

## **Differences in Extinction of an Appetitive Instrumental Response in Female Inbred Roman High- (RHA-I) and Low- (RLA-I) Avoidance Rats**

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An experiment was conducted with the goal of exploring strain differences between female inbred Roman High and Low Avoidance rats (RHA-I, and RLA-I, respectively) on acquisition and extinction of a food-rewarded running response in a straight alley. Acquisition proceeded faster in the less emotional RHA-I and Wistar rats (used as controls) than in the more emotional RLA-I rats. However, extinction proceeded slower in RHA-I rats than in RLA-I and Wistar rats. This strain-based asymmetry on instrumental performance between acquisition and extinction is discussed in terms of strain differences in locomotor activity, associative flexibility and emotional reactivity.

Inter-individual differences in physiology and behaviour have been repeatedly found in animals and human beings. These differences seem to critically influence organisms' adaptive capacity for coping with challenging situations, and seem to be conditioned by environmental as well as genetic factors (Steimer & Driscoll, 2005). One of the behavioural inter-individual differences that have been widely explored in rodents refers to their ability to avoid aversive stimuli. Within this context, different strains of rats have been genetically selected based on performance divergences in foot-shock avoidance behaviour. The Swiss sublimes of Roman High-

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\* This research was supported by MCYT, Ministerio de Ciencia y Tecnología, Spanish grants to C. Torres (SEJ2004-03231/PSIC) and A. Fernández-Teruel and A. Tobeña (Dept. Psychiatry & Forensic Medicine, UAB; SAF2003-03480; and "EURATools" n° LSHG-CT-2005-019015). Correspondence concerning to this article should be addressed to Carmen Torres, Departamento de Psicología, Universidad de Jaén, 23071 Jaén, Spain. E-mail: mctorres@ujaen.es

Avoidance (RHA/Verh) and Roman Low-Avoidance (RLA/Verh) rats (derived from the original RHA and RLA rats developed by Broadhurst and Bignami in 1965) were genetically selected by mating the animals based on their rapid vs. poor acquisition of a two-way active avoidance behaviour in the shuttlebox (Driscoll & Bättig, 1982), some of these rats being continued as inbred at the Autonomous University of Barcelona (Escorihuela, et al., 1997). As a result of this selection clear behavioural RHA/RLA differences have been found, these differences probably being the result from a complex interaction among differences on anxiety/emotionality, in locomotor activity and novelty/reward seeking, and in active vs passive coping styles (Giorgi, Piras & Corda, 2007; Steimer & Driscoll, 2005).

Recent research in our laboratory has extended the exploration of the emotional divergences observed between RHA-I and RLA-I rats to situations in which the aversive outcome is obtained through an unexpected reduction (e.g. successive negative contrast –SNC-) in the amount or value of the outcome used as reinforcer (for a review see Papini, Wood, Daniel, & Norris, 2006). Thus, appetitive and aversive instrumental SNC effects have been found to be greater in female RLA-I rats as compared to RHA-I (Rosas et al., 2007; Torres et al., 2005).

The main goal of the present experiment was to analyze whether the SNC strain differences described above may be also found during the extinction of an appetitive instrumental response previously learned, a situation that has been shown to induce aversive emotional responses similar to those observed in SNC situations (Amsel, 1992; Mackintosh, 1974).

## METHOD

**Animals.** Twenty-seven female rats were used (9 RHA-I, 9 RLA-I and 9 Wistar –groups RHA, RLA, and Wistar, respectively). Their weight ranged from 220g to 300g at the start of the experiment. Animals were individually housed with water continuously available, and deprived to 80% of ad lib feeding weight. Room temperature was kept to about 20°C. Animals were maintained under a 12L-12D cycle with lights in at 8:00 am. All testing sessions were performed between 9:00 am and 14:00 pm. The experiment was carried out according to E.U. guidelines on the use of animals for research (86/609/EU). One of the RLA-I rats was eliminated because of an error on data recording.

**Apparatus.** The test apparatus was a straight 120 length x 11 width x 14 high cm runway divided into three sections separated by cardboard guillotine doors. The “start” section measured 20 cm; the running section measured 80 cm; and the goal section measured 20 cm. The food reward was 45-mg pellets (Noyes formula P; Research Diets, Inc.). Time to run through the runway was manually recorded by using a stopwatch (see Rosas et al., 2007, for details).

**Procedure.** Rats were taken to the experimental room in sets of six, spacing trials about 12 min during all the experimental sessions. This inter-trial interval was kept constant throughout the experiment. Three days of habituation to the apparatus preceded training. On the fourth day, each animal was placed in the start box with the start box door closed and the goal box door opened. The start box door was then opened and the rat was allowed to run down the runway to obtain the food reward (12 pellets). When the rat reached the goal box, the goal box door was quietly closed by the experimenter and a stopwatch was started. The rat was given a maximum of 30 s to eat the food and of 20 s to complete the trial. Each session consisted of six trials per day. A learning criterion of 2 consecutive sessions with a mean latency equal to or under 3 s was used. Each animal begun extinction on the following day to the one in which it reached the acquisition criterion. Extinction lasted for 6 days and was identical to training phase with the exception that no reward was provided at the goal-box (see Rosas et al., 2007, for more procedural details).

**Dependent variable and data analysis.** Sessions to criterion and latency on reaching the goal section were used as dependent variables on acquisition and extinction, respectively. Preliminary analyses were conducted to test the homogeneity of the variance using the Levene test. When the homogeneity assumption was broken, data were converted to log (base 10). When the homogeneity assumption was not filled after the log transformation, non-parametric analyses were used (Kruskal-Wallis and Kolmogorov-Smirnov). The rest of the analyses were conducted using ANOVA. Where appropriate, post-hoc comparisons were made using Bonferroni test. For all statistical analyses, alpha was set at 0.05.

## RESULTS

The mean  $\pm$ SEM number of sessions needed to reach the acquisition criterion was as follows: group RHA=  $3.56 \pm 0.18$ ; RLA=  $10.13 \pm 1.54$  and Wistar=  $5.22 \pm 0.55$ . Levene's test for homogeneity of the variance was statistically significant,  $F(2, 23) = 9.56$ , even when data were transformed to log,  $F(2, 23) = 3.79$ . Thus, raw data were analyzed using non-parametric statistics. Difference among groups on the number of trials to criterion was significant, Kruskal-Wallis Chi-Square = 16.26,  $df = 2$ . Subsequent pairwise comparisons using the Z of Kolmogorov-Smirnov found that RLA-I rats needed more sessions to reach the acquisition criterion than RHA-I rats,  $Z = 2.06$ . Differences between RLA-I and Wistar rats were close to significance,  $Z = 1.32$ ,  $p = .06$ . RHA-I and Wistar rats did not differ between them,  $Z = 1.18$ ,  $p = .12$ .

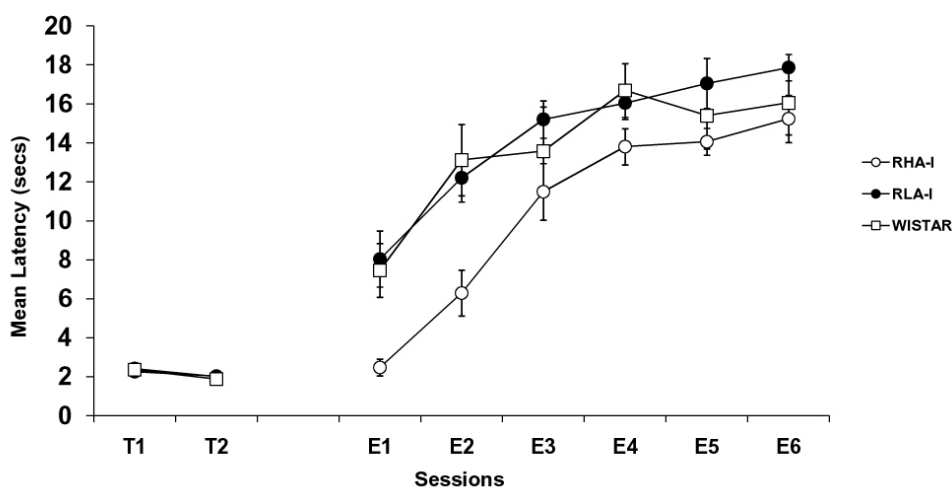
Figure 1 presents the mean latency to reach the goal-box during the last two days of training (T1 and T2) and the six days of extinction in groups High, Low, and Wistar. Not surprisingly given that rats were trained to a criterion, groups did not differ at the end of acquisition. Levene's test for homogeneity of the variance was not statistically significant,  $F(2, 23) = 1.54$  and  $.46$  for T1 and T2, respectively. Subsequently, a 3 (Group)  $\times$  2 (Day) ANOVA was conducted revealing a significant main effect of Day,  $F(1, 23) = 37.64$ . No other main effect or interaction was significant,  $F_s < 1$ .

Levene's test for homogeneity of the variance was statistically significant on extinction trials 1, 3 and 5, *Smallest*  $F(2, 23) = 3.82$ . This problem disappeared when data were converted to log, *Largest*  $F(2, 23) = 3.25$ . A 3 (Group)  $\times$  6 (Day) ANOVA conducted with the extinction data (log latency) found significant main effects of Group,  $F(1, 23) = 5.51$  and Day,  $F(5, 115) = 53.96$ . Most important, there was a significant Group  $\times$  Day interaction,  $F(1, 115) = 5.26$ . Subsequent analyses found that the simple effect of Group was significant in Days 1 and 2,  $F_s(2, 25) = 11.19$  and  $8.10$ , respectively. Post-hoc comparisons found that latency in RHA-I rats was smaller than in RLA-I and Wistar rats during Days 1 and 2. The simple effect of Group was not significant after Day 2,  $F_s(2, 25) = 1.25$ .

## DISCUSSION

The present study analyzed performance of RHA-I, RLA-I and Wistar rats in an appetitive instrumental task where animals were unexpectedly exposed to the omission of a 12 pellets-reward previously presented in the goal-box of a straight-alley. Higher performance observed in the RHA-I strain in comparison to the RLA-I strain during the acquisition of the

runway response replicated performance divergences previously obtained in our laboratory with a similar instrumental runway task in which response latency, rather than sessions to criterion, was used as dependent variable. These divergences resemble the strain differences observed in tasks where appetitive reinforcers such as drugs of abuse or saccharine solutions are used, and could be related to an enhanced mesolimbic dopaminergic transmission and/or to a higher locomotor activity in the RHA-I strain (see Rosas et al., 2007).



**Figure 1.** Mean latency (secs) to reach the goal-box during the last two days of training (T1 and T2) and the six days of extinction in groups High, Low, and Wistar. Bars denote standard errors of the mean.

As opposed to the results obtained during the acquisition phase, RHA-I rats showed slower extinction of the instrumental response than RLA-I and Wistar rats, suggesting greater resistance to extinction in RHA-I than in the other strains. These RHA/RLA differences could be explained on the basis of putative strain divergences in cognitive flexibility, rather than on behavioural divergences emotionally based. In this regard, Flaherty (1996) suggested that the early reaction to the reward omission might be considered as cognitive in nature, and that this reaction would precede the emotional response. From this perspective, it could be hypothesized that RHA-I animals showed more resistance to extinction because of a reduced

associative-reversal capacity or because of a lower ability to process the associative change that occurred during the extinction phase. In agreement with this hypothesis, it has been reported that RLA-I rats show an enhanced performance with respect to the RHA-I strain in a variety of tasks related to working memory and associative learning (for review see Fernández-Teruel et al., 2006). These behavioural differences could be related to an enhanced vulnerability to neurochemical imbalances in the RHA reward seeking system, as several studies seem to indicate (Guitart-Masip et al., 2006, 2008).

Alternatively, behavioural, pharmacological and neuroanatomical studies suggest that the complete omission of an expected reinforcer triggers emotional reactions that can influence performance of instrumental responses (see Papini et al., 2006, for review). These emotional reactions may underlie the RHA/RLA behavioural differences observed in the present study. The omission of an expected reward during the extinction phase could have induced frustration and freezing reactions in the more fearful RLA-I rats, precluding the occurrence of the instrumental response and therefore facilitating extinction (see Papini, 2003). These emotional reactions would be attenuated in the less fearful RHA-I group, determining its greater behavioural persistence and resistance to extinction. This explanation would also predict faster extinction in RLA-I rats than in Wistar rats, given that RLA-I rats are more emotionally reactive than Wistar rats. The fact that extinction proceeded equally fast in those two strain of rats could be considered an argument against the explanation of slower extinction in RHA-I rats in terms of lower emotional reactions to no reinforcement. However, it is possible to argue that the speed of extinction in Wistar rats was high enough as to precluding detection of additional differences in RHA-I rats because of a ceiling effect in performance.

Accordingly, the explanation of the present results in terms of strain emotional differences may be considered plausible at this point. However, additional studies will be needed in order to clarify whether associative, emotional or both processes are implied in RHA-RLA differences observed during the extinction of an appetitive instrumental response.

## RESUMEN

**Diferencias en la extinción de una respuesta instrumental apetitiva en ratas Romanas consanguíneas hembra de Alta y Baja Evitación.** Se realizó un experimento con el objetivo de explorar las diferencias de ejecución entre ratas Romanas consanguíneas de Alta y Baja Evitación (RHA-I y RLA-I, respectivamente) en la adquisición y la extinción de una respuesta de carrera recompensada con comida en un laberinto recto. La adquisición fue más rápida en las ratas menos emocionales RHA-I y Wistar (utilizadas como controles) que en las ratas más emocionales RLA-I. Sin embargo, la extinción fue más lenta en las ratas RHA-I que en las ratas RLA-I y Wistar. Esta asimetría en la actuación instrumental en función de la cepa entre la adquisición y la extinción se discute en términos de diferencias de cepa en la actividad locomotora, en la flexibilidad asociativa y en la reactividad emocional.

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(Manuscript received: 4 February 2008; accepted: 21 May 2008)