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Stress and risk avoidance by exploring rats: Implications for stress management in fear-related behaviours

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ABSTRACT

Animals display protective patterns of behaviour. Adoption of such protective patterns requires the assessment of risks posed by the environment and subsequent avoidance of high-risk locations. We hypothesized that adverse experiences lead to formation of a context-specific memory, thus changing the pattern of exploratory behaviours in response to specific cues associated with this experience. Here we examined exploratory movement patterns and compared approach/avoidance behaviours in rats following a stressful experience in the same versus a different environment. Using an open table exploration task, we compared exploratory movements in rats that were either stressed by restraint in a different environment or stressed in the test environment. The following day rats were allowed to explore the open table environment. Different test situations were provided in which rats were given access to a refuge or a large visual object as placed in the vicinity to the open table. The results indicate that stressed rats avoid the specific location associated with a stressful experience. However, when provided with a refuge in this location the salience of the stressful memory is reduced and the refuge will become a preferred location. Exploratory patterns and avoidance behaviours were correlated with corticosterone levels. Behavioural changes were not related to dendritic morphology in the medial prefrontal cortex (layer III). The results provide insights into risk avoidance strategies in rats and suggest that environmental intervention can reverse stress-related changes in behaviour.

1. Introduction

Animals adopt protective patterns of behaviour in their natural habitat and in laboratory settings (Blanchard et al., 2001; Bonsignore et al., 2008; Brown, 1996; Brown et al., 1999; De Boer and Koolhaas, 2003; Pyke et al., 1977). Avoiding the areas of high danger is a common strategy that serves to minimize the risks in the environment (Blanchard et al., 2001; Bonsignore et al., 2008; Lima, 1985; Lima et al., 1985; Lipkind et al., 2004). The exploratory strategy adopted by animals represents a trade-off between the benefits, such as access to food resources, and the potential risks (Lima, 1985; Lima et al., 1985; Whishaw et al., 1992, 2006). The adoption of a protective pattern of behaviour to enhance the benefit of exploration while minimizing its risks requires the assessment of risks in the environment (Blanchard et al., 2011). Examination of risk assessment strategies includes exposing animals to a situation in which stressful events conflict with the experience of a favourable environment and modulate subsequent approach and avoidance strategies.

The laboratory examination of exploratory behaviour and emotionality in the open field began with Hall's (1934) study of emotional behaviour of rats on an open table (Hall, 1934; Halliday, 1966, 1968). A series of subsequent studies indicated that exploratory locomotion of rats might become organized to their point of entry, home bases, local objects or their memorized locations (Eilam and Golani, 1989; Hines and Whishaw, 2005; Nemati and Whishaw, 2007).

The analysis of exploratory behaviour in an open field is widely used as a well-studied paradigm to model anxiety-like and stress-related behaviours in animals (Costall et al., 1989; D'Aquila et al., 2000; Halliday, 1966; Eilam et al., 2011; Lipkind et al., 2004). The purpose of the present study was to examine the fear-related pattern of exploratory behaviour in rats in relation to the location of the stressor. The behavioural changes were evaluated in relation to circulating corticosterone values and morphology of pyramidal neurons in the medial prefrontal cortex (Zilles, 1985). This area is vital to contextual learning of emotions and fear memory and it is involved in the modulation of stress responses (Corcoran and Quirk, 2007; Cook and Wellman, 2004; Onge and Floresco, 2010; Radley et al., 2004). Female rats were used because they organize their exploration patterns in reference to their point of entry in an open table paradigm (Nemati and Whishaw, 2007). It was anticipated that the exploratory profile reflects contextual memory of adverse

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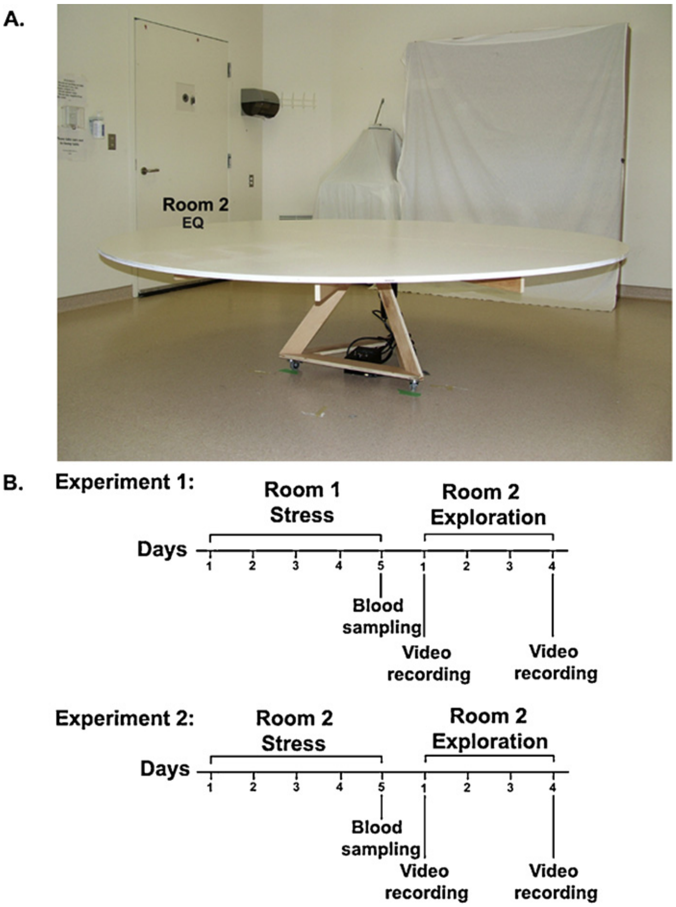

Fig. 1. Testing condition and apparatus in Experiments 1 and 2. (A) Rats explored a circular open table in a large testing room starting from entry quadrant (EQ). (B) Experiment 1: exploratory behaviour of rats was video recorded in Room on the first and last days of four consecutive exploration days starting the day after blood sampling following 5 days of control or stress treatments in a different room (Room 1). Experiment 2: exploratory behaviour of rats was video recorded on the first and last days of four consecutive exploration days in Room 2 starting the day after they received their control or stress treatments at the same location.

experience and that environmental intervention can reverse such stress-related patterns of behaviour.

2. Materials and methods

2.1. Animals

Thirty-seven adult female Long–Evans rats were used in the experiments, 90 days old and weighing 250–300 g at the beginning of the experiment. Rats were raised at the University of Lethbridge vivarium and housed in groups of two or three in Macrolon cages with sawdust bedding and ad libitum food and water. The colony room temperature was maintained at 20–21 °C and 30% humidity, with a 12/12 h light/dark cycle. Experimental procedures were approved by the University of Lethbridge Animal Care Committee in accordance to guidelines set forth by the Canadian Council of Animal Care.

2.2. Open field

The open field and testing environment are illustrated in Fig. 1A. The open table was a 244-cm diameter white round wooden table, elevated 64 cm above the floor (Nemati and Whishaw, 2007). The open table was located in a large testing room surrounded by a number of cues including a paper towel dispenser, switches, and posters on the wall. Very large salient objects, including a bookcase, and a sink were covered with white sheets to make them less conspicuous. No local cue was presented near the table in the first or second experiments, and a cage (entry quadrant) and/or a large visual cue (adjacent to the entry quadrant) was presented to the animals in Experiment 3. In order to minimize the local olfactory cues the table was cleaned with soap and water following each trial for each rat.

2.3. Stress treatment

Mild stress was induced by restraint for 20 min a day for 5 consecutive days. Animals were placed in a transparent Plexiglas cylinder (5 cm inner diameter) that maintained them in a standing position without compression of the body (Metz et al., 2005). The restraint was considered mild compared to other experiments (McEwen, 1999, 2000). Ventilation was possible through perforated ends of the container. Rats in the control group were placed in a Plexiglas transportation tub in the same room and for the same period of time.

2.4. Test conditions

Rats were tested in the following cue conditions:

(1) *No local object*: Room lights were on and no object was placed on or near the table.
(2) *Cage*: The cage was a Plexiglas box (20 cm × 12 cm × 25 cm) with a 4 cm × 4 cm entrance, facing the center of the table, placed on the edge of the table.
(3) *Large visual object*: A large visual object (48 cm × 48 cm × 52 cm black box), oriented toward the table was placed 20 cm away from the edge of the table.

2.5. Experimental design

Three experiments were conducted to assess the response to various cue conditions as described in the following.

Experiment 1: Rats in the stress group ($n = 6$) underwent restraint stress and those in the control group ($n = 6$) were placed in a transportation tub every day for 20 min in Room 1. Both containers and transportation tubs were covered from three sides to minimize the appearance of visual cues. Blood samples were collected immediately after the last restraint session on the fifth day of the experiment. On the sixth day of the experiment, rats were placed on the edge of an open table at the entry quadrant where they started to explore the environment for four consecutive days. The exploratory movements of rats were video taped in the first (on day 1) and last exploratory session (on day 4) for 20 min (Fig. 1B; Experiment 1).

Experiment 2: In the second experiment the procedure was similar to Experiment 1, except that rats in the stress ($n = 7$) and control ($n = 6$) groups were placed in the restraint containers or in the transportation tubs, respectively, within the testing environment. The containers and tubs were covered from three sides to prevent that rats learn the visual cues near the entry quadrant during the five stress sessions. The entry quadrant was the area from which rats started their exploration the day after the last stress session. Rats were tested for four consecutive days (Fig. 1B; Experiment 2).

Experiment 3: The restraint and control procedures were similar to Experiment 2 except that a large visual object was presented near the entry quadrant. Stressed ($n = 7$) and control ($n = 5$) rats were allowed to explore the open table with a different condition each day. The exploration started from inside a cage placed in the entry quadrant of the open table with no other local object near the