The Roman high- and low-avoidance rats differ in the sensitivity to shock-induced suppression of drinking and to the anxiogenic effect of pentylenetetrazole

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**ABSTRACT**

The Roman high- (RHA) and low-avoidance (RLA) outbred rat lines are selected for respectively rapid vs. poor acquisition of active avoidant behavior. Emotional reactivity appears to be the most prominent behavioral difference between the two lines, with RLA rats being more fearful/ anxious than their RHA counterparts. Accordingly, here we show that shock-induced inhibition of drinking behavior in the Vogel's test is significantly more pronounced in RLA than RHA rats. Thus, unpunished drinking activity is similar in both lines (38.1 ± 0.9 and 36.4 ± 0.6 licking periods/3 min in RLA and RHA rats, respectively), whereas under punished conditions (0.05–1.00 mA electric shocks delivered through the drinking tube) a more robust decrease in drinking behavior is observed in RLA vs. RHA rats. Moreover, fear-related behaviors like freezing and self-grooming are more frequent in RLA than RHA rats throughout the test. Similar results are obtained with the inbred RHA-I and RLA-I rats, which have been selected and bred through brother/sister mating of the outbred lines. In keeping with the above findings, we also show that, compared with their RHA counterparts, the outbred RLA rats are similarly responsive to the anticonflict effect of diazepam but more responsive to the proconflict effect of pentylenetetrazole in the Vogel's test. Collectively, these results reveal another behavioral trait distinguishing RHA from RLA rats and add experimental support to the view that the Roman lines/strains are a valid genetic model for the study of the neural underpinnings of fear/anxiety- and stress-related behaviors.

**1. Introduction**

Anxiety is an alerting signal that warns of impending danger thereby allowing the individual to deal with a threat. The activation of the sympathetic system that accompanies the feeling of anxiety is a clear demonstration of its utility. Thus, the characteristic symptoms of anxiety, such as tachycardia, hyperreflexia, and the reduction of the blood flow in viscera in favor of an increment in muscle and brain, are useful to tackle adverse situations that occur in everyday life. However, when the anxiety becomes inappropriate in terms of intensity and duration, it can be considered as a pathology because it interferes with everyday life.

Clinical anxiety is divided into several disorders distinguished from one another by the nature of the stimulus provoking the anxiety and by the responsiveness to different classes of anxiolytic drug treatments, suggesting discrete neurobiological etiologies (American Psychiatric Association, 2014). Anxiety disorders represent some of the most common and proliferating health problems worldwide (Wong and Licinio, 2001, 2004) and frequently co-occur with depression (Merikangas et al., 2003). Importantly, persons with both anxiety and depression experience more disability and distress than persons with a single disorder (Merikangas et al., 2003; Cairney et al., 2008). There is mounting evidence that pathological anxiety is a result of complex interactions among environmental factors (i.e., the economic and socio-cultural context) and the genetic and epigenetic backgrounds (McEwen, 2000).

The current pharmacological treatments of anxiety disorders have limited efficacy in a significant percentage of cases because of side effects that reduce the patient’s compliance (Holmes et al., 2003). For this reason, the development of more effective therapeutics necessitates a clear
comprehension of the causes of pathological anxiety. In this context the development of valid animal models, that mimic some features of the anxious phenotype, is a major challenge for neuropsychiatric research (Cryan and Holmes, 2005). One of these models is represented by the Roman low- (RLA) and high-avoidance (RHA) rats, which were bidirectionally selected from the original Wistar stock for, respectively, poor vs. rapid acquisition of the two-way active avoidance response in a shuttle-box (Brodhurst and Bignami, 1965; Driscoll and Bättig, 1982; Giorgi et al., 2007). Along the selection procedure of the Roman lines many other behavioral traits have been segregated. Thus, RHA rats display a robust sensation/novelty seeking profile, behave as proactive copers in the face of aversive conditions, and show high impulsivity and an innate preference for natural and drug rewards (Steimer and Driscoll, 2003; Escorihuela et al., 1999; Fernández-Teruel et al., 1992, 2002a; Fattore et al., 2009; Manzo et al., 2014; Moreno et al., 2010; Sanna et al., 2014); on the other hand, RLA rats behave as reactive copers, display a strong activation of the hypothalamus–pituitary–adrenal axis when exposed to stressors, and are prone to display stress-induced depression-like behavior (Díaz-Morán et al., 2012; López-Aumatell et al., 2009a, 2009b; Carrasco et al., 2008; Steimer and Driscoll, 2003, 2005; Piras et al., 2010, 2014). Notably, emotional reactivity-related traits (e.g. anxiety, fearfulness, stress sensitivity) rather than learning ability are the most prominent behavioral differences between the two lines, with RLA rats being more fearful/anxious than their RHA counterparts (Driscoll et al., 1998, 2009).

In an effort to identify the genetic bases responsible for the divergent emotionality profiles seen in these rats by means of quantitative trait loci analysis (Fernández-Teruel et al., 2002b), two inbred Roman strains, RLA-I and RHA-I, have more recently been selected and bred through brother/sister mating of the respective outbred lines (Driscoll et al., 1998; Escorihuela et al., 1999). Most behavioral traits that distinguish the outbred RLA and RHA lines have been preserved in their inbred counterparts (Driscoll et al., 1998, 2009). However, it remains to be determined whether the higher sensitivity of the outbred RLA vs. RHA lines to the conflict involved in the shock-induced suppression of drinking (Ferré et al., 1995), is also observed in the inbred RLA-I rats as compared with their RHA-I counterparts.

On the basis of the above mentioned findings, the aim of the present study was two-fold: (i) to compare the inhibition of drinking in the RHA-I and RLA-I strains with that observed in the outbred RHA and RLA lines, using a wide range of electric shock intensities in the Vogel’s conflict test, a well validated paradigm of fear and anxiety (Vogel et al., 1971), and (ii) to examine the anxiolytic effect of diazepam and the anxiogenic effect of pentylenetetrazol (PTZ) (Corda et al., 1983; Corda and Biggio, 1986) on the RHA and RLA lines in the conflict test.

2. Materials and methods

2.1. Animals

Male outbred (i.e. RHA and RLA) and inbred (i.e. RHA-I and RLA-I) rats were used throughout. Animals were 3 month old and weighed 300–380 g at the beginning of the behavioral tests. Outbred Roman rats were obtained from the colony established in 1998 at the University of Cagliari, Italy, (Giorgi et al., 2005) whereas the inbred Roman rats were obtained from the colony established in 1997 at the Autonomous University of Barcelona (Driscoll et al., 1998; Escorihuela et al., 1999).

Animals were housed in groups of 4 per cage and maintained in a temperature- and humidity-controlled environment (23 ± 1 °C and 60 ± 10%, respectively) under a 12 h light-dark cycle (light on at 8:00 a.m.) for 15 days before the beginning of the experiments. Water and standard laboratory food were available ad libitum. To reduce stress due to manipulation, contact with the animal house maintenance personnel was limited to a single attendant and bedding in the home cages was never changed either the day before or on the day of the experiment. All the behavioral tests were performed between 10:00–15:00 h and according to the guidelines of the European Communities Directive of September 22, 2010 (2010/63/EU) and the Italian Legislation (D.L. March 4, 2014, n. 26).

2.2. Conflict-punishment procedure

2.2.1. Apparatus

The rat to be tested was placed in a clear Plexiglas box (28 × 20 × 20 cm) with a stainless steel grid floor. This chamber was enclosed in a sound-attenuated and ventilated box. Water was provided through an electrically shielded stainless steel drinking tube extending 2 cm into the box, 3 cm above the floor. The unshielded tip of the drinking tube and the grid floor were connected to a constant current shock generator and to a drinkometer. The drinkometer output and the shock generator were interfaced with relays, controlled by a personal computer, which delivered one shock lasting for 0.5 s, for every 3 s of cumulative drinkometer output. This 3 s cumulative drinking time was termed “licking period”. All behavioral equipment was purchased from Lafayette Instruments (Lafayette, IN, USA).

The test was carried out as described by Vogel et al. (1971) with minor modifications (Corda et al., 1983) and consisted of two sessions: habituation and conflict.

2.2.2. Habituation session

Rats were kept in their home cages in the animal house without the drinking bottle for ~24 h for all the experiments with the exception of the pentylenetetrazol (PTZ) test, in which the water deprivation period lasted ~48 h. The animals were then transferred to the test room, where each rat was placed in the Plexiglas test box for 15 min. During this time period the shock generator was turned off, the rats had free access to the drinking tube and could explore the test box. At the end of this session animals were transferred to their respective home cages without the drinking bottle and brought back to the animal house. Notably, to prevent dehydration of the rats used in the PTZ test, these animals had water access in their home cages for 15 min, 24 h after the beginning of the water deprivation period.

2.2.3. Conflict session

The day after the habituation session, the rats were transferred again to the test box that they had explored for 15 min on the previous day; therefore, in this condition the animals approached the tube of the water bottle and began to drink very quickly. Importantly, the total water deprivation time was ~48 h for all the experiments (with water access for 15 min after 24 h) except for the PTZ test, in which rats were water deprived for ~70 h (with water access for 15 min after 24 and 48 h). After 10 s of continuous drinking, a 3 min unpunished session (i.e., with the shock generator turned off) was started. This session was followed by a 3 min punished session in which the generator delivered an 0.5 s electric shock to the rat’s tongue every 3 s of cumulative drinking (i.e., a licking period). The punished session started when the rat received the first electric shock upon completing the first licking period. To evaluate whether the outbred and inbred Roman rats differ in their responsiveness to the intensity of the electric shock, different groups of rats were treated with increasing shock intensities (0.05, 0.10, 0.20, 0.30, 0.50, 0.60, 0.80, and 1.00 mA). Each rat was used only once.

The following behavioral measures were recorded from each rat during the punished sessions: (1) number of approaches to the drinking tube, (2) latency to the first approach to the drinking tube, (3) number of freezing episodes, and (4) number of grooming bouts.

The anticonflict effect of diazepam was evaluated delivering 0.5 s electric shocks of intensities that suppressed drinking by approximately 80%: 1.0 mA for RHA rats and 0.8 mA for their RLA counterparts, respectively. The proconflict effect of PTZ was assessed delivering 0.5 s electric shocks of low intensities which induced only a marginal suppression of drinking: 0.3 mA for RHA rats and 0.1 mA for RLA rats.
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