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Comparative effects of stressors on behavioral and neuroimmune responses of fawn-hooded (FH/Wjd) and Wistar rats: Implications for models of depression

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ABSTRACT

Patients with depression and rodent models of depression show increased cytokines and activated microglia. Fawn Hooded (FH/Wjd) rats have long been used as a model of depression based on their depressive-like behaviors, high basal corticosterone levels and altered serotonergic levels, but little is known about the neuroimmune function in this model. To test whether depressive-like behaviors relate to dysfunction of the neuroimmune system, depressive-like behaviors in the forced swim test (FST) and corticosterone (CORT) response to the swim test were compared in male Fawn Hooded versus Wistar rats, and cytokine levels in plasma and brain and plasma CORT in response to lipopolysaccharide (LPS, an endotoxin that activates the neuroimmune system) or 1 h restraint were measured. Fawn Hooded rats had more depressive-like behaviors in the FST (decreased swim time and increased immobility) and increased overall plasma CORT compared with Wistar rats. Additionally, Fawn Hooded rats exhibited blunted brain and plasma cytokine response to LPS compared with Wistar rats, an effect that might be related to the blunted plasma CORT response to LPS. No strain differences were found on these measures in response to restraint stress. These results suggest that Fawn Hooded rats have a depressive-like phenotype potentially more closely associated with serotonin dysregulation and a dysregulated HPA axis and remain a relevant model for further defining the role of these systems in depressive conditions.

1. Introduction

Depression is a common disorder with approximately 7.6% of Americans surveyed experiencing depression during the two weeks prior to assessment (Pratt and Brody, 2014). Though depression is one of the most common psychiatric disorders, it has one of the lowest heritability estimates (Cardno et al., 1999). There are many different antidepressant treatments currently used to treat depression and these treatments target different systems within the brain from serotonin to glutamate to the neuroimmune system (Andrade, 2018; Kohler et al., 2014; Machado-Vieira et al., 2017). Interestingly, all treatments developed for depression thus far, no matter the putative mechanisms of action, seem to be effective in only some individuals with depression (Ruhe et al., 2006). Additionally, some dysfunctions that are targeted by drug treatments, like neuroimmune dysfunction, are not consistently found in all depressed patients, and therefore patients with neuroimmune dysfunction are potentially a subpopulation of depressed patients (Hodes et al., 2015; Kohler et al., 2017). Altogether this information suggests that there is etiologic heterogeneity in depressed populations. Finding one model to represent all depressed patients might be unrealistic and it might be more realistic to have separate models related to specific dysfunctions.

The Fawn Hooded (FH/Wjd) rat has been suggested as a genetic animal model of depression (Overstreet et al., 2007) because this substrain exhibits high immobility in the forced swim test (FST) (Hall et al., 1998; Rezvani et al., 2007; Rezvani et al., 2002; Tizabi et al., 2009), high corticosterone levels (Aulakh et al., 1993; Aulakh et al., 1988) and serotonin dysfunction (Aulakh et al., 1994; Hulihan-Giblin et al., 1993). While the FH/Wjd model has provided many insights into the potential genetic, pharmacological, and behavioral factors involved in depression, these rats have not been extensively studied for neurobiological factors known to be sensitive to stress—a hypothesized contributor to the etiology of depression. The present work sought to determine if the FH/Wjd model is an appropriate model for stress-related aspects of

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depression by confirming behavioral findings and expanding the focus on the neurobiological and hormonal domains.

Two neurobiological foci of the stress response are the hypothalamic–pituitary–adrenal axis (HPA), and the neuroimmune system. Individuals with depression have HPA dysregulation reflected in higher basal cortisol which can be associated with blunted response to stress, exaggerated HPA responses during recovery from stress, and altered responses to dexamethasone (Belvederi Murri et al., 2014; Burke et al., 2005a; Burke et al., 2005b; Lopez-Duran et al., 2009; Morris et al., 2012). Results vary across studies with factors such as age and time of blood collection. FH/Wjd rats have elevated basal HPA activity, as measured by corticosterone levels (Aulakh et al., 1993). The closely related FH/Har substrain has an exaggerated response to stress (Aulakh et al., 1993; Hall et al., 2001) (the response to stress has not been measured in the FH/Wjd substrain). Stress in rodent models of depression has been shown to cause a neuroimmune response including increased cytokine levels and microglia activation (Hinwood et al., 2013; Kreisel et al., 2014; Menard et al., 2016; Wohleb et al., 2011), which can be reversed by antidepressants (Kreisel et al., 2014). Individuals with depression can have elevated blood cytokines (Menard et al., 2016; Sluzewska et al., 1995; Tsao et al., 2006), and activated microglia have been found in postmosterm brains of humans with depression and in live human patients studied with imaging technology (Bayer et al., 1999; Menard et al., 2016; Setiawan et al., 2015; Steiner et al., 2008). Also patients with depression have an elevated immune response to challenges such as stress, vaccines or cytokines (Christian et al., 2010; Fagundes et al., 2013; Glaser et al., 2003; Miklowitz et al., 2016; Pace et al., 2006). The present work sought to further define the behavioral and corticosterone responses to swim stress in FH/Wjd rats and a comparator strain, Wistar rats, and to define the neuroimmune response to challenge with the classic endotoxin, lipopolysaccharide.

2. Materials and methods

2.1. Animals

Male Fawn Hooded (FH/Wjd) and Wistar rats (originally from Charles River, Raleigh, NC) obtained from breeding colonies at the University of North Carolina at Chapel Hill served as subjects. Rats were weaned at 21–23 days of age and housed in groups of 1–5 (1–3 for swim tests) until testing began. All single housed rats showed behaviors within two standard deviations from the mean and were thus included in the data. Subjects from multiple cages were used in all studies. Rats were on a 12:12 h light cycle and all experiments were done during the light part of the cycle. All animal work was approved by the University of North Carolina Institutional Animal Care and Use Committee.

Ten FH/Wjd and ten Wistar rats were used to gather behavioral data for the swim test. An additional cohort of 11 FH/Wjd and 14 Wistar rats used for the swim test had blood collected via tail nick at 30 mins post-test as this time point has been shown to have peak CORT response (Fig. 1a) (Connor et al., 1997). To measure basal plasma CORT levels seven FH/Wjd and five Wistar rats had their blood collected at the same time of day using the same procedure. These rats remained undistributed in their home cages until blood collection. There were 18 FH/Wjd and 18 Wistar rats used for cytokine assays. Outliers were excluded leaving cortex samples N = 4–6 per group; hypothalamus samples N = 3–5 per group; Blood samples N = 4–6 per group. The first experimental group, the controls, remained in their home cages until tissue collection. The second experimental group received an intraperitoneal injection of 125 µg/kg of lipopolysaccharide (LPS, an endotoxin that activates the neuroimmune system). This dose of LPS was chosen because this dose has been shown to have behavioral effects and a strong cytokine response in the brain (Breese et al., 2008; Whitman et al., 2013). The last experimental group received 1 h restraint stress in a decapicone (Braintree scientific, Braintree, MA). Four hours post-stress or post-injection rats were sacrificed by rapid decapitation (Fig. 1b). The four hour time point was chosen as brain tissue collected four hours post-stress or post-LPS injection have elevated cytokine levels, however at this time point CORT response to restraint stress is expected to have returned to nonstressed levels (Knapp et al., 2016; Vecchiarelli et al., 2016; Whitman et al., 2013). Trunk blood was collected while brains were rapidly removed and placed in ice cold phosphate buffered saline (PBS). Brains were then dissected on ice, immediately frozen on dry ice, and stored in −80C until homogenized. All blood was collected into tubes containing 0.1 M heparin (pH 7.4). Then blood was spun at 2300 *g* for 10 min, and the supernatant was frozen at −80C until use for corticosterone assays and cytokine ELISAs.

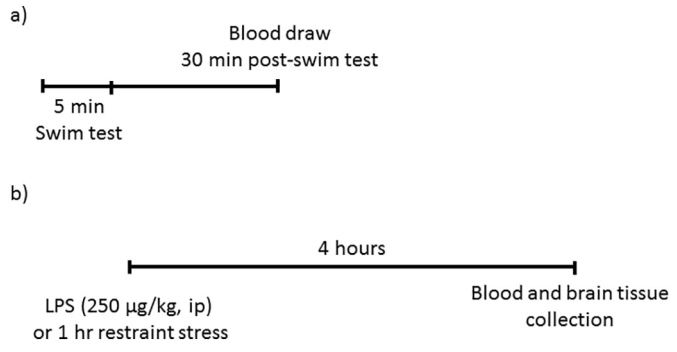

Fig. 1. a) Diagram of timing used for swim test and blood collection rats. b) Timing use for rats that were exposed to LPS or stress before tissue collection.

2.2. Forced Swim Test (FST)

The FST used in this experiment was a modified Porsolt swim test, which replicated the methods used previously to determine FH/Wjd behavior on the FST (Porsolt et al., 1977; Tizabi et al., 2009). The tanks used for the FST were Plexiglas cylinders measuring 20.0 cm in diameter and 40.0 cm in height. Water temperature was kept at 25 °C for all experiments. The water level for the swim tests was adjusted individually for each rat depending on weight/size, i.e. the water level was set such that the rats could not place their hind paws on the floor of the tank but their tails could reach the bottom (Overstreet, 2012). During each 5-min swim, behaviors were scored in real time, recorded, and rescored by a second rater at a later date. Inter-rater reliability was 95%. Rats were scored using criteria in Table 1 (Cryan et al., 2005; Detke et al., 1995; Mague et al., 2003).

2.3. Corticosterone (CORT) measurements

Plasma CORT levels following the swim test were assessed in duplicate with radioimmunoassay (RIA) using I^{125}-RIA kits Rat and Mouse (MP Biomedicals, Orangeburg, NY). Samples were diluted 1:200. Radioactivity was assessed relative to standard curves with an LKB

Table 1
Behavioral criteria for the FST.

Climbing	"Making forceful thrashing movements with its forelimbs directed against the walls of the cylinder"
Swimming	- Nose pointed upwards "Actively making swimming movements that caused it to move within the center of the cylinder"
Immobile	- Nose horizontal - Often, tail pulled off bottom "Only movement necessary to keep its head above water"
Diving	- Characteristic posture; or - Movement of one limb to maintain balance "If it swam below the water, toward the bottom of the cylinder"