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Learning strategy is influenced by trait anxiety and early rearing conditions in prepubertal male, but not prepubertal female rats

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ABSTRACT

Rodents solve dual-solution tasks that require navigation to a goal by adopting either a hippocampus-dependent *place strategy* or a striatum-dependent *stimulus–response strategy*. A variety of factors, including biological sex and emotional status, influence the choice of learning strategy. In these experiments, we investigated the relationship between learning strategy and anxiety level in male and female rats prior to the onset of puberty, before the activational effects of gonadal hormones influence these processes. In the first experiment, prepubertal male rats categorized as high in trait anxiety at 26 days of age exhibited a bias toward stimulus–response strategy at 28 days of age, whereas age-matched females exhibited no preference in strategy regardless of anxiety level. In the second experiment, male and female rats were separated from their dams for either 15 or 180 min per day during the first 2 weeks of life and tested on a battery of anxiety and cognitive tasks between 25 and 29 days of age. Prolonged maternal separations for 180 min were associated with impaired spatial memory on a Y-maze task in both prepubertal males and females. Furthermore, prolonged maternal separations were linked to elevated anxiety and a bias for stimulus–response strategy in prepubertal males but not females. Alternatively, brief separations from dams for 15 min were associated with intact spatial memory, lower levels of anxiety, and no preference for either learning strategy in both sexes. These results provide evidence of sex-specific effects of trait anxiety and early maternal separation on the choice of learning strategy used by prepubertal rodents.

1. Introduction

When learning to navigate their environments, humans and rodents alike execute distinct cognitive strategies that are mediated by interacting brain structures (Iaria, Petrides, Dagher, Pike, & Bohbot, 2003; McDonald & White, 1994; Packard & McGaugh, 1996). Hippocampus-based navigational strategy, known as *place strategy*, is dependent upon the spatial relationships between salient visual cues in the surround and a goal (McDonald & White, 1994). Alternatively, the striatum guides the expression of *response* and *stimulus–response strategies*, which are based on either proprioceptive cues that signal specific body movements toward a goal (Packard & McGaugh, 1996), or on salient visual cues proximal to a goal that mark its location (McDonald & White, 1994). Various extrinsic and intrinsic factors affect the choice of learning strategy used by rodents, including cue salience, training level, biological sex, hormonal profile, and emotional status (Chang & Gold, 2004; Hawley, Grissom, Barratt, Conrad, & Dohanich, 2012; Hawley, Grissom, & Dohanich, 2011; Hawley, Grissom, Patel, Hodges, & Dohanich, 2012; Kanit et al., 2000; Korol, 2004; Korol, Malin, Borden, Busby, & Couper-Leo, 2004; Packard & Goodman, 2012; Packard & McGaugh, 1996; Pleil & Williams, 2010; Restle, 1957; Tunur, Dohanich, & Schrader, 2010; Wingard & Packard, 2008).

When rats are tested on a task that can be solved by either place or response strategy, adult males typically prefer place strategy, particularly early in training (Chang & Gold, 2003; Packard & McGaugh, 1996). The preference for a place strategy by adult male rats does not appear to be contingent upon the presence of testicular hormones, which indicates that this strategy preference is established during development (Gibbs, 2005; Hawley, Grissom, Barratt et al., 2012). In contrast, there is a robust relationship between ovarian hormonal status and learning strategy in adult female rodents (Daniel & Lee, 2004; Korol et al., 2004; Pleil & Williams, 2010). When estradiol levels are high, during the proestrous stage of the estrous cycle or following administration of exogenous estradiol, female rodents are more likely to prefer a place strategy (Korol, 2004). Conversely, when estradiol levels are low, adult females prefer a response strategy (Korol, 2004).

Heightened anxiety influences the choice of learning strategy by routing cognitive control away from the hippocampus and toward the striatum (Packard, 2009). Adult male rats exhibited a preference

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for a response learning strategy upon administration of anxiogenic drugs, which heighten state anxiety (Elliott & Packard, 2008; Packard & Wingard, 2004). Interestingly, natural or trait anxiety also influenced hippocampus-dependent learning and memory (Hawley, Grissom, & Dohanich, 2011; Herrero, Sandi, & Venero, 2006) and choice of learning strategy (Hawley, Grissom, & Dohanich, 2011). Adult male rats that expressed higher levels of trait anxiety exhibited poorer spatial memory on hippocampus-dependent versions of a water-maze task (Herrero et al., 2006) and a Y-maze task (Hawley, Grissom, & Dohanich, 2011), and were biased toward a stimulus–response strategy on a dual-solution version of a visible platform water maze task (Hawley, Grissom, & Dohanich, 2011). Therefore, anxiety impairs performance when only a place strategy can be used to solve a spatial task but shifts learning strategy from place to response or stimulus–response strategy on dual-solution tasks.

Although a link between anxiety and learning strategy has been documented in male rodents, this relationship has not been investigated in females. However, elevations in anxiety associated with chronic stress during adulthood impaired performance on a variety of cognitive tasks in males, but did not impair and even enhanced performance in females (Barha, Pawluski, & Galea, 2007; Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Kosten, Lee, & Kim, 2006; Lehmann, Pryce, Bettschen, & Feldon, 1999; Wigger & Neumann, 1999). Furthermore, evidence from our laboratory indicated that later cognitive performance was impaired by daily separations for 180 min from dams during the first 2 weeks of life in prepubertal males compared to males separated daily for 15 min (Frankola et al., 2010). In contrast, the performance of prepubertal females was either unaffected or even enhanced by 180 min of daily maternal separation. Whether sex differences in cognitive function associated with prolonged maternal separation in early life correspond with a shift in learning strategy preference remains to be determined.

Gonadal hormones exert organizational effects on brain development during critical periods of prenatal and early postnatal life, and subsequently affect brain function by exerting activational effects in adulthood. Studying sex differences prior to puberty provides a unique developmental window during which circulating gonadal hormones are nominal in both males and females, and not yet cyclical in females (Korenbrot, Huhtaniemi, & Weiner, 1977; Leibowitz, Akabayashi, Alexander, Karateyev, & Chang, 2009; MacKinnon, Puig-Duran, & Laynes, 1978; Whitney & Clark, 2001). Therefore, investigations during this period allow for an understanding of the organizational influence of gonadal hormones, prior to the onset of their activational effects following puberty.

In two experiments presented here, we examined the interaction between biological sex and trait anxiety on learning strategy preference in prepubertal rats between 25 and 29 days of age. In the first experiment, unmanipulated male and female rats were tested to determine if higher levels of trait anxiety were associated with a bias toward a stimulus–response strategy on a dual-solution visible platform water maze task (McDonald & White, 1994). In a second experiment, a daily maternal separation paradigm during the first 2 weeks of life was implemented to heighten prepubertal levels of trait anxiety. For both experiments, we predicted that higher levels of anxiety would be linked to the use of stimulus–response strategy in prepubertal males, but not females.

2. Materials and methods

2.1. Experiment 1: Sex differences in the effects of trait anxiety on learning strategy

2.1.1. Subjects

Male and female Long-Evans hooded rats were obtained from Harlan, Inc. (Indianapolis, IN) and mated, yielding 10 litters of pups. If birth occurred before 17:00 h, that day was defined as Postnatal Day 0 (PND 0). Within 24 h of birth, litters were culled to 10–12 pups. The resulting 51 male and 48 female pups were used as subjects in Experiment 1. Breeder rats and their offspring were housed in a climate-controlled facility under a 12:12-h light/dark cycle (lights on at 07:00 h) with free access to food and water. Pups were weaned at PND 21 and group housed by sex and litter. Behavioral tests were administered in the same sequence for each rat. Rats were transferred to the testing room 30 min prior to each procedure and remained undisturbed to habituate to the staging area of the testing environment. All procedures were approved by the Tulane University Institutional Animal Care and Use Committee in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (1996). The animal care and use program of Tulane University is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.1.2. Open field test

Anxiety was tested at PND 26 in an open field constructed of black Plexiglas (90 × 90 × 45 cm) under low lighting. Each rat was placed in the corner of the open field and allowed a single exploration trial of 5 min. The floor and walls of the field were cleaned thoroughly with 70% ethanol and air-dried after each trial to remove olfactory cues. Trials were recorded by an overhead video camera for later analysis. The image of the field was divided into 16 equally sized squares (4 inner and 12 outer) by a transparency superimposed over a video monitor. Entry into a square was scored when a rat crossed the borders of a designated square with all four paws. Scoring was conducted by experimenters blind to the sex of the rat. Anxiety was indicated by calculating the percentage of time rats spent in the 4 inner squares of the open field. Activity was measured by calculating the total number of squares entered during the 5-min test.

2.1.3. Visible platform water maze task (VPWM)

Learning strategy was determined in accordance with previously validated procedures (Bizon, Han, Hudon, & Gallagher, 2003; Janis, Bishop, & Dunbar, 1994; Kim, Lee, Han, & Packard, 2001; McDonald & White, 1994). A white circular pool, 180 cm in diameter, was filled to a depth of 26 cm with water made opaque by the addition of non-toxic white tempera paint (Crayola, Inc., Easton, PA), and surrounded by extra-maze cues of varying shapes and sizes. The temperature of the water was maintained at approximately 25 °C. A visible black platform, 9.5 cm in diameter, projected 2 cm above the water surface and was located 30 cm from the wall of the pool. One day prior to testing, at PND 27, rats were placed in the pool without a platform present for a 1-min habituation swim. Twenty-four hours later, at PND 28, rats were trained on the task with the visible escape platform located in the southwest quadrant of the pool. Eight training trials were conducted with the rat entering from each of four cardinal points in a pseudorandomized order. Each rat was allowed 30 s to mount the platform where it remained for an additional 10 s (McDonald & White, 1994). If a rat failed to mount the platform in 30 s it was guided to the platform. Training trials were separated by an inter-trial interval of 5 min during which rats were dried and warmed under heat lamps. Learning was indicated by increasingly shorter path lengths as training progressed. Following the completion of training trials, the platform was moved to the opposite quadrant of the pool (northeast) and a probe trial was conducted in which rats entered the pool from the south, at a location most distal from the relocated visible platform. During the probe trial, rats that swam directly to the newly relocated visible platform were categorized as stimulus–response learners and rats that initially returned to within 5 cm of the location of the platform during training trials were