Behavioural Processes 136 (2017) 20–27

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Individual and sex differences in high and low responder phenotypes

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ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form 3 January 2017
Accepted 9 January 2017
Available online 11 January 2017

Keywords:
Behavioral test
Anxiety
Depression
Fear
Locomotor activity
Memory

ABSTRACT

Individual differences in responses to a novel environment are an important tool to predict predisposition to neuropsychiatric disorders. One way to examine individual differences involves classifying animals based on locomotion in a novel context. In this study we focused on individual and sex differences by categorizing female and male mice as high (HR) or low responders (LR) on the basis of open field locomotion. We then assessed whether groups differed on behavioral measures of spontaneous alternations, anxiety, depression and contextual fear conditioning. In the Y-maze, we observed no differences across HR/LR or sex on spontaneous alternations, but HR displayed more locomotion. HR male mice showed less anxiety-like behavior in the light-dark test but not the elevated plus maze. We observed no differences in the forced swim test across HR/LR, although males exhibited greater depression-like behavior overall. HR mice exhibited less contextual fear memory compared to LR regardless of sex. Principal component analyses suggested sex-specific patterns of behaviors across tests, with female responses within individual tests tending to load together. In females anxiety- and depression-like behaviors explained a large part of the variance observed across tests in our battery, whereas male behavior was primarily explained by variables related to locomotion.

1. Introduction

One of the major challenges in behavioral neuroscience is to understand the role of individual differences in predicting vulnerability to disease as well as responses to pharmacological interventions (Pawlak et al., 2008). One approach used to analyze individual differences in rodent studies involves exposing animals to a novel environment and assessing behavioral responses to novelty. In this regard, animals can be categorized as high (HR) or low (LR) responders on the basis of locomotor activity in a novel environment (Antoniou et al., 2008; Kabbaj et al., 2000; Kazlauckas et al., 2005; Piazza et al., 1989). This categorization has been shown to predict differences in learning (Antoniou et al., 2008; Görisch and Schwarting, 2006; Kazlauckas et al., 2005), stress responsiveness, as well as anxiety, depression (Jama et al., 2008; Kabbaj et al., 2000; White et al., 2007), and drug-related behavior (Hooks et al., 1991; Kalinichev et al., 2004; Piazza et al., 1990; Suto et al., 2001). For instance, studies have suggested that HR/LR phenotypes predict self-administration of amphetamines (Piazza et al., 1990) and cocaine (Hooks et al., 1991), namely, HR exhibit increased locomotion to amphetamines and cocaine, and also acquire self-administration of these drugs at lower doses than LR (Hooks et al., 1991; Piazza et al., 1989). Similar associations have been found between locomotor activity and acquisition of drug self-administration for morphine (Deroche et al., 1993; Kalinichev et al., 2004), ethanol (Hoshaw and Lewis, 2001; Nadal et al., 2002) and nicotine (Suto et al., 2001). HR rats also show lower levels of anxiety-like behavior in the light/dark test and elevated plus maze relative to LR (Kabbaj et al., 2000; White et al., 2007), an effect some authors suggest may reflect preference for novelty-seeking because, when given a choice, HR prefer novel and more stressful conditions (Dellu et al., 1993; Kabbaj et al., 2000; White et al., 2007). Other studies report less learned helplessness and anhedonia in HR relative to LR (White et al., 2007; Stedenfield et al., 2011), or no differences (Antoniou et al., 2008; Jama et al., 2008) in depression-like behaviors. Novelty-induced locomotion has also been associated with performance in explicit tests of learning, such as spatial learning, attention and emotional memory (Antoniou et al., 2008; Matzel et al., 2006, 2003). Together, these studies suggest that novelty-induced locomotion in rodents may predict specific responses in some behaviors that are relevant to neuropsychiatric disorders in humans.

Describing behavioral differences based on novelty-induced locomotion has proven a useful tool in male rodent studies but

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http://dx.doi.org/10.1016/j.beproc.2017.01.006
0376-6357/

evidence is lacking with regard to its predictive utility in females. Several studies show that females respond differently than males to cognitive, stressful and emotional experiences (Johnston and File, 1991; Keeley et al., 2005; Kokras and Dalla, 2014; Maren et al., 1994; Shors, 1998). For example, females show greater exploration, have a stronger preference for novelty compared with males (Lynn and Brown, 2009), and show lower levels of anxiety (Johnston and File, 1991). Similarly, sex differences have been described in spatial learning (Jonasson, 2005; Keeley et al., 2005; Bettis and Jacobs, 2009), and both cued and contextual fear conditioning (Cossio et al., 2016; Maren et al., 1994; Wiltgen et al., 2001). Interestingly, few studies have described sex-dependent behavioral differences based on locomotion in a novel environment. Some studies have examined the effects of sex on depression-like behavior (Pitychoutis et al., 2011) and drug-associated behaviors (Davis et al., 2008; Klebaur et al., 2001; Sell et al., 2005) as a function of HR/LR classification. However, a report of sex differences in HR/LR across behavioral domains is currently lacking.

The purpose of this study was to determine sex-dependent and individual differences in a battery of experimental tasks as a function of locomotor responses to a novel environment. We examined behavioral responses in a battery of tests in mice categorized into high (HR) and low responder (LR) phenotypes. We assessed whether HR and LR mice differ in exploratory behaviors, spontaneous alternations, anxiety, depression and associative fear learning. Two principal component analyses were undertaken also investigating the relationship between sex and behavior patterns across the test battery.

2. Methods

2.1. Animals

Thirty-five male and twenty-six female C57BL/6 mice weighing 23–29 g (Harlan, Mexico) at 8 weeks of age were used. Mice were maintained at 22 ± 2 °C on a 12-h light-dark cycle (lights on at 6:00 am) housed in groups of 3–4. Animals had free access to water and food. Behavioral testing occurred during the light period. All experiments were conducted in accordance with the National Institutes of Health regulations for the care and use of laboratory animals and INDICASAT policies.

2.2. Experimental procedures

All animals were exposed to the behavioral tests in the battery in the same sequence, one test per day, in the following order: open field, Y-maze spontaneous alternation, elevated plus maze, light/dark test, forced swim test, fear conditioning and context retention test. Mice were returned to their home cage at the end of each test. All mazes, chambers and related materials were cleaned with 10% ethanol alcohol between tests.

2.2.1. Tracking system

Behavioral data for all tests were obtained and analyzed with automated tracking software (ANY-maze Version 4.83x; Stoelting Company, Wood Dale, IL, USA) installed on a computer with digital video cameras mounted on the ceiling above each instrument.

2.2.2. Open field test

Locomotor activity in the open field was used to assess baseline exploratory behavior and categorize the animals in one of two different phenotypes: high (HR) and low (LR) responders (Antoniou et al., 2008; Kabbaj et al., 2000; Piazza et al., 1989). The open field consisted of a square open field (50 cm × 50 cm) constructed of white acrylic with walls of 30 cm height. The floor was divided into four central and 12 peripheral squares. Each mouse was taken from its home cage and placed into a corner, facing the wall, and was allowed to explore freely for five min. A line crossing was recorded when an animal moved across a line on the floor of the open field including central areas (Prut and Belzung, 2003), and the total line crossings was used as an index of baseline locomotor activity (Crawley, 1985). Based on the median number of lines crossed, mice were divided into groups of high responders (score above the median) and low responders (score under the median). Male and female medians were calculated separately. In addition, the time spent in central and peripheral areas was recorded (Bethancourt et al., 2011; Prut and Belzung, 2003).

2.2.3. Y-maze continuous spontaneous alternation

The Y-maze was constructed of white acrylic with three identical arms (40 × 9 × 6 cm) at a 120° angle from each other. Animals were placed at the center and allowed to freely explore the three arms for five min during which the sequence of entries into the arms was recorded (Hughes, 2004). The number of alternations was recorded also. Mice tend to explore a new arm of the maze rather than return to a previously visited arm, and performance in the Y-maze assesses the tendency of mice to alternate arms on successive opportunities (Hughes, 2004).

2.2.4. Elevated plus maze

The elevated plus maze is a widely used behavioral test to assess anxiety-like behavior and the effects of pharmacological agents on anxiety responses (Hogg, 1996; Walf and Frye, 2007). The apparatus, constructed of acrylic, consists of four arms originating from a central platform forming a cross. It has two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm), and 35 cm high walls. The apparatus was elevated 50 cm from the ground on a black acrylic stand. The procedure was similar to that described by Pellow et al. (1985). Briefly, test sessions began by placing the animal at the center facing towards an open arm, and allowing it to freely explore the maze for five min. The number of entries and time spent in the closed and open arms were recorded. Increases in the number of open arm entries and time spent in open arms are used as an index of anxiolytic behavior (Pellow et al., 1985; Walf and Frye, 2007).

2.2.5. Light/dark test

The apparatus used for the light/dark test was based on that described by Britton and Bethancourt (2009). One compartment (34 × 47 × 27 cm) was illuminated by a lamp in the ceiling (336 lx), while the other (25 × 47 × 27 cm) was not illuminated and was encased by a dark cover. The compartments were separated by a centrally positioned opening (10 × 10 cm) at the floor level. Mice were kept in a dark holding room for one hour, and then taken from the room in a dark container to a dark testing room (Bourin and Hascoët, 2003; Hascoët and Bourin, 1998). Mice were placed at the center of the light compartment, facing the opening, and allowed to move freely in the apparatus for five min (Bourin and Hascoët, 2003; Britton and Bethancourt, 2009; Hascoët and Bourin, 1998). The time spent in each compartment and the number of transitions between compartments was measured.

2.2.6. Forced swim test

The forced swim test has been widely employed for screening antidepressant activity in rodents (Petit-Demouliere et al., 2005; Porsolt et al., 1977). This model is based on the evaluation of immobility as a measure of behavioral despair, which rodents adopt after being placed in a condition from which they cannot escape. The animal is considered to be immobile when it ceases to struggle and remains floating in an upright position making small movements to keep its head above the water (Porsolt et al., 1977). Mice were individually forced to swim in an open cylindrical container (height: