

## Successive negative contrast in one-way avoidance learning in female roman rats

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### Abstract

The inbred RLA (Roman Low-Avoidance) and RHA (Roman High-avoidance) rat strains have been psychogenetically selected for rapid (RHA) vs. extremely poor acquisition (RLA) of two-way active avoidance. As a consequence of this selective breeding, RLA animals exhibit a higher level of emotionality that can be observed in many anxiety models. The present study was conducted in order to analyze the performance of female RLA, RHA and Wistar rats in a behavioral test of anxiety that involves the reduction of the magnitude of an expected reward: the negative contrast effect that is obtained in one-way avoidance learning by reducing the time spent in the safe compartment. To this aim, three groups of animals (30–1/RLA, 30–1/RHA and 30–1/W) were trained to avoid an electric foot-shock administered in a “danger” compartment, by running from this compartment to a “safe” compartment. We observed an impairment of the avoidance response when time spent in the safe compartment was reduced from 30 to 1 s, when 30–1/RLA and 30–1/W groups were compared with control groups that were trained with a constant safe time (1–1/RLA and 1–1/W, respectively). We also obtained significant differences between 30–1/RLA and 30–1/RHA groups in the postshift phase. These results indicate that RLA rats respond more negatively to the frustration triggered by the reduction in time spent in the safe compartment, suggesting that animal models based on negative contrast effects can be useful tools for studying the genetic basis of anxiety.

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### 1. Introduction

How rats respond to a given reward often depends on their prior experience with other rewards. They compare the quantity and quality of the present reward with that of other rewards within their realm of experience, and respond accordingly [1]. An illustration of adjustment to the incentive value of a reward is the successive negative contrast paradigm (SNC). This effect basically consists of a behavioral suppression that is observed in animals shifted from a high to a lower magnitude of reinforcement, when

compared with the behavior of animals that receive the lower magnitude of the reward throughout the experiment. SNC has been used in a variety of testing situations, including operant runway [2], maze [3], instrumental aversion [4], as well as consummatory [5], and Pavlovian [6] tasks, and has been suggested to be partially the result of a negative emotional response (anxiety, frustration or disappointment) triggered by a denial of reward expectancies [7–9]. The involvement of negative affective states in SNC is supported by further behavioral [10–12], pharmacological [13–15] endocrinological [16] and neuroanatomical studies [1,17–19], and SNC has been considered to be an animal model for studying the psychobiological basis of anxiety [7,9,20]. Although SNC has been shown across

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different species [21], few studies have been interested in systematically studying behavioral differences among genetically selected strains of rodents. In this context, Flaherty and Rowan [22] tested Syracuse Low-Avoidance and Syracuse High-Avoidance rats (selectively bred for differences in performance in active avoidance) in a consummatory SNC task, showing that Syracuse Low-Avoidance rats exhibited a substantially greater contrast than Syracuse High-Avoidance rats. Similarly, Rowan and Flaherty [23] found differences in consummatory contrast in the Maudsley strains, with the Maudsley Reactive rats showing a smaller SNC effect than the Maudsley Non-reactive rats. According to these studies, strains of rats psychogenetically selected for differences in fearfulness could be potentially useful for analyzing those theoretical explanations of SNC based on emotional processes.

The Swiss sublines of Roman High-Avoidance (RHA/Verh) and Low-Avoidance (RLA/Verh) rats have been initially selected and bred for rapid (RHA/Verh) vs. extremely poor (RLA/Verh) acquisition of active, two-way avoidance behavior [24], using stock from the original RHA and RLA rats [25]. A large body of evidence shows that, as a consequence of this selection, RLA/Verh rats are characterized by more pronounced, anxious reactions to novel, conflict and threatening situations, less novelty-seeking behaviors, and a blunted response to addictive drugs when they are compared with their RHA/Verh rats counterparts (e.g. [26–28]). Briefly, when exposed to a novel environment (e.g. open-field, holeboard, or elevated plus-maze), RLA/Verh animals show greater behavioral and neuroendocrine indices of anxiety than RHA/Verh rats, such as increased defecation, freezing and self-grooming, decreased exploration, and a higher activation of the HPA axis [29,30]. Behavioral differences have also been observed when animals are exposed to fear-conditioning stimuli. RLA/Verh rats show a more pronounced acoustic startle reflex, and enhanced freezing in classical fear conditioning tests [26,31,32]. These results consistently indicate that the RLA/Verh line presents higher emotionality and reactivity to a variety of stressful situations [33]. However, these genetically based differences in emotionality have not been tested in animal models of anxiety related to frustrative non-reward, such as extinction procedures or the SNC described above.

The main aim of the present experiment was to study the performance of RHA/Verh and RLA/Verh rats (referred to in this study as RHA and RLA), and outbred Wistar rats in a one-way avoidance learning SNC task [4]. This test consists of a danger compartment in which the rat receives a warning signal followed by an electric foot-shock, and a safe compartment in which the warning signal or the shock never appear. Subjects placed in the danger compartment can run to the safe compartment when the warning signal is turned on, thus avoiding the shock. From modern “two factors” and “homeostatic opponent process” theories [34,35], this avoidance response could be considered as a

mixture of flight from fear and approach to safety, the weight of each component being a function of the relative time spent in the danger and safe compartments, respectively (see Ref. [36] for review). In this context, SNC can be obtained by reducing the time spent by the animals in the safe compartment. Once subjects acquire the avoidance response with the time spent in the safe compartment being 30 s (preshift phase), this time is reduced to 1 s (postshift phase). The performance of this group is compared to that of a control group in which the time spent in the safe compartment remains constant (1 s) during both preshift and postshift phases. A previous study [4] showed that the performance of the shifted group on the postshift phase was worse than in the nonshifted group. This behavioral impairment implies an aversive emotional reaction triggered by a reduction in magnitude of an expected reward (in this case, the time spent in the safe compartment), as it has been consistently shown that this SNC effect can be attenuated, or even abolished, by injecting GABAergic anxiolytic compounds such as diazepam [14,37,38] or thiopental sodium [20]. Given that SNC in one-way avoidance learning has been consistently obtained in female Wistar rats [4,14,20,37,38], female RHA, RLA and Wistar rats were used in the experiment we present below.

## 2. Method

### 2.1. Subjects

Twenty female RHA, 20 female RLA (from the colony of inbred RHA and RLA rats maintained at the Autonomous University of Barcelona), and 20 female Wistar rats (from the University of Granada experimental animal stock), at about 90 days of age at the start of the experiment, weighing 190–230 g, were used. Animals were housed individually with food and tapwater ad lib. Room temperature was kept to about 20 °C with a 12-h light/dark cycle. Training took place during the light phase, and the period needed to complete the whole experiment was 4 weeks. In order to avoid the influence of the oestrus cycle on the performance of rats in the behavioral test, the day of testing was counterbalanced across groups, in such a way that every testing day rats belonging to each of the 6 experimental groups were submitted (counterbalancing also the time of the day across groups) to the avoidance task.

### 2.2. Apparatus

A Leticia one-way avoidance chamber was used. It consisted of two equal compartments 27 cm long × 25 cm wide × 28 cm high, made of black Plexiglas. The compartments were separated by a 0.5 cm-thick partition 25 cm wide × 28 cm high, with a square 9 × 9 cm hole at floor level and a removable automatic gate to allow movement between compartments. Only the danger compartment was

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