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Research report

Rapid avoidance acquisition in Wistar–Kyoto rats

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ABSTRACT

The relationship between trait stress-sensitivity, avoidance acquisition and perseveration of avoidance was examined using male Wistar–Kyoto (WKY) and Sprague–Dawley (SD) rats. Behavior in an open field was measured prior to escape/avoidance (E/A) acquisition and extinction. E/A was assessed in a discrete trial lever-press protocol. The signal-shock interval was 60 s with subsequent shocks delivered every 3 s until a lever-press occurred. A 3-min flashing light safety signal was delivered contingent upon a lever-press (or failure to respond in 5 min). WKY rats displayed phenotypic low open field activity, but were clearly superior to SD rats in E/A performance. As avoidance responses were acquired and reached asymptotic performance, SD rats exhibited "warm up", that is, SD rats rarely made avoidance responses on the initial trial of a session, even though later trials were consistently accompanied with avoidance responses. In contrast, WKY rats did not show the "warm up" pattern and avoided on nearly all trials of a session including the initial trial. In addition to the superior acquisition of E/A, WKY rats demonstrated several other avoidance features that were different from SD rats. Although the rates of nonreinforced intertrial responses (ITRs) were relatively low and selective to the early safety period, WKY displayed more ITRs than SD rats. With removal of the shocks extinction was delayed in WKY rats, likely reflecting their nearly perfect avoidance performance. Even after extensive extinction, first trial avoidance and ITRs were evident in WKY rats. Thus, WKY rats have a unique combination of trait behavioral inhibition (low open field activity and stress sensitivity) and superior avoidance acquisition and response perseveration making this strain a good model to understand anxiety disorders.

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1. Introduction

Trait behavioral inhibition (BI), typified by reserved response or inactivity in the face of novel social and nonsocial situations [44,45], is a risk factor for anxiety disorders in children and adults [8,9,41,83,84,89,52,65,39]. The extreme behavioral withdrawal in BI is associated with enhanced stress reactivity, polymorphism of the corticotropin releasing hormone (CRH) gene [91,90] and increased reactivity within the hypothalamic pituitary adrenal axis [100]. Although BI is expressed in social situations, BI is a risk factor for the spectrum of anxiety disorders [17,36,1,65].

The translation of subject vulnerabilities to actual anxiety disorders is a function of environmental exposures (physical and emotional challenges), coping success and learning [58,59]. This is particularly apparent for posttraumatic stress disorder (PTSD), which can be traced to an event or series of experiences. Avoidance and avoidant behaviors are expressed along the trajectory from traumatic experience to the development of PTSD [32,64,63,66,51]. The growth of avoidance and avoidant behaviors distinguish between those who develop PTSD and those who recover from the traumatic experience and do not develop PTSD [32,66]. Thus, the growth and perseveration of avoidance behaviors appear integral to the development and maintenance of anxiety disorders. Accordingly, risk for anxiety disorders may reflect enhanced sensitivity to avoidance and avoidance contingencies.

Avoidance acquisition is a process of associating fear with warning signals and maintenance of responding through fear reduction [60,60,24,23,102]. Fear is important during the initial stages of avoidance acquisition [92]; removal of the amygdala impairs early acquisition but not avoidance maintenance or retention [99,78,85]. Avoidance acquisition depends on a distributed network which includes the cingulate cortex, thalamus, and hippocampus [98,27,33–35,50,77]. A byproduct of exposure to avoidance contingencies is the development of nonreinforced responses. In the absence of specific response contingencies, nonreinforced responses (intertrial responses, ITRs) are thought to reflect the

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emotional state or appreciation of the learning context in a molar sense [61].

Animal models offer the possibility to examine the relationships between trait stress reactivity, avoidance performance and the development of anxiety. Whereas fearful temperament expressed in nonhuman primates bears a striking resemblance to human BI in behavioral and neurobiological terms [46–49], sensitivity to avoidance learning has not been specifically addressed. Rodent models have not been informative. Historically, there is an inverse relationship between trait emotionality and active avoidance acquisition and its perseveration. Flinders rats bred for cholinergic hypersensitivity and displaying BI in the open field [67] are impaired in acquisition of active avoidance relative to cholinergic insensitive and outbred control strains [68]. Maudsley high and low reactive rats, selectively bred for high and low open field defecations, respectively, do not differ in avoidance [10]. Thus, trait BI in rats has not been associated with superior avoidance acquisition or its perseveration. Conversely, rats selectively bred for superior avoidance performance (e.g., Syracuse-high (SHA) and Roman-high avoidance (RHA) rats) are not inhibited by novel situations and the open field. It is their low avoidance counterparts (SLA and RLA, respectively) that display greater emotionality in the open-field test and tests of novelty and social interaction [16,94].

The Wistar–Kyoto (WKY) rat may be an exception. The Wistar–Kyoto rat, originally bred as a normotensive comparison strain for the spontaneously hypertensive rat, demonstrates trait BI. WKY rats display decreased activity and withdrawal in novel social [29,76] and nonsocial challenges [75]. Extensively studied by Paré and colleagues, WKY rats show low activity in the open field [75,87], sensitivity to ulcer formation [71], hyperresponsive peripheral and central stress responses [69,4,103], and learning and memory alterations [29]. Often cited as an animal model of depression [56,43,101,82,26,53,12], the behavioral signs are also in keeping with anxiety [12,57,22,55,70]. The dominant behavioral pattern of WKY rats is freezing and accordingly they exhibit superior passive avoidance [74]. As acknowledged by Paré, trait BI creates difficulty attributing nonmovement to learning. Early assessments of active avoidance performance of WKY rats suggested poorer shuttle box avoidance [96] and lever-press avoidance [6], but did not include an outbred strain for reference. Therefore, the present study examined acquisition and extinction of an active lever-press avoidance response in WKY rats compared to male Sprague–Dawley (SD) rats.

Avoidance and extinction were compared with a modification of discrete trial lever-press avoidance. A lever-press is not among the species-specific defense reactions of rats [11,18]; thus intrinsic reactions have the potential to compete with acquisition of a lever-press. However, lever-press avoidance is not influenced by overall activity. Acquisition of lever-press avoidance is affected by factors such as the shock intensity [19,7], duration of safety signal [13], and prior motivational state [31,15,14,2]. The procedural modification to use brief intermittent shock as the unconditional stimulus allows the rat to examine alternative behavioral responses within the shock–shock interval [5]. Thus, freezing and general shock-induced activity has less influence on the overall rate of acquisition.

From the early research on active avoidance in WKY rats, one would expect slower avoidance acquisition in WKY rats compared to SD rats. This finding would be in keeping with numerous studies examining the relationship between trait BI and avoidance. However, a case could be made for the converse. Superior passive avoidance and the association between BI and anxiety also suggested superior lever-press avoidance could be observed in WKY rats. To verify BI in WKY rats, all rats were tested in the open field. We then compared WKY and SD rats in several phases of training: acquisition training (10 sessions), extinction training in the absence of footshock (13 sessions), and extinction training in the absence of the footshock and safety signal (5 sessions).

2. Materials and methods

2.1. Animals

Male SD rats (60 days of age) were obtained from Charles River (Kingston, NY) and male WKY rats were obtained from Harlan Sprague–Dawley (Indianapolis, IN). Rats were housed in single cages in sound-attenuating chambers (12:12 light cycle, lights on 0600). Upon arrival, all rats had at least 2 weeks to acclimate to their living conditions prior to the start of testing. Rats had free access to water and food in their home cages. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) in accordance with AAALAC standards.

2.2. Open field test

Open field activity was evaluated consistent with previous work [88]. Briefly, a rat was placed in the center of a circular open field under bright light conditions with masking white noise. Latency to leave the center and segments crossed were scored by observers blind to the experimental manipulations. The arena was wiped with a soapy solution between the testing of rats.

2.3. Lever-press escape/avoidance (E/A)

Training was conducted in four otherwise identical operant chambers (30 cm × 25 cm × 30 cm) (Coulbourn Instruments, Langhorn, PA) enclosed in four sound-attenuated boxes, each with an observation hole in the front door. The operant chambers have a clear Plexiglas front with a door. One wall of the chamber is fitted with a lever (10.5 cm above the floor), a cue light (14 W, 20.5 cm above the grid floor) and a speaker (26 cm above the grid floor). On the opposing wall, a light (14 W, 26 cm above grid floor) is constantly lit for general illumination. The unconditional stimulus (US) was a scrambled 1.0-mA electric foot-shock delivered through the grid floor from a shocker (Coulbourn Instruments, Langhorn, PA). The warning signal (WS) was a 1000-Hz 75-dB tone (10 dB above background noise).

Escape/avoidance training consisted of 20 trials in daily sessions separated by 2–3 days (3 sessions/week). Each session began with a 60-s stimulus-free period. A trial commenced with the delivery of the auditory warning signal. A lever-press during the warning signal, but before 60 s had elapsed, prevented the delivery of a shock, triggered a 3-min safe period signaled by a flashing light, and was scored as an ‘avoidance’. A lever-press during the warning signal, but after 60 s elapsed, terminated shock delivery, triggered a 3-min safe period signaled by a flashing light, and was scored as an ‘escape’. In the absence of a lever-press, a maximum of ninety-nine 0.5-s shocks were delivered on a fixed interval 3-s schedule leading to the automatic end of a trial and beginning of the safe period. A rat that failed to emit a lever-press response by the end of the fourth training session was removed from the study.

There were several phases of training. During acquisition, training proceeded as described above. Acquisition continued until both group performances were greater than 60% avoidance with no more than 5% variation between sessions. There were two phases of extinction. During the first phase, the shock was omitted; a lever-press turned off the warning signal and initiated the signaled safety period. During the second extinction phase, the safety signal was omitted in addition to the shock; a lever-press only terminated the warning signal. Extinction continued until there was less than 5% variation between sessions. Although shocks were omitted, responses during the first 60 s of the warning signal were designated as ‘avoidance’, and those with latencies greater than 60 s were designated as ‘escape’.

2.4. Testing schedule

SD ($n = 8$) and WKY ($n = 8$) rats were examined in the open field prior to escape/avoidance training. Escape/avoidance training consisted of 10 sessions of acquisition, followed by 13 sessions of extinction without the shock, and 5 sessions of extinction without the shock and safety signal. One WKY rat was omitted from the study due to its failure to emit a single lever-press response during the first four sessions (local IACUC standard).

2.5. Data analysis

Data from the open field test were analyzed using a t-test for independent groups. For escape/avoidance training, several measures were obtained. The session data were tabulated on a trial basis for shocks received, whether an escape response was emitted, whether an avoidance response was emitted, response latency, and lever presses for each minute of the safety period (intertrial responses, ITRs). Inferential statistics were applied only to the data corresponding to response latency and ITRs. For trials which ended without a lever-press, the maximum value of 396 s was substituted. Post hoc analyses of significant interactions were elaborated with