance after pargyline (MAO inhibitor) treatment.

We have found significant relations between neurochemistry findings and impulsivity measures only in RHA-I rats. The individual data of the RHA-I showed in nucleus accumbens that reduced levels of 5-HIAA were related with impulsive responding in the 5-CRST task; and in the striatum increased levels of DA, DOPAC and 5-HT activity ratio were associated with preference for larger delayed rewards (or decreased impulsivity). These results seem to be consistent with *in vivo* studies that associate decreasing 5-HT with impulsive responding (Harrison *et al*, 1997; Winstanley *et al*, 2004). However, post-mortem studies revealed no relation between DA and 5-HT and their metabolites in nucleus accumbens and dorsal striatum with impulsive premature responses (Dalley *et al*, 2002; Puumala and Sirviö *et al*, 1997).

Although, high impulsive subjects in the 5-CRST task exhibited increased DA activity in other brain areas: the anterior cingulate, prelimbic and infralimbic cortices (Dalley *et al*, 2002). The reason for these discrepancies may be due to procedural differences, the most prominent being from the selection of a specific animal strain. In summary, the RHA-I rats, characterized as more impulsive than RLA-I, revealed higher levels of 5-HT and lower levels of DA under basal conditions; however, correlations found in RHA-I rats showed that lower levels of 5-HT were associated with an increase in impulsivity while higher levels of DA were associated with a decrease in impulsivity. These apparent contradictory results could be related to the complexity of the 5-HT and DA involvement in impulsivity (Dalley *et al*, 2008 for review). *In vivo* neurochemical studies may allow a clearer understanding of the role of monoamines in the impulsive phenotype showed in RHA-I rats.

The current results provide a pattern of consistent behavioral and neurochemical differences between the RHA-I and RLA-I rat strains, where RHA-I rats exhibited a high impulsive phenotype across different measures for impulsive behavior and also in an OCD model. High levels of impulsivity are a cause and/or consequence of many disabling psychopathological disorders in children and adults, including OCD, attention-deficit hyperactivity disorder, schizophrenia, antisocial behavior, and addictive behaviors. Thus, neurobehavioral studies targeting exploration of the different forms of impulsivity may have a broader relevance for future treatments of these psychopathologies. The present study is the first to directly characterize these Roman rat strains as a valid model for studying the different aspects of impulsive behaviors and implies their usefulness as animal models of human psychopathological disorders.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Figure Legends

Figure 1 The mean (\pm S.E.M.) water intake (panel A) and total licks (panel B) in FT-60s across 20 sessions of SIP. (*) Statistical analyses indicate significant differences between RHA-I and RLA-I from that session onwards. (#) Significant differences from session 1.

Figure 2 The mean (\pm S.E.M.) number of choices of the large reward on the delay-discounting task between strains. (*) Statistical analyses indicate significant differences in delay between RHA-I and RLA-I.

Figure 3 5-CSRT task acquisition of RHA-I and RLA-I rats. The data are the cumulative number of sessions \pm S.E.M. required to reach each training stage (see Methods section for details on the training procedure).

Figure 4 Performance of RHA-I and RLA-I on the 5-CSRT task (SD = 1): percent of accuracy (A), percent of omissions (B), number of premature responses (C), and number of perseverative responses (D). RHA-I rats made more premature responses, while low-avoidance rats made a significant percentage of omissions. Data are means \pm S.E.M.; (*) statistical analyses indicate significant differences between RHA-I and RLA-I.

Figure 5 Latency to correct response, incorrect response, and reward RHA-I and RLA-I on 5-CSRT task (SD = 1). Data are means \pm S.E.M.; (*) statistical analyses indicate significant differences between RHA-I and RLA-I.

Figure 6 DOPAC+HVA/DA and 5-HIAA/5-HT turnover ratios (pmol/mg tissue) in striatum and nucleus accumbens (NAc) in RHA-I and RLA-I rats. Data are means \pm S.E.M.; (*) statistical analyses indicate significant differences between RHA-I and RLA-I.

Figure 7 Correlations for RHA-I rats between neurochemical measures and impulsivity assessment. Premature responses on the 5-CSRT task and levels of 5-HIAA in NAc (A), number

of choices of the large reward on the delay-discounting task (delay 40) and 5-HIAA/5-HT levels in striatum (B), number of choices of the large reward on the delay-discounting task, delay 20 (—) and delay 40 (---) and levels of DA and DOPAC in striatum (C and D).

Table 1 Monoamine concentration levels (pmol/mg tissue) in striatum and nucleus accumbens (NAc) in RHA-I and RLA-I rats. Data are means \pm SEM; (*) statistical analyses indicate significant differences between RHA-I and RLA-I.

FIGURES

Figure 1

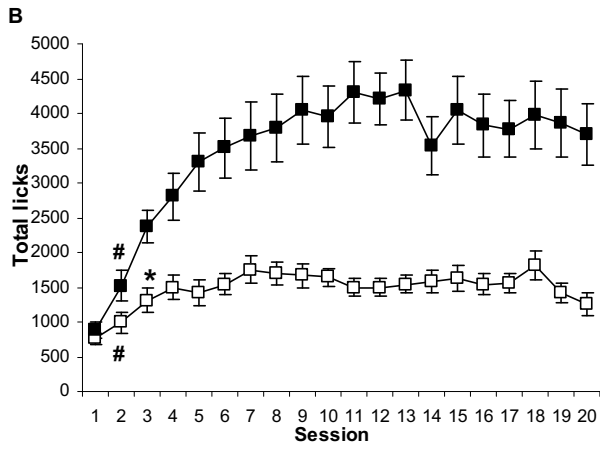
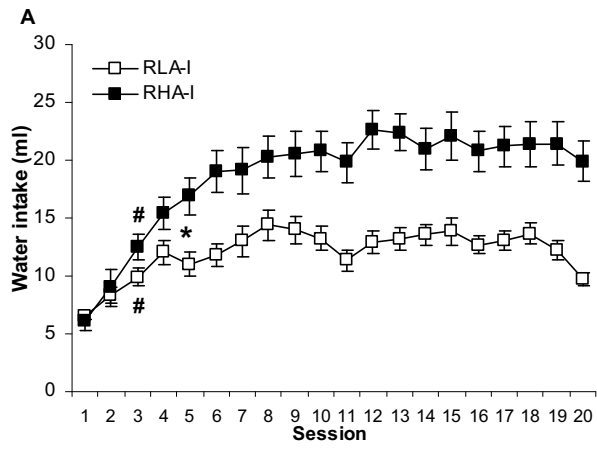


Figure 2

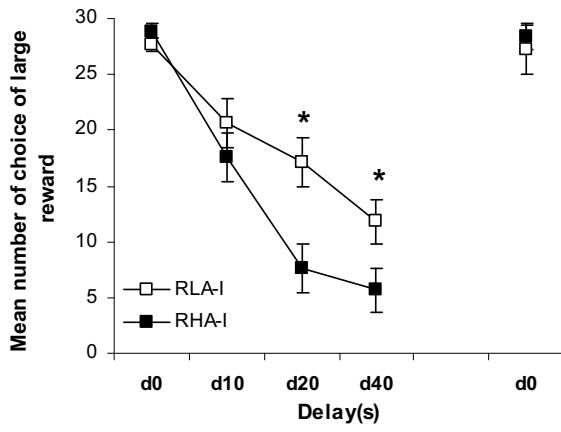


Figure 3

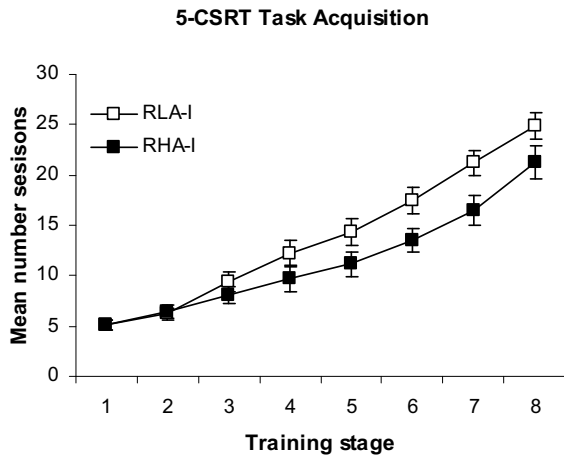


Figure 4

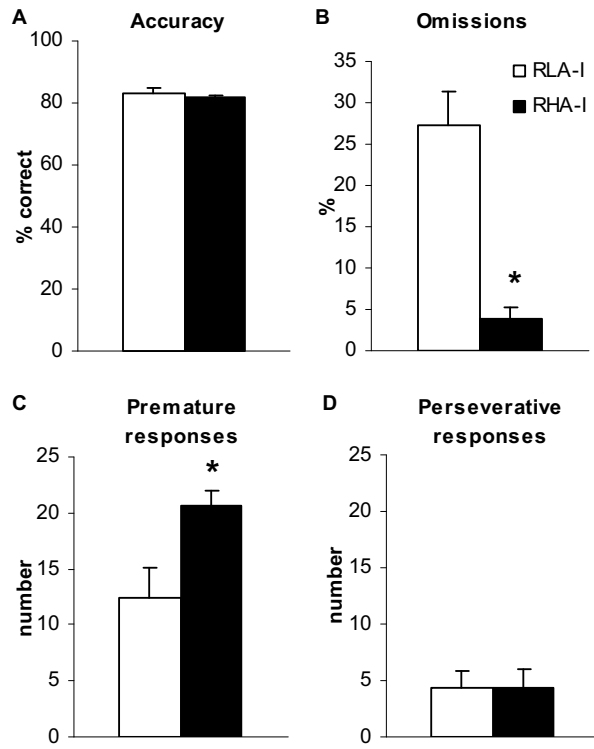


Figure 5

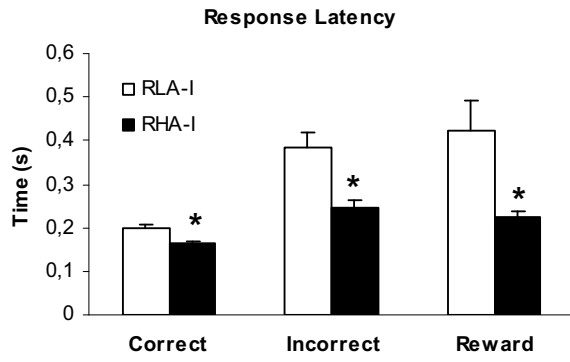


Figure 6

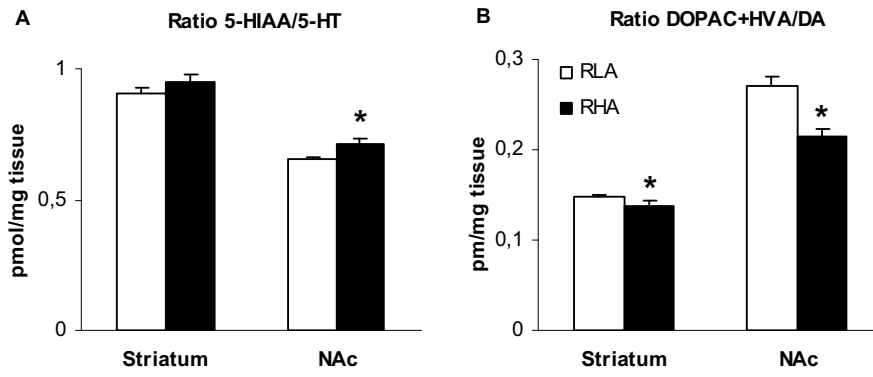
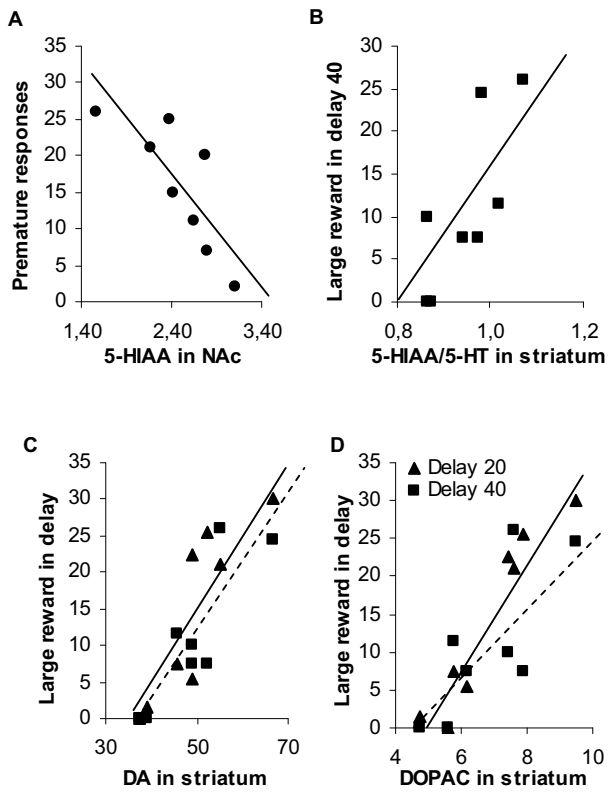


Figure 7



TABLES

Table 1

	Striatum		NAc	
	RLA-I	RHA-I	RLA-I	RHA-I
5-HT	1.81 ± 0.10	2.84 ± 0.15*	2.74 ± 0.22	3.51 ± 0.26*
5-HIAA	1.64 ± 0.08	2.58 ± 0.09*	1.79 ± 0.14	2.48 ± 0.18*
NE	0.29 ± 0.02	0.57 ± 0.08*	3.07 ± 0.28	6.03 ± 0.76*
DA	51.20 ± 2.84	49.22 ± 3.46	16.86 ± 1.44	16.69 ± 2.04
DOPAC	7.58 ± 0.50	6.84 ± 0.57	4.45 ± 0.38	3.60 ± 0.55
HVA	3.30 ± 0.27	2.84 ± 0.21	1.53 ± 0.16	1.26 ± 0.12