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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

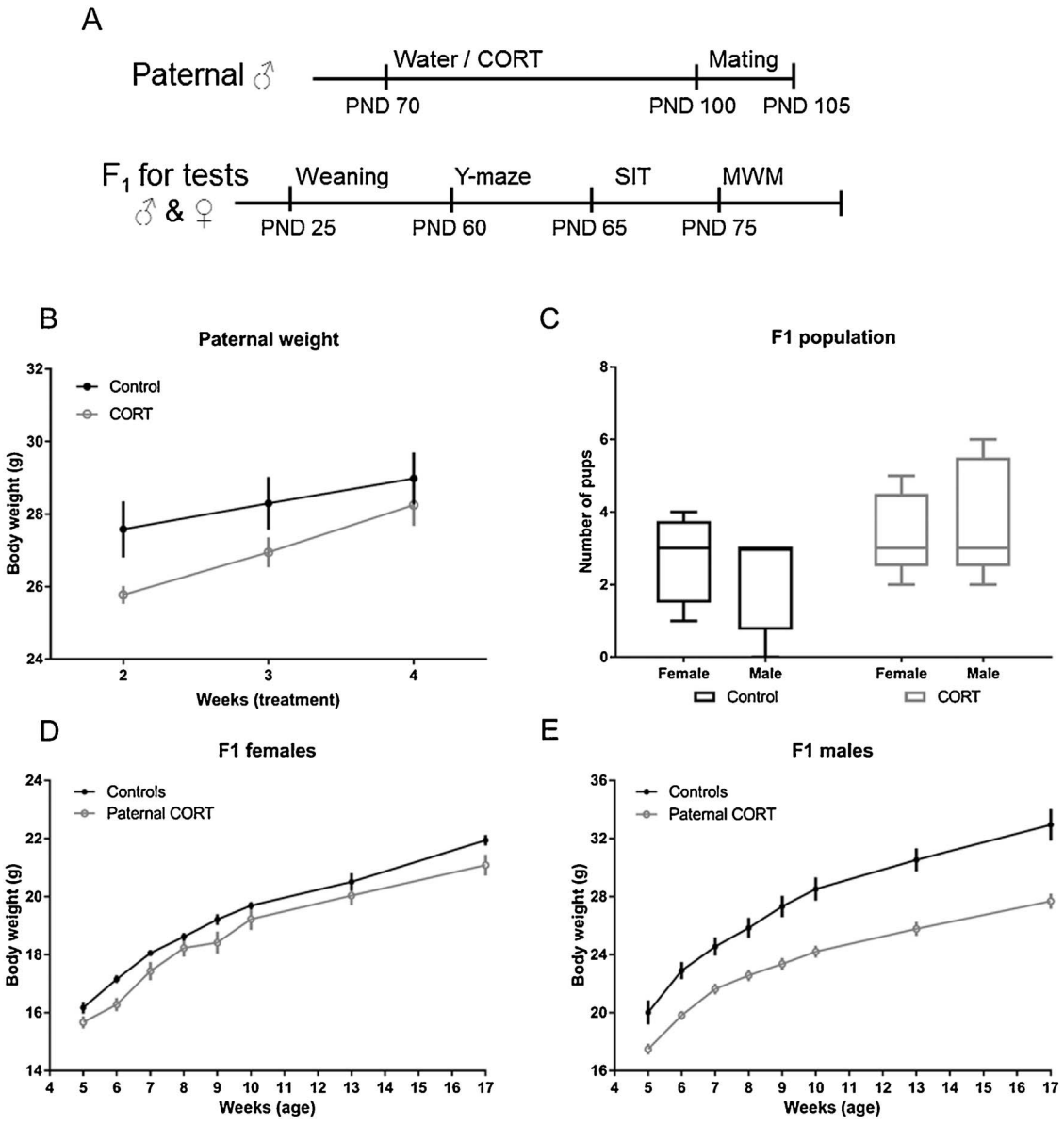

Fig. 1. Study design, paternal weight, litter size and offspring body weights. (A) Diagrammatic representation of the experimental design. 16 adult male C57BL/6 mice were randomly allocated into groups of four mice per cage for water controls and CORT-treated mice. 4 weeks later, male mice in both groups were each mated with a naïve female for five days. F1 female and male offspring were tested from the age of 8 weeks in the Y-maze, social interaction (SIT), and the Morris water maze (MWM). (B) CORT treatment had no effect on paternal body weights (n = 8 per group). Values represent mean ± SEM. (C) Distribution of female and male pups born to each litter. Box plot whiskers represent minimum and maximum. (D, E) Female and male offspring of paternal CORT treatment displayed reduced body weight throughout the study. n = 8 control and paternal CORT females; n = 6 control males and n = 10 paternal CORT males. Values represent mean ± SEM.

previously described (Mo et al., 2014). The maze is a Y-shaped apparatus made of light-coloured Perspex with three arms (10 × 30 × 17 cm; San Diego Instruments, CA, USA) with a visual cue placed at the end of each arm. The individual arms were designated as home, familiar or novel arm, separated by a central neutral zone. In the training trial, mice were placed facing the wall of the home arm and explored the maze for 10 min with only the home and familiar arms accessible while the novel arm was blocked. After a 1-h interval in the home cage, the blockage was removed and the test trial was conducted where the mouse was placed in the home arm and explored the entire maze for 5 min. In order to reduce olfactory cues, the maze floor was covered with the bedding from the home cage of the tested mice (bedding was left untouched between the training and test trials) and was replaced when a new box of mice was tested. The arena was cleaned with 70% ethanol and dried with paper towels between each box to minimize olfactory confounds. Both trials were automatically monitored using TopScanLite 2.00 (CleverSys Inc., VA). Duration and latency for each arm were recorded and analysed as well as the total distance moved in each trial. The first minute in the test trial was excluded from the duration analysis in order to control for anxious behavior characterised by delayed latency to leave the home arm. Novel-arm preference index (PI) was calculated for the test trial in the following way: time spent in the novel arm/[average time spent in other two arms], where a preference of 1 indicates no preference. Spending more time in the novel arm is indicative of intact memory of the two familiar arms.

2.4.3. *Morris water maze*

Mice were tested using a Morris water maze version as we previously described (Rogers et al., 2016b). The circular pool measured 1.2 m in diameter and 0.5 m in height was located in a room with a number of high-contrast 2D distal cues as well as several 3D cues in

order for mice to be able to navigate and learn the location of the hidden platform (Rogers et al., 2016a). The pool was filled with water at a temperature of 22 ± 2 °C that was made opaque with 50 ml nontoxic white paint to conceal a circular platform (10 cm in diameter) placed 0.5 cm beneath the water surface. Each mouse was trained for 6 consecutive days at 4 trials per day (a different starting point for each trial was used in each day) separated into 4 blocks of 8 mice of the same sex with alternated paternal treatment each time. Mice were allowed to search for the platform location for a maximum of 60 s and if they did not find its location they were gently guided to it. Mice were video recorded in each trial and their time to platform was recorded. Once on the platform, mice were left for 30 s in order to promote spatial mapping of its location. Afterwards, mice were removed and placed in holding containers underneath heating lamps for approximately 15 min before their next trial.

A day after the 6 days of learning, a retention probe was performed, where the platform was removed from the pool and mice were allowed to swim for 60 s starting from a novel position. Preference for target quadrant, number of platform crossings and proximity from platform were automatically monitored using TopScanLite 2.00 (CleverSys Inc., VA) and analysed in blocks of 15 s.

2.4.4. Morris water maze search strategy analysis

Search strategy analysis was performed using time-tagged xy-coordinates derived from TopScanLite using a MATLAB algorithm (Mathworks, Natick, MA, USA) as we previously described (Rogers et al., 2016a). The algorithm quantifies the strategy mice employ during each trial to find the platform into seven different strategies which are either non-spatial (egocentric) or spatial (allocentric), hippocampus-dependent approaches (Rogers et al., 2016a). Indication of hippocampal-dependent spatial learning is presented as the percentage of spatial search strategy in each trial. Effects sizes for paternal treatment were valued as odds ratios (ORs) with 95% confidence intervals (CIs).

2.5. Statistical analysis

For comparisons between groups, either Student's *t*-test, two-way analysis of variance (ANOVA) or repeated-measure (RM) two-way ANOVA were used where appropriate. Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., LA Jolla, CA). For the search strategy analysis (binary outcome), we performed a logistic regression model using Stata v13.0 (StataCorp, College Station, TX) as we previously described (Rogers et al., 2016a). All data are presented as mean ± SEM unless stated otherwise. The threshold for significance was set at $P < 0.05$.

3. Results

Male mice were administered CORT or water for 4 weeks before mating with females. During that period, mice were weighed at three different time-points. Comparison between controls and CORT-treated males by two-way repeated-measures ANOVA revealed a significant effect of time ($F_{(2,28)} = 39.01$, $P < 0.0001$) but without differences in body weight between control mice and CORT-treated mice and no CORT x time interaction (CORT: $F_{(1,14)} = 2.514$, $P = 0.1352$; interaction: $F_{(2,28)} = 3.013$, $P = 0.0653$) (Fig. 1B).

3.1. Paternal treatment with CORT alters offspring body weights

In the F1 offspring, 20 mice were born to control fathers (from 4 successful matings), and 36 mice were born to CORT-treated fathers (from 5 successful matings). Two-way ANOVA revealed that litter sizes were not affected due to paternal CORT ($F_{(1,14)} = 2.738$, $P = 0.1202$) and there were no differences in the number of male or female pups ($F_{(1,14)} = 0.005657$, $P = 0.9411$) and no paternal CORT x sex interaction ($F_{(1,14)} = 0.4582$, $P = 0.5095$). From a week after weaning and until the end of all behavioral experiments, female and male offspring were weighed weekly unless a behavioral experiment was conducted at the time. Two-way repeated-measures ANOVA revealed an expected significant effect of time ($F_{(7,98)} = 219.2$, $P < 0.0001$), a significant paternal CORT treatment effect ($F_{(1,14)} = 4.860$, $P = 0.0447$) and no paternal CORT x sex interaction ($F_{(7,98)} = 0.5964$, $P = 0.7574$) on body weights of the female offspring (Fig. 1D). Interestingly, male offspring of CORT fathers exhibited a strikingly significant decrease in their body weight compared with controls ($F_{(1,14)} = 29.78$, $P < 0.0001$) together with significant effects of time ($F_{(7,98)} = 282.2$, $P < 0.0001$) and paternal CORT x time interaction ($F_{(7,98)} = 4.759$, $P = 0.0001$) (Fig. 1E).

Offspring of both sexes were behaviorally assessed at 8 weeks of age in the three-chamber social interaction test, the Y-maze, and the Morris water maze. Since we have previously found a sex-specific anxiety phenotype in this model (Short et al., 2016), we analysed female and male offspring independently.

3.2. Paternal CORT administration had no influence on offspring behavior in the social interaction test

Distance travelled during the test was not affected by paternal CORT for both female ($t_{(14)} = 0.3107$, $P = 0.7606$) (Fig. 2A) and male offspring ($t_{(14)} = 1.789$, $P = 0.0953$) (Fig. 2B). Interaction time of the test mouse with the stranger was not influenced by paternal CORT treatment for both female ($t_{(14)} = 0.1921$, $P = 0.8504$) (Fig. 2C) and male offspring ($t_{(14)} = 0.1203$, $P = 0.9060$) (Fig. 2D). The ratio of time spent in the test chamber to the empty chamber also did not significantly differ between the groups in both females ($t_{(14)} = 1.685$, $P = 0.1141$) (Fig. 2E) and males ($t_{(14)} = 1.262$, $P = 0.2277$) (Fig. 2F).

3.3. Paternal CORT treatment had an influence on offspring exploratory behavior while short-term memory remained unaffected

While female offspring of paternal CORT did not differ in the total distance travelled ($t_{(14)} = 0.3941$, $P = 0.6994$) (Fig. 3A), male offspring of paternal CORT travelled significantly further than controls ($t_{(14)} = 2.396$, $P = 0.0311$) (Fig. 3B). Male offspring of paternal CORT were also quicker to leave the home arm showing reduced latency compared with controls ($t_{(14)} = 2.915$, $P = 0.0113$) (Fig. 3D), whereas no effects were found in female offspring ($t_{(14)} = 0.7071$, $P = 0.4911$) (Fig. 3C). We further analysed the preference index for time spent in the novel arm after excluding the first minute of the test in order to control for the delayed latency to leave the home arm. Offspring of both sexes were not affected by paternal CORT treatment in the novel arm preference index (females: $t_{(14)} = 0.07359$, $P = 0.9424$; males: $t_{(14)} = 1.143$, $P = 0.2724$), suggesting that paternal CORT had no effect on offspring spatial short-term memory (Fig. 3E and F).

3.4. Search strategy selection in the Morris water maze was not influenced by paternal CORT treatment

During the training phase of the task, female and male offspring of both groups improved their performance by decreasing their latency to the platform (females: $F_{(5,70)} = 9.294$, $P < 0.0001$; males: $F_{(5,70)} = 19.49$, $P < 0.0001$), but there was no effect of paternal CORT (females: $F_{(1,14)} = 0.3197$, $P = 0.5808$, Fig. 4A; males: $F_{(1,14)} = 0.02716$, $P = 0.8715$, Fig. 4B) or day x paternal CORT interaction (females: $F_{(5,70)} = 0.5480$, $P = 0.7393$; males: $F_{(5,70)} = 1.787$, $P = 0.1268$). Moreover, our analysis of the search strategy selection demonstrated that the percentage of mice using hippocampal-dependent learning increased by more than 50% per day during the training for all mice (OR 1.56, 95%CI 1.40–1.75, $P < 0.0001$), regardless of paternal treatment (OR 1.23, 95%CI 0.69–2.21, $P = 0.483$) and with a parallel strategy in both sexes (OR

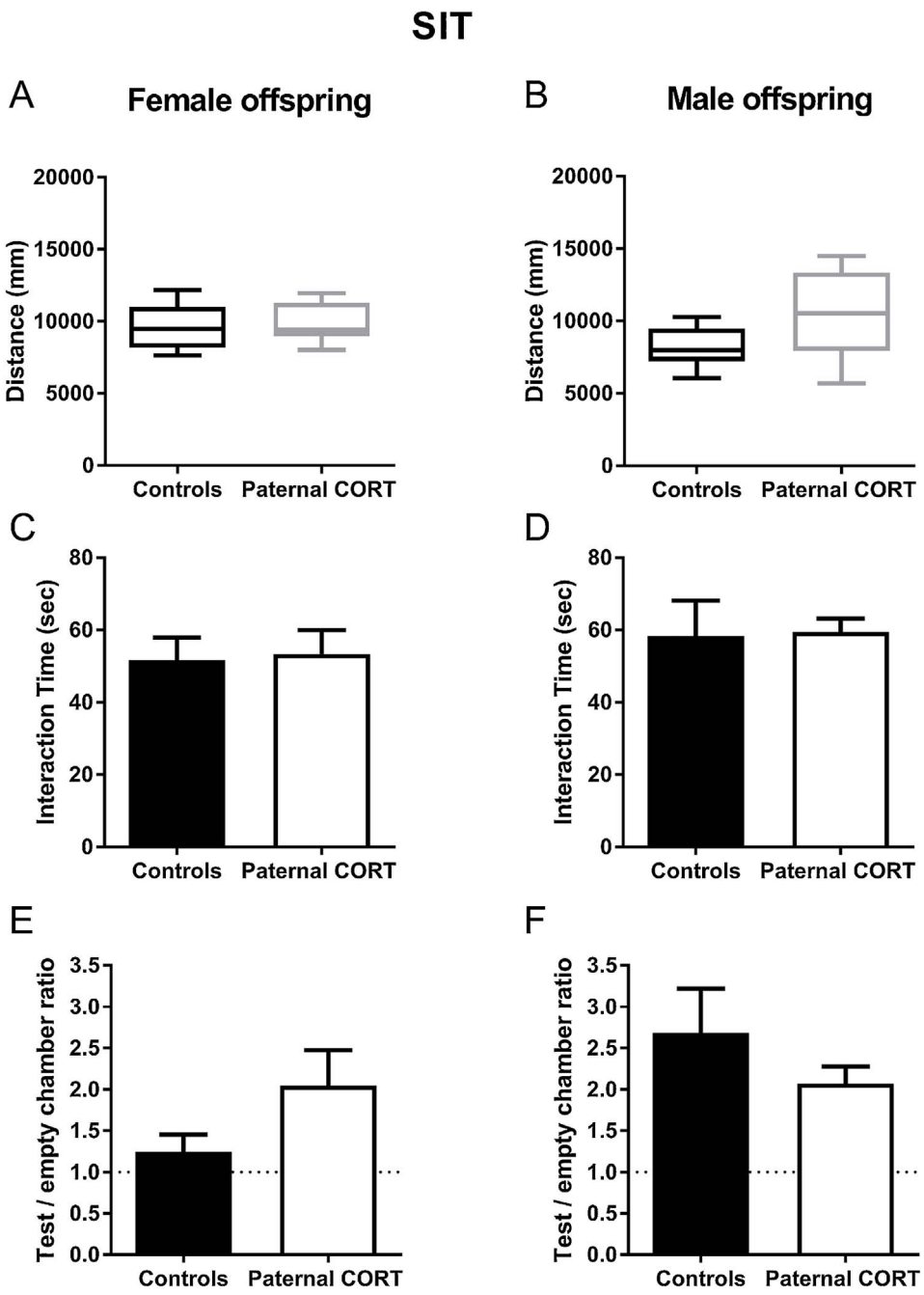

Fig. 2. Offspring performance in the social interaction test (SIT). (A, B) Total distance travelled in the social interaction test for female and male offspring. Box plots represent interquartile range with 95% CI. (C, D) Paternal CORT treatment had no effect on female and male offspring interaction time with a stranger mouse. (E, F) Ratio of time spent in test chamber and empty chamber was not altered due to paternal CORT treatment in both female and male offspring. n = 8 control and paternal CORT females; n = 6 control males and n = 10 paternal CORT males. Bar plots represent mean ± SEM.

0.99, 95%CI 0.56–1.78, $P = 0.986$) (Fig. 4C and D).

3.5. Female offspring of paternal CORT show reduced memory retention in the Morris water maze

In the retention probe, female offspring of control fathers demonstrated a clear preference for the target quadrant compared with the other quadrants (15.91 s, 95%CI 11.08–20.74). In contrast, female offspring of CORT-treated fathers failed to exhibit target quadrant preference (11.82 s, 95%CI 6.71–16.93). Since the confidence intervals overlapped with the 7.5 s of chance, these results suggest impairment in long-term spatial memory due to paternal CORT (Fig. 5A) (see Supplementary Fig. S1A for duration in all quadrants). Likewise, control females demonstrated significantly more crossings of platform location than paternal CORT ($t_{(14)} = 2.949$, $P = 0.0106$) (Fig. 5E) and proximity to platform location was, on average, 25% lower, although this was not significant ($t_{(14)} = 1.905$, $P = 0.0776$) (Fig. 5C). These results demonstrate that paternal corticosterone administration resulted in female offspring with impaired retention memory compared with controls. Paradoxically, control male offspring did not show target quadrant preference (9.64s, 95%CI 4.807–14.47), while male offspring of CORT-treated fathers showed a preference (13.42s, 95%CI 9.181–17.67) (Fig. 5B) (see Supplementary Fig. S1B for duration in all quadrants). However, proximity to platform was not affected by paternal CORT ($t_{(14)} = 1.191$, $P = 0.2533$) (Fig. 5D), nor platform crossings ($t_{(14)} = 0.9211$, $P = 0.3726$) (Fig. 5F).

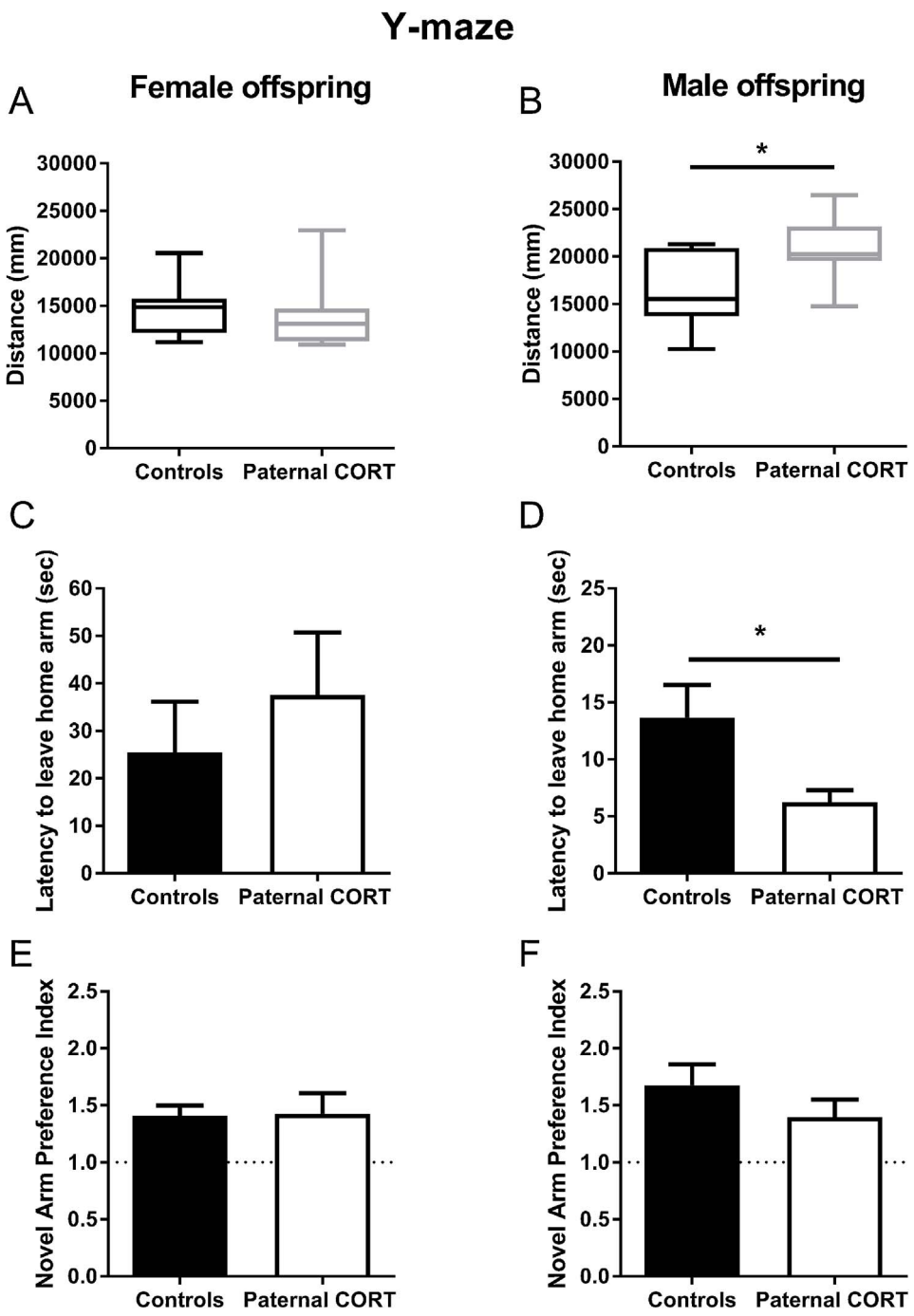

Fig. 3. Effects of paternal CORT treatment on offspring behavior in the Y-maze. (A, B) Total distance travelled in the Y-maze for female and male offspring. Box plots represent interquartile range with 95% CI. (C, D) Latency to leave the home arm for female and male offspring. (E, F) Preference index for duration in the novel arm, of female and male offspring. n = 8 control and paternal CORT females; n = 6 control males and n = 10 paternal CORT males. Bar plots represent mean ± SEM. *P < 0.05.

4. Discussion

This study has found that paternal treatment with corticosterone resulted in offspring with decreased body weights with a marked effect on males. We show that offspring sociability was not altered following paternal CORT administration. Moreover, there were no changes in short-term spatial memory in the Y-maze. Paternal CORT treatment resulted in impaired cognitive performance of the female offspring in the retention probe day of the Morris water maze, while there were no differences in the learning strategies of the mice. These findings suggest that paternal dysregulation of the HPA axis could alter offspring cognitive performance in a sex-specific manner, which complements and extends recent discoveries regarding the transgenerational impacts of paternal stress.

While not significant, there was a trend for CORT administration to reduce fathers' body weights, which was later recovered by the 4th week. We previously showed that body weights of adult male mice were reduced in the first week of oral CORT administration but returned to the same levels as controls from second week until the end of the study (Mo et al., 2014). An additional study also reported similar results using this model (Karatsoreos et al., 2010). These results are consistent for this model, demonstrating that while there is an initial reduction in body weights due to CORT treatment, they are the same as controls prior to conception. However, while interaction was not significant

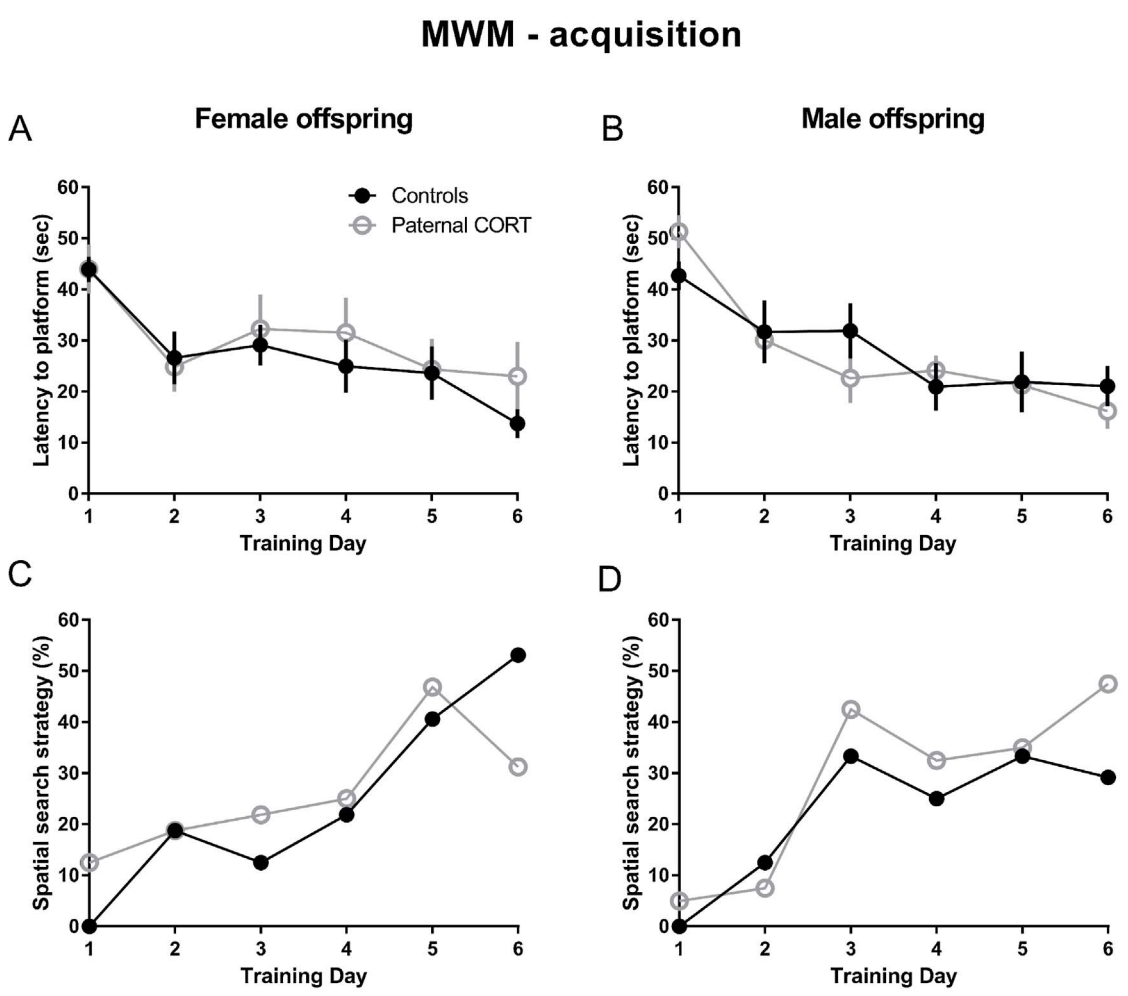

Fig. 4. Paternal CORT had no effect on offspring spatial acquisition training and search strategy selection in the Morris water maze. (A, B) Female and male offspring latency to platform during training phase. Values represent mean ± SEM. (C, D) Female and male offspring spatial search strategy selection during training phase. Values represent mean. n = 8 control and paternal CORT females; n = 6 control males and n = 10 paternal CORT males.

(P = 0.0653), body weight changes in the first week of CORT intake could still affect the spermatogenesis cycle of mice and thus influence the offspring.

In this study, we show that paternal CORT treatment significantly reduced offspring body weights, which is comparable with other transgenerational inheritance studies. Maternal separation combined with unpredictable stress (MSUS) has also resulted in offspring with lessened body weights (Weiss et al., 2011) and, in rats, ancestral exposure to chronic stress resulted in reduced offspring body weights at birth (Yao et al., 2014). Interestingly, we previously demonstrated that paternal exposure to enriched environment had the opposite effect, as male offspring displayed elevated body weights (Yeshurun et al., 2017). Taken together these results suggest that a stressful versus an enriched environment may directly impact on a common biological pathway that modulates offspring body weight. Another explanation would be that these biological pathways are separate but converge onto a common factor which controls the weight of offspring.

We observed that male offspring of CORT-treated fathers show increased exploratory behavior compared with controls by increased total distance travelled and reduced latency to leave the home arm in the Y-maze. Behavioral performance in the Y-maze is based on a rodent's innate exploratory drive and is therefore sensitive to the effects of stress (Conrad et al., 2004). Furthermore, we have previously observed that male offspring of CORT-treated fathers show augmented anxiety-like behavior (Short et al., 2016). Therefore, results of the current study in the Y-maze, which show hyperactivity and physical agitation, could be explained as increased anxiety-like behavior.

While results observed in the Y-maze could result from the modified affective behavior of the male offspring, impaired memory retention of the female offspring in the Morris water maze is novel and reveals another pathway of influence for paternal HPA-axis dysregulation, and might be sex-specific in nature.

We found impaired memory retention of the female offspring in the Morris water maze which is a novel result and reveals another pathway of influence by which HPA-axis dysregulation can affect cognitive performance, even when the offspring were not exposed to the CORT treatment themselves. It is well established that stress exposure has an impact on long-term memory, which is mediated by glucocorticoid receptors and brain-derived neurotrophic factor (BDNF) (Finsterwald and Alberini, 2014). Furthermore, CORT administration results in decreased Nr3c1 (glucocorticoid receptor) and corticotropin-releasing factor (Crh) expression levels in the hippocampus (Lee et al., 2010). Previous studies using the CORT administration model of HPA-axis dysregulation demonstrated deficits in learning acquisition (Olausson et al., 2013) and fear memory extinction (Gourley et al., 2009) in both rats and mice respectively. In addition, oral CORT treatment resulted in reduced BDNF levels in the hippocampus of male mice, which was associated with reduced motivational performance (Gourley et al., 2008). Moreover, both exposure to chronic stress and CORT treatment induced retention impairment in the Morris water maze (de Quervain et al., 1998). In addition, transgenic mice overexpressing Crh or with impaired glucocorticoid receptor (GR) function exhibited deficits of

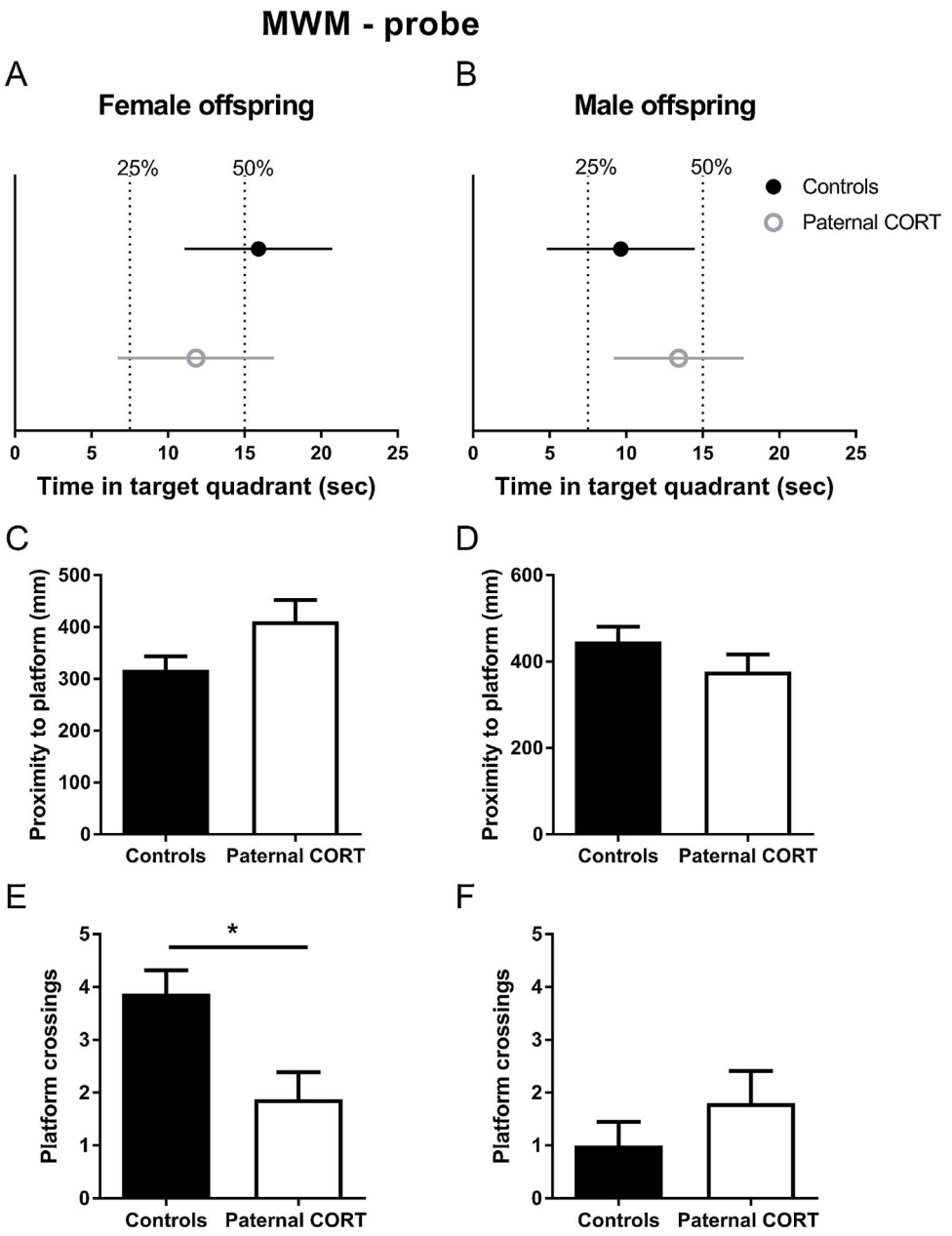

Fig. 5. Female offspring of CORT-treated fathers show reduced retention probe performance in the Morris water maze. (A, B) Female and male offspring target quadrant preference during retention probe. Values represent mean ± 95%CIs. (C, D) Female and male offspring proximity to platform. (E, F) Female and male offspring number of platform crossings. n = 8 control and paternal CORT females; n = 6 control males and n = 10 paternal CORT males. Bar plots represent mean ± SEM. *P < 0.05.

memory acquisition in the hidden-platform test (Heinrichs et al., 1996; Rousse et al., 1997). In a recent study using CORT administration as a model for HPA-axis dysregulation, short-term episodic memory in the novel object recognition test, associative memory in contextual fear conditioning and spatial memory in both the Morris water maze and Barnes maze were all impaired due to CORT (Darcet et al., 2014). As the balance between stress resilience and vulnerability is regulated by Crh (Cramer et al., 2015), an interesting follow-up for the current study would be to examine offspring Crh expression levels in the hypothalamus as well as the glucocorticoid receptor, Nr3c1.

Here we show that female offspring of CORT-treated fathers showed reduced performance in the retention probe of the Morris water maze, but without differences in the search strategy selection compared with controls. These results suggest that non-cognitive processes influenced the task performance and the changes observed are not due solely to altered learning capabilities but are likely to be determined by other aspects of cognitive function. In other words, female offspring of CORT-treated fathers employ the same search strategies as controls but show decreased memory retention, as navigation and escape in the Morris water maze require other cognitive processes apart from spatial learning, such as motivation (Kapadia et al., 2016). The Morris water maze is aversive and depends on motivation behavior, and is thus more prone to stress than other mazes (Morris, 1984). Moreover, exploratory drive could be another possible explanation for poor performance in the water maze (Kapadia et al., 2016). Therefore, further investigations should be carried out in order to elucidate whether the female offspring show lessened retention memory due to specific cognitive impairments or due to other non-cognitive factors.

Unpredictably, male offspring of control fathers have not shown a significant preference for the target quadrant in the Morris water maze. However, male offspring of CORT-supplemented fathers demonstrated target quadrant preference, although this preference was less than 50% on average. In addition, both controls and paternal CORT males exhibit low levels of platform crossing suggesting that both groups have failed

to perform in the retention probe. It is well established that chronic stress affects memory differently in male and female rats (Luine, 2002). Moreover, in the Morris water maze, sexual dimorphism is not uncommon and females usually perform better than male mice (Jonasson, 2005). Transgenerational epigenetic effects are dependent on the sex of both the parents and the offspring (Bale, 2015). The sex differences observed in our study might occur due the involvement of epigenetic modifications in the X chromosome but not the Y, the effects of sex hormones, and/or differential gene expression in the placenta in response to the sex of the fetus (Bale, 2015). Hence, our results complement and extend the literature demonstrating sexually dimorphic effects on offspring following paternal stress interventions (Pang et al., 2017). Nevertheless, reduced performance in the retention probe for males of both groups should be taken into consideration, as robust conclusions cannot be drawn from this data. Together with our previous evidence that only male offspring demonstrated altered anxiety-like behavior due to paternal CORT (Short et al., 2016), this suggests a sexually dimorphic dissociation of the cognitive and affective impacts of paternal HPA-axis dysregulation.

We show that paternal HPA-axis dysregulation transgenerationally altered female offspring memory retention. Previous studies show that CORT administration in pregnant dams of rats results in longer fear memory retention in their offspring (Callaghan and Richardson, 2012) and altered fear extinction (Bingham et al., 2013). However, while these modifications in memory retention could be due to effects *in utero* or altered maternal behavior, our results suggest a role for non-Mendelian inheritance, as the fathers only contribution to the offspring in the present study was encapsulated in the sperm. Moreover, we previously showed that maternal licking and grooming behavior towards the pups was not altered when mothers were mated with CORT-treated fathers and mating frequency was comparable to those of control males, suggesting an epigenetic mode of inheritance via alterations in the male germ cells (Short et al., 2016). Therefore, the present study is an important follow-up to the cumulative literature on the effects of transgenerational inheritance on offspring behavior. Cognitive performance has been shown to be affected by parental stress, which reduced spatial memory performance in the Barnes maze in second-generation male offspring via the male linage (Morgan and Bale, 2011). However, in an additional study from the same group, offspring of chronically stressed fathers have not shown differences in the Barnes maze compared with controls (Rodgers et al., 2013). In contrast, a model of paternal social defeat stress resulted in induced depression and anxiety-like behaviors in the offspring (Dietz et al., 2011). Furthermore, maternal separation combined with unpredictable stress (MSUS) transgenerationally modified offspring affective behavior (Franklin et al., 2010) and social behavior (Franklin et al., 2011), possibly via small noncoding RNA (sncRNA) changes in the sperm (Gapp et al., 2014). Modifications in long but not short-term memory as well as altered fear conditioning and modified synaptic plasticity have been demonstrated using the same paradigm (Bohacek et al., 2014). In the same study, changes in brain-specific gamma subunit of protein kinase C (Prkcc), which is implicated in synaptic plasticity and memory performance, were observed. In addition, DNA methylation of the Prkcc promoter was altered in the offspring hippocampus and in the sperm of the fathers. Nevertheless, our CORT administration model is substantially different from the chronic stress or MSUS paradigms, as it directly targets the HPA axis and does not affect the fathers' behavior (Pang et al., 2017; Short et al., 2016). Hence, by directly manipulating the CORT levels, we may avoid activating other neuroactive hormones including adrenalin, adrenocorticotropic hormone and Crh, in contrast to the other models of stress (Karatsoreos et al., 2010). Moreover, our laboratory has demonstrated that the transgenerational effects of CORT treatment are likely to be mediated via sncRNA alterations in the paternal sperm (Short et al., 2016). Therefore, we can speculate that in the current study, changes in the offspring can also be attributed to sncRNA changes in sperm. However, as this is a relatively new and rapidly evolving field, the precise mechanisms mediating this transgenerational epigenetic inheritance are yet to be fully elucidated.

5. Conclusions

Paternal CORT administration had a significant influence on memory retention of the female offspring. These findings complement and extend previous studies in our laboratory and others, focusing on the transgenerational influence of the environment in both rodent model studies and human epidemiological analyses. However, further investigations of the mechanisms behind these effects are needed in order to understand the transgenerational effects of paternal stress and develop preventative approaches as well as therapeutic interventions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.05.014.

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