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# Elevated paternal glucocorticoid exposure modifies memory retention in female offspring

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ABSTRACT

Recent studies have demonstrated that behavioral traits are subject to transgenerational modification by paternal environmental factors. We previously reported on the transgenerational influences of increased paternal stress hormone levels on offspring anxiety and depression-related behaviors. Here, we investigated whether offspring sociability and cognition are also influenced by paternal stress. Adult C57BL/6J male mice were treated with corticosterone (CORT; 25 mg/L) for four weeks prior to paired-matings to generate F1 offspring. Paternal CORT treatment was associated with decreased body weights of female offspring and a marked reduction of the male offspring. There were no differences in social behavior of adult F1 offspring in the three-chamber social interaction test. Despite male offspring of CORT-treated fathers displaying hyperactivity in the Y-maze, there was no observable difference in short-term spatial working memory. Spatial learning and memory testing in the Morris water maze revealed that female, but not male, F1 offspring of CORT-treated fathers had impaired memory retention. We used our recently developed methodology to analyze the spatial search strategy of the mice during the learning trials and determined that the impairment could not be attributed to underlying differences in search strategy. These results provide evidence for the impact of paternal corticosterone administration on offspring cognition and complement the cumulative knowledge of transgenerational epigenetic inheritance of acquired traits in rodents and humans.

## 1. Introduction

Exposure to stressful life events can cause a variety of mental health conditions, in part because the brain structures which are involved in cognitive and emotional functions are sensitive to the stress hormone corticosterone (CORT) (Lupien et al., 2009, 2007). Rodent models of chronic stress and CORT administration have demonstrated impaired cognitive performance, including spatial memory formation and retrieval, and stress-related psychopathologies (Finsterwald and Alberini, 2014). It has been shown that glucocorticoids are vital for memory consolidation and hippocampal glucocorticoid receptors are important in long-term memory formation (Chen et al., 2012). Furthermore, oral administration of corticosterone to male mice for 4 weeks impairs their cognitive performance including episodic memory in the novel object recognition test (NORT), associative memory in contextual fear conditioning, and spatial learning and memory in the Morris water maze (MWM) and Barnes maze (Darcet et al., 2014). Moreover, corticosterone injections before puberty reduced time exploring a juvenile rat during the social interaction test in adult rats (Veenit et al., 2013).

In recent years, various studies have shown that paternal exposure to stress can influence the progeny of the affected parents as well (reviewed in Babenko et al., 2015; Bale, 2015; Skinner, 2014; Szyf, 2015; Toth, 2014). Children of World War II veterans displayed increased PTSD-like behaviors without being exposed to the combat themselves (Rosenheck, 1986). Additionally, transgenerational effects of stress were observed in children of both male and female Holocaust survivors as they presented elevated PTSD symptoms and altered cortisol levels alongside epigenetic regulation of the glucocorticoid receptor gene (Yehuda et al., 2014, 2001; Yehuda and Bierer, 2007). Rodent models have confirmed the potential effects of paternal trauma on the offspring and investigated the role of epigenetic alterations in the sperm as a possible mechanism of inheritance. Paternal stress produced robust changes in the microRNA content of the sperm and decreased hypothalamic-pituitary-adrenal (HPA) axis response (ele-

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vated CORT levels in the blood after stress) in offspring (Rodgers et al., 2013), while environmental enrichment, which reduces the effects of stress, increased HPA-axis response in progeny of the enriched males (Yeshurun et al., 2017).

It has been proposed that deficits in social behaviors are developed due to a combination of heritable and environmental factors (Champagne, 2010), and this premise has been explored in recent experimentation. Male offspring of fathers exposed to early life maternal separation combined with unpredictable stress (MSUS) spent less time interacting with a stranger mouse in the social interaction test, had abnormal social memory in the social recognition test and altered response to social defeat (Franklin et al., 2011). Furthermore, male rats exposed to the endocrine disruptor, Vinclozolin, sired progeny with impaired social behavior and memory in addition to altered response to stress (Crews et al., 2012). Female offspring of mothers, fathers, or both, exposed to social stress (alternated cage mates twice a week) displayed increased anxiety in the elevated-plus maze (EPM) and in the open field (OF) test, and reduced interaction time with a juvenile mouse in the social interaction test. These behavioral and physiological changes occurred in the offspring even when they were cross-fostered to dams (Saavedra-Rodríguez and Feig, 2013). Furthermore, our laboratory has demonstrated that oral corticosterone administration of male mice before conception altered the patterns of ultrasonic vocalization during maternal separation of their juvenile male offspring (Short et al., 2016). In addition, offspring of MSUS-treated fathers showed increased anxiety, social defeat behavior and impaired long-term memory associated with reduced long-term potentiation (LTP) and elevated long-term depression (LTD) in the hippocampus (Bohacek et al., 2014). This converging evidence strongly suggests that paternal stress affects different phenotypes in the offspring and that this can occur in a sex-specific manner.

We aimed to investigate whether paternal corticosterone treatment would affect offspring sociability as well as spatial learning and memory. Corticosterone oral administration was chosen as it provides a clean approach, selectively controlling the levels of circulating stress hormone with minimal intervention, unlike paradigms of physical stressors (Gourley and Taylor, 2009). Previous work using this method found that while CORT administration had no behavioral effects on male mice, their offspring demonstrated an increase in anxiety-like phenotypes (Short et al., 2016). Since CORT has been shown to modulate memory formation and social behavior, we hypothesized that CORT administration to fathers would reduce offspring cognitive performance and social interaction.

## 2. Materials and methods

### 2.1. Mice

7-week-old male C57BL/6 mice were purchased from the Animal Resources Centre (Murdoch, WA, Australia) and housed in the core animal facility in ventilated cages (Tecniplast, Sealsafe PLUS Mouse IVC Green Line; 20 × 39 × 16 cm) with *ad libitum* food and water. Mice were group-housed and maintained on a 12-h light/dark cycle (lights on at 0700H). Mice were weighed once a week and the cage bedding was changed weekly. All procedures were approved by the Florey Institute of Neuroscience and Mental Health Animal Ethics Committee in accordance with the recommended guidelines set by the National Health and Medical Research Council (NHMRC) of Australia.

### 2.2. Oral corticosterone administration

After 3 weeks of acclimation, 16 male mice were randomly separated into 2 groups. 8 mice received CORT-supplemented water (25 μg/ml; Sterloids Inc., Newport, RI, USA; as described in Gourley and Taylor, 2009). 8 control mice received untreated water and bottles were replaced twice a week at the same time as the CORT-treated mice. CORT was dissolved by increasing the pH with NaOH and was left at a pH of 12 for 3 h. Once dissolved, HCl solution was added in order to return to a pH of 7.3. The CORT solution was made fresh twice a week and was stored at 4 °C. Fluid intake for both groups was determined by weighing the drinking bottles twice weekly, prior to replacing the bottles. The 4 weeks duration of treatment was chosen in order to cover the spermatogenic cycle of the mouse (Oakberg, 1956), thus ensuring that all mature and developing sperm were exposed to the treatment for the entire cell cycle.

### 2.3. Breeding

After four weeks of treatment, male mice were weighed and moved to a new cage to be single-housed. 10-week-old naïve C57BL/6 females were introduced in the afternoon for pair-mating with *ad libitum* food and water. After 5 days, males were removed and females were single-housed until they littered down.

Pups were toe-clipped 7–10 days after birth and boxes were replaced. On postnatal day 25, offspring were weaned and divided into new standard-housing boxes (15 × 30 × 12 cm). Every box comprised 3–5 mice of the same sex and same paternal treatment with ad libitum food and water. Behavioral testing began when offspring were 8 weeks of age and was performed on both sexes.

### 2.4. Behavioral testing

All tests were performed during the light phase of the light/dark cycle, completing before 1700H. Mice were acclimatised to the room for at least 1 h before commencement of each test. From 8 weeks of age, offspring were tested on the two-trial Y-maze for spatial memory, and the social interaction test (SIT). Tests were performed on a separate day with at least two resting days. At 11 weeks of age, mice were tested in the Morris water maze (MWM), which was performed last in order to avoid any impact of the stressful experience of forced-swim on the other tests. All analysis was collected using automatic tracking to eliminate the possibility of experimenter bias and experimenter was blinded to the treatment group. The same mice were used for all the experiments. See Fig. 1A for experimental design diagram.

#### 2.4.1. Social interaction test

We used the social interaction task for the assessment of sociability according to Yang et al. (2011). The test was performed in a rectangular, three-chamber box made of Perspex (also known as Plexiglas) (42 × 39 × 11 cm) and covered with a transparent Perspex lid. The box consisted of a smaller central chamber (12 × 39 cm) that is connected to the two other side chambers (15 × 39 cm) via a small rectangular opening (4.5 cm). Each side chamber contained a rectangular wire cage (15 × 10 × 10 cm), which was positioned next to the upper wall of the chamber. Each cage was divided by a mesh into 6 × 10 cm and 9 × 10 cm areas.

In the habituation trial, each test mouse was placed in the central chamber and could explore the apparatus with two empty cages for 10 min. Immediately after the test mouse was removed, a stranger mouse of the same sex was placed in one of the cages (alternated between each mouse) at the smaller part of the cage (6 × 10 cm), which is next to the central chamber, while the other side contained an empty cage. The test mouse was reinserted into the central chamber immediately after to explore the apparatus for 5 min (test trial). During the test trial mice were automatically monitored using TopScanLite 2.00 (CleverSys Inc., VA) for the overall distance and duration in each chamber, while interaction time with the stranger mouse was manually recorded by an experienced scorer blind to the experimental group.

#### 2.4.2. Y-maze

We used the Y-maze test to assess short-term spatial memory based on the innate tendency of mice to explore a novel environment as we