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Cryptic sexual dimorphism in spatial memory and hippocampal oxytocin receptors in prairie voles (*Microtus ochrogaster*)

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ABSTRACT

Sex differences are well documented and are conventionally associated with intense sex-specific selection. For example, spatial memory is frequently better in males, presumably due to males' tendency to navigate large spaces to find mates. Alternatively, monogamy (in which sex-specific selection is relatively relaxed) should diminish or eliminate differences in spatial ability and the mechanisms associated with this behavior. Nevertheless, phenotypic differences between monogamous males and females persist, sometimes cryptically. We hypothesize that sex-specific cognitive demands are present in monogamous species that will influence neural and behavioral phenotypes. The effects of these demands should be observable in spatial learning performance and neural structures associated with spatial learning and memory. We analyzed spatial memory performance, hippocampal volume and cell density, and hippocampal oxytocin receptor (OTR) expression in the socially monogamous prairie vole. Compared to females, males performed better in a spatial memory and spatial learning test. Although we found no sex difference in hippocampal volume or cell density, male OTR density was significantly lower than females, suggesting that performance may be regulated by sub-cellular mechanisms within the hippocampus that are less obvious than classic neuroanatomical features. Our results suggest an expanded role for oxytocin beyond facilitating social interactions, which may function in part to integrate social and spatial information.

1. Introduction

How animals use and remember space is very important in determining survival and reproductive success. In fact, although it is generally underappreciated, space use underlies most of the recognized theories that attempt to explain the evolution of mating systems (e.g., Emlen and Oring, 1977). Consider that individuals must effectively navigate space to locate resources, shelter, and potential mates. Several studies have demonstrated that spatial distribution of resources has a profound effect on mating systems (Lott, 1984). Veritably, the distribution of resources impacts how individuals disperse, thus impacting density and accessibility of mates. Therefore, space is a crucial element on which mating decisions and mating behaviors depend.

The ecology and life history of a species has a profound impact on how individuals use space. A proficiency in navigating space and a capacity for spatial memory should therefore be essential for reproductive success, and differential selective pressures have the potential to create striking differences in tactics between and within species. However, reproductively successful strategies should not be defined by the same behaviors across species. In many polygynous species like the meadow vole (*Microtus pennsylvanicus*), male reproductive success is largely determined by the number of females they can encounter and fertilize. Mating among meadow voles, for example, occurs in scramble competition, which forces males to locate and acquire females within a critical period of receptivity (Spritzer et al., 2005). In cases such as this, males tend to occupy much larger home ranges than females, and hence some have argued that polygynous males should be better at spatial tasks than females (Ostfeld, 1990). Indeed, male meadow voles are better in spatial performance tests than females (Galea et al., 1995; Gaulin and Fitzgerald, 1989, 1986; Jacobs et al., 1990). In contrast, monogamous species such as the pine vole (*Microtus pinetorum*) maximize reproductive success by mating with one female and aggressively guarding her to protect paternity (Fitzgerald and Madison, 1983). It has been argued that the equitable mating seen in monogamous species alleviates the sex-specific selective pressures that drive sexual dimorphism (Andersson, 1994). In this case, sexually monomorphic species should not only be similar in their outward morphological phenotypes (exophenotype), but also show minimal or

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no differences in their behavioral tendencies and neural phenotypes (endophenotypes) (c.f., John and Lewis, 1966). Furthermore, home range size for males and females among monogamous species is usually equitable, as can be seen in monogamous species of voles (Getz and Hofmann, 1986), for example. Supporting this view, comparative studies have shown a relative absence of sex differences in spatial performance in pine (*M. pinetorum*) and prairie (*M. ochrogaster*) voles when compared to meadows voles (Gaulin and Fitzgerald, 1989, 1986; Jacobs et al., 1990). These data have been interpreted as supporting the hypothesis that sex-specific selection – selection that acts differently on males and females and acts as a driver of sexual dimorphism – promotes sex differences in cognition only in mating systems where sex-specific selection is strong. For instance, sex-specific selection creates sex biased gene expression, which results in sexual dimorphism (Cheng and Kirkpatrick, 2016).

It is difficult to ignore the hippocampus when considering spatial navigation and memory. This brain structure is implicated in spatial cognition across a wide array of taxonomic groups (Eichenbaum et al., 1992). Within and between species, the relative and absolute size of the hippocampus is often directionally proportional to the capacity for spatial memory performance (Sherry et al., 1989). For example, the relative volume of hippocampi in food-caching birds is larger than non-caching conspecifics (Krebs et al., 1989). Even within humans, there is evidence that increased hippocampal volume is correlated with spatial ability (Maguire et al., 2000).

Beyond anatomy of the brain, neuromodulators in the hippocampus also affect spatial cognition. Oxytocin (OT) for example, is a mammalian nonapeptide that regulates a suite of social behaviors including pair bonding, aggression, parental care, and social recognition (Donaldson and Young, 2008). Moreover, this hormone has specifically been implicated in the modulation of mating decisions and tactics (Ophir et al., 2012). Yet, before the more recent emphasis for its role in sociality, trust, mating behavior, and 'love' (Baumgartner et al., 2008; Ferguson et al., 2002; Kosfeld et al., 2005; Zeki, 2007), OT was studied in the context of learning behavior (de Wied, 1980; Mühlethaler et al., 1984) and, notably, for its role in memory within the hippocampus (Engelmann et al., 1996). Indeed, OT impacts several forms of learning, and in most of these cases, OT appears to diminish, inhibit, or interfere with learning (Bohus et al., 1978; Kovács et al., 1979; Popik and van Ree, 1998). Understanding the oxytocinergic system is therefore an important component to understanding the neural basis of memory and how this aspect of behavior fits into the larger context of behavioral ecology and natural behavior.

Some have theorized that mating systems that foster relatively strong sex-specific selection pressures will foster more sex differences in behavior, morphology, and cognitive processes (Jacobs, 1995). If the principles acting to promote sexual dimorphism do indeed impact cognitive processes, and if how animals use and navigate space is vital for maximizing reproductive success, then males and females should utilize space differently. This hypothesis is largely supported by studies investigating sex differences in spatial ecology (Ims, 1987; Ostfeld, 1985; Pasch and Koprowski, 2006). Such thinking has led to the idea that sex-specific selective pressures on spatial ability and the hippocampus are absent in monogamous voles, where males do not appear to compete by increasing the size of their home ranges relative to females (Sherry et al., 1992). There is some evidence for this; wild caught meadow voles show a sex difference in relative hippocampal volume, whereas pine voles do not (Jacobs et al., 1990). However, this line of reasoning overemphasizes the relative size of space use (i.e., quantity), and underemphasizes the nature of the space use (i.e., quality). Indeed, it ignores the fact that even within the same area of space, the demands on how males and females use space likely differs. For example, as theory predicts, even monogamous males might prioritize spatial cognition to maximize reproductive success, and/or to mate guard by excluding neighboring conspecifics, whereas females might use space primarily to find survival enhancing resources, and possibly occasional additional mates (i.e., when they are sexually receptive) (Okhovat et al., 2015; Ophir, 2017; Ophir et al., 2012, 2008; Phelps and Ophir, 2009; Zheng et al., 2013). Such a scenario should lead to (presumably quite subtle) selective pressures that differentially prepare monogamous males and females to take advantage of space differently, thus maintaining some degree of dimorphism in spatial cognition. If such differences exist in classic monomorphic species, we expect that they are more likely to be observable at the endophenotype.

In this study, we propose that the challenges facing males and females of monogamous species differ substantially, despite the relatively relaxed sexual selective pressures that presumably act on them. If this is true, then it would be erroneous to dismiss ecologically driven sex-specific cognitive demands that are likely operating at more nuanced or subtle levels (i.e., 'cryptic sexual dimorphism'). We hypothesize that sex-specific cognitive demands are present even when the sexes are monomorphic, socially monogamous, and maintain territories of the same size in nature. To this end, we investigated whether prairie voles, which exhibit these characteristics (Gaines and Johnson, 1982; Heske and Ostfeld, 1990) demonstrate quantifiable sex differences in spatial learning ability and neural correlates of spatial learning and memory. Specifically, we analyzed spatial memory performance, hippocampal volume and cell density, and hippocampal oxytocin receptor expression in male and female prairie voles.

2. Materials and methods

2.1. Subjects

Fifteen wild caught or F1 unrelated pairs derived from Urbana-Champaign, Illinois were co-housed and allowed to breed naturally. At 21 days olds, pups were weaned from each breeding pair and housed with same-sex siblings. Individuals were housed in polycarbonate cages (28 × 18 × 13 cm) and kept on a 14: 10 h light-dark cycle. All animals were given Rodent Chow (Harland Teklad, Madison, WI, USA) and water ad libitum. Twelve unrelated male and twelve unrelated female pups were used as subjects. All voles were sexually mature (60–90 days) and inexperienced, and female voles were sexually unreceptive at the time of testing. For the duration of the behavioral testing, subjects were housed singly in polycarbonate cages.

2.2. Behavioral testing

Spatial memory was assessed using the Morris water maze (Morris, 1984) (Fig. 1a). The apparatus consisted of a 1000 L tank (measuring 140 cm in diameter and 59 cm tall), a submerged platform (11.5 cm in diameter, and 3 cm below the water surface), and room dividers (171 cm tall) surrounding the tank. The water temperature was maintained between 28 and 32 °C throughout testing. To conceal the location of the platform, the water was made opaque using non-toxic white paint powder (Fresco Tempera Paint, Rich Art, Northvale NJ). Visual cues were placed on the room dividers, to assist the subjects in navigating to the platform location. To analyze swimming performance, the apparatus was divided into four quadrants (Fig. 1b). The submerged platform was defined in the Noldus EthoVision XT 8.5 software package (Noldus, Leesburg, VA) by two zones. The first zone covered the entire platform area, which measured 11.5 cm in diameter (area: 103.87 cm^2). The second zone covered only the centermost point of the platform, which measured 7.19 cm in diameter (area: 40.55 cm^2) (see Fig. 1b). Thus, the center area encompassed 39% of the entire platform. Duration (the amount of time spent in the platform area) and frequency (the number of visits to an area) were measured within the defined quadrants and designated platform and inner platform area.

Spatial learning and memory testing in the Morris water maze was conducted over a series of 5 days, with one trial in the morning and one in the afternoon (each successive daily trial was separated by at least 1 h). The first nine trials (testing spatial learning) each lasted up to

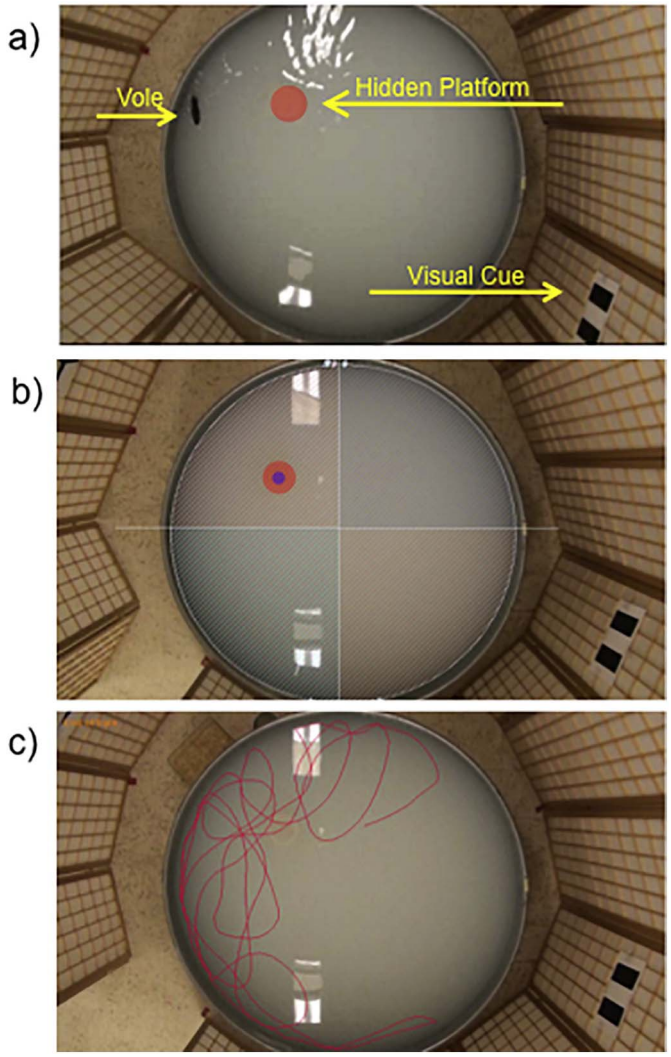

Fig. 1. (a) The apparatus of the Morris water maze test consisted of a 1000 L tank, a submerged platform (red), and room dividers surrounding the tank. (b) Quadrants and zones within the Morris water maze used to analyze swimming performance. The platform area was divided into two zones: the entire platform area (red), and only the centermost point of the platform (blue). (c) Track visualization in red depicts the swim path of a single participant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2 min; the final trial (testing spatial memory) lasted 1 min. In each of the learning trials, subjects were placed in the maze at a randomized start position. The subjects freely swam in the apparatus, and were retrieved upon successfully locating the platform. If the subjects did not locate the platform within 2 min, the subject was removed and the trial was recorded as unsuccessful. At end of unsuccessful trials, the subject was guided to the platform prior to removal. We used 2 min as a cut off time for unsuccessful trials because this is the standard for water maze testing (Vorhees and Williams, 2006) and anything longer in duration would be unethical. We measured the latency to locate the platform as an assessment of learning in trials 1 through 9. An unsuccessful trial received the maximum latency of 120 s. During the memory trial, the platform was removed and subjects were given 1 min to swim in the tank. We assessed memory by measuring the amount of time the subject spent in the quadrant of the tank previously containing the platform (Fig. 1b). Additionally, we quantified how accurate subjects were in locating the exact position where the platform was previously stationed (Fig. 1b). All trials were video recorded using a Sony SR-120 camcorder (Sony, New York City, NY, USA). All video recordings of behavior were analyzed using EthoVision XT 8.5.

2.3. Volume measurements and cell density

Following the completion of behavioral tests, subjects were euthanized by CO_2 suffocation and brains were immediately extracted. The brains were flash-frozen using powdered dry ice, and stored at − 80 °C. Brains were later cryosectioned coronally into four sets with 20 µm thickness and were mounted at 100 µm intervals on SuperfrostPlus slides (Fisher Scientific, Atlanta, GA). Brain slides were then stored at − 80 °C.

Our aim was to investigate sex differences in the neuroanatomy of the hippocampus, with a special interest in the oxytocin receptor (OTR) expressing areas of this structure. We therefore focused our measures of hippocampal neuroanatomy and OTR (see below) on the dorsal hippocampus (Fanselow and Dong, 2010) from the most anterior point of the hippocampus to the level of the periaqueductal gray, at which point we stopped sectioning the brain. OTR is primarily expressed in the dorsal regions of CA1 and CA3 from about the level of − 1.92 to − 3.60 mm bregma on the anterior/posterior axis (Fig. 2a). By focusing on this portion of the hippocampus, we attained a robust estimate of co-localized hippocampal neuroanatomy and OTR density.

To obtain hippocampus volume measurements, one of the sets of frozen brain sections was stained using cresyl violet (Fig. 2b). Brains were thawed and air-dried overnight, bathed in a series of 100% EtOH, 95% EtOH, 75% EtOH for 2 min. The slides were then bathed in 0.5% cresyl violet with 1 M acetyl acetate for 90 min and then re-hydrated in a reverse series of EtOH baths (75%, 95%, 100%) for 2 min. Finally, slides were washed in Citrisolv (Fisher Scientific) for 2 min, and air-dried. The slides were then prepared with permount (Electron Microscopy Sciences, Hatfield, PA), coverslipped, and allowed to cure for 2 days.

Once slides were stained, we photographed them using a bright field transmitted light microscope (Leica DM5500) mounted with a monochrome CCTV camera (Leica DFC3000 G). Images of the left and right side of the hippocampus were captured at 2.5 × magnification and a composite image was created using a motorized platform (Leica, EK 75 × 50 Pilot) and Leica software (Leica Application Suite Advanced Fluorescence 3.2.0). Surface area measurements of the hippocampus were taken using the software ImageJ (NIH, Bethesda, MD). An estimation of overall hippocampal volume was calculated using each surface area measurement along the anterior-posterior axis. Section volume was calculated by taking the sum of each surface area section multiplied by the inter-section thickness (100 µm and 20 µm for the final section) (Fig. 2c).

We assessed cell density within the hippocampus for males and females to estimate the amount of OTR expression per cell. A modified version of the fractionator sampling method (Akdogan et al., 2002; West, 1993) was used to calculate cell density using the same set of brains from the volumetric analysis. After cresyl violet staining, photos were taken using our Leica microscope at 10 × magnification. A subset of tissue sections were selected for imaging to ensure cell densities correlated with the areas of visible autoradiography expression. Our modified fractionator method focused on the x-axis and y-axis only for estimating cell density. Typically, the z-axis is included with a sectioned thickness of at least 50 µm, as anything less increases miscalculations of cell counts due to tissue shrinkage (West, 2012). Although our tissue sections were cut at 20 µm, we assumed that any tissue shrinkage that might have resulted in an overestimation of our cell counts would be the same across all subjects because there is no obvious sex difference in tissue quality and because we handled and processed male and female brains identically. Thus, our comparison of male and female cell density calculations should be unaffected by our modification of the fractionator method.

After obtaining our images, they were sharpened twice with the Leica Application Suite software, and exported for analysis in ImageJ. An unbiased counting frame (West et al., 1991) was established that aligned the top left and bottom right corner with CA1 and the dentate

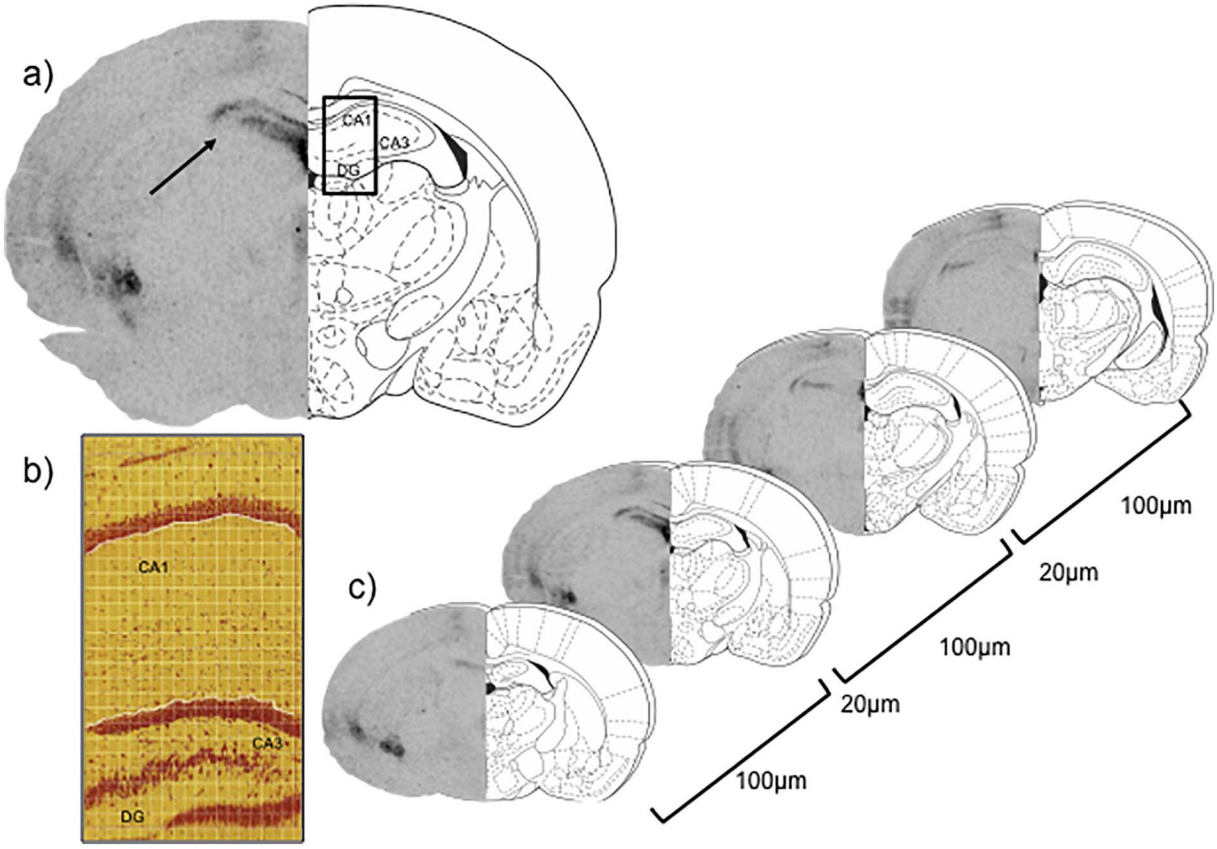

Fig. 2. (a) Coronal section of brain tissue showing hippocampal oxytocin receptor autoradiogram (arrow, left) and atlas (right). Hippocampal sub-regions, CA1, CA3 and dentate gurus (DG) are labeled. The box indicates magnified section for panel b. (b) Cresyl violet stained bright-field image of hippocampus with an overlaid grid for calculating cell density. Pseudocoloring provided contrast for visualization purposes only. (c) Cartoon example demonstrates our method of volume estimation (see Methods). The actual values provided in the figure (totaling 380 μm) do not accurately represent the total distance from the beginning of the hippocampus to the point at which we stopped collecting data (i.e., the level of the periaqueductal gray).

gyrus respectively (Fig. 2b). A 25 × 20 square grid was superimposed onto the image of the section. An unbiased count (West, 1993) of the number of cells per box was obtained using the established fractionator method for systematic random sampling (Gundersen, 1986; Gundersen et al., 1988; West et al., 1996). Briefly, every other box was counted across the rows of the grid. The starting box alternated each row between immediately adjacent and one box adjacent to the left border of the counting frame. Within each box, the top and left lines were considered 'acceptance lines' and the bottom and right lines were considered 'rejection lines'. A cell must have been visible inside the acceptance and rejection lines to be counted, and a cell touching any part of the rejection line was not counted. The total number of cells were summed and then divided by the total number of boxes counted to calculate a cell density (cells per box).

2.4. Autoradiography

Another set of the same brains was used to visualize oxytocin receptor (OTR) density in the hippocampus (Fig. 2). We evaluated OTR because receptor density is relatively stable in adults (Ophir et al., 2013) and because receptors are often regarded as the targets of selection (Ketterson and Nolan, 1999). To visualize OTR, we labeled brain tissue with ^{125}I labeled ornithine vasotocin analog ([^{125}I]-OVTA); NEX 254, PerkinElmer; Waltham, MA) using our established protocol for autoradiography (Ophir et al., 2009). The radiolabeled slides were exposed to Biomax MS film (GE Healthcare, Buckinghamshire, UK) for 72 h alongside ^{125}I microscale standards (American Radiolabeled Chemicals, St. Louis, MO).

Films were developed and digitized using a Microtek Scanner (Microtek, Santa Fe Springs, CA) and then scored using ImageJ. Localization of OTR expression in the hippocampus was identified using the rat brain atlas (Paxinos et al., 2009) and cresyl violet stained sections as guides. We measured optical density for the hippocampus for each bilateral section. We also measured nonspecific binding on each section by measuring the background levels of cortex (bilaterally) in areas that do not express OTR. OTR density was estimated by converting the optical density of exposed film into disintegrations per minute (dpm) in tissue equivalence (TE) estimated from 1 mg in rat brain (dpm/mg TE). The optical density for each section was then averaged and adjusted to represent specific binding by subtracting nonspecific binding from total binding.

2.5. Statistical methods

All statistical models mentioned in the Results section were run in JMP, Version 12 (SAS Institute Inc., Cary, NC) and effect size estimates were calculated using the JMP add-in "Calculate Effect Size".

3. Results

3.1. Spatial learning and memory

A linear mixed model was used to analyze spatial learning performance by comparing the latency to reach the platform over training for males and females. In the model, latency to platform was the response variable and fixed effects were sex, trial, and sex by trial. Individual subjects were included as random effects to account for multiple responses, and the model also controlled for swim velocity. Our data

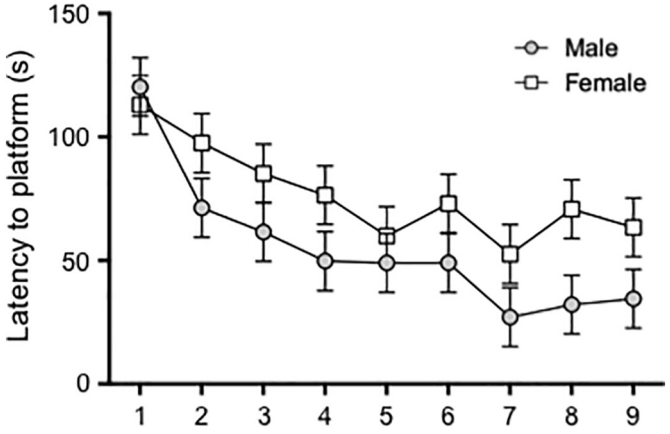

Fig. 3. Marginal mean (± SE) latency in seconds (s) to reach the platform throughout all nine learning trials for males (gray circles) and females (white squares) in the Morris water maze.

showed that males consistently found the location of the platform faster and performed better in the spatial learning test ($F_{1,22} = 4.25$, $p = 0.05$, d = 0.467; Fig. 3).

We controlled for swim speed in the mixed model, indicating that this main effect of sex was not due to males being more active than females, which is consistent with previous findings that activity does not predict sex differences in maze performance (Gaulin et al., 1990). Not surprisingly, we also found a significant trial effect ($F_{8,175} = 11.23$, $p < 0.0001$, $\eta^2 = 0.221$) indicating that all animals improved over training. No trial by sex interaction was found ($F_{8,175} = 0.89$, $p = 0.52$, $\eta^2 = 0.18$), indicating that learning rates of males and females were similar. Thus, although rates of learning may be relatively similar between males and females, males show consistently shorter latencies to find the hidden platform across trials during spatial learning indicating they were more efficient at locating the platform than females.

Next, we assessed sex differences for performance in the memory test (time spent swimming in the quadrant from which the platform was removed in trial 10). We found no sex difference in time spent in correct quadrant using this gross assessment of memory (two-tailed Student's *t*-test; $t_{(22)} = 0.41$, $p = 0.68$, d = 0.166; Fig. 4a). Similarly, we found no differences in the frequency with which males and females entered the quadrant (Generalized Linear Model [GLM] with a poisson distribution and a log link $B = -0.079$, $SE = 0.085$, $p = 0.35$; Fig. 4c). Although focus on the quadrant is a common measure of spatial memory, it is a very rough measure that does not necessarily capture the full accuracy of spatial memory. We therefore next quantified spatial memory accuracy to find the exact location of the platform by measuring the duration of time and frequency of visits to the site where the platform was previously located within the quadrant, and to the place where the center of the platform was previously located (see Fig. 1). These measures indicated that males tended to spend more time in the area where the platform had been placed (two-tailed; $t_{(22)} = 1.89$, $p = 0.07$, d = 0.771; Fig. 4b). Moreover, males visited the platform location (GLM; $B = -0.326$, $SE = 0.171$, $p = 0.04$; Fig. 4d) and inner platform location more frequently (GLM, $B = -0.972$, $SE = 0.534$, $p = 0.02$; Fig. 4e). We did not compare the duration of time over the center point of the platform because time swimming over a single point essentially amounted to count (i.e., frequency) data. Males were better than females at finding the platform's previous location; 11 of 12 males compared to only 8 of 12 females swam over the platform location. Furthermore, 7 of 12 males compared to 1 of 12 females swam over the inner platform location. Taken together, these data indicate that males demonstrate more precise spatial learning and spatial memory than females, a result that can only be seen when memory is measured on a fine scale.

3.2. Hippocampal volume, OTR density, and cell count

Male and female absolute hippocampus volume did not differ (two-tailed, $t_{(8)} = 0.84$, $p = 0.42$, d = 0.167; Fig. 5a). It is well known that male and female prairie vole body size does not differ (Mean ± SE; Male 124.0 ± 7.9 mm, Female 123.62 ± 8.1 mm, Heske and Ostfeld, 1990). Nevertheless, we measured body length and volume of the thalamus (using the same method to calculate estimated hippocampal volume) in a GLM to compare relative hippocampal volume between males and females. The total thalamus was chosen as a reference site to determine relative volume because it is a stable region of the brain indicative of total brain volume. In this model, the response variable was relative hippocampus volume, and the fixed effects were body length, sex, and thalamus volume. Similar to absolute volume, we found no sex difference in relative hippocampal volume ($F_{(1,14)} = 0.69$, $p = 0.42$, $\eta^2 = 0.026$; see Fig. 5b). In contrast to volume measurements, males expressed significantly less hippocampal oxytocin receptor density than females ($t_{(20)} = 2.42$, $p = 0.025$, d = 1.037; Fig. 5c). Lastly, we compared male and female hippocampal cell density within the same areas known for OTR expression to estimate OTR density per cell. We found no difference in cell densities between sexes ($t_{(18)} = 0.3418$, $p = 0.74$, d = 0.156; Fig. 5d). These data indicate that males express fewer oxytocin receptors per cell in OTR-expressing regions of the hippocampus.

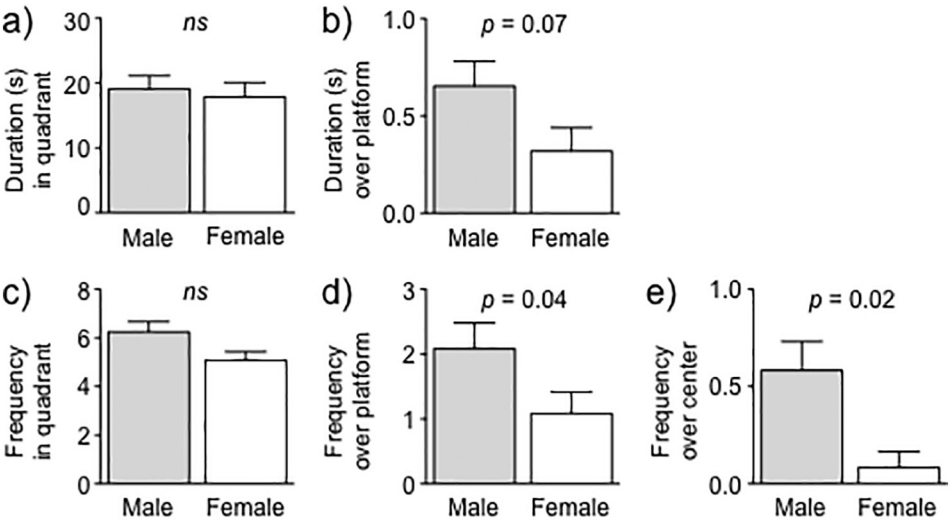

Fig. 4. Mean (± SE) time in seconds (s) subjects spent swimming (a) in the platform-containing quadrant of the water maze, and (b) in the area specifically where the hidden platform was located. Panels c–e present the mean (± SE) number of times subjects swam in the platform-containing quadrant of the water maze (c), in the area where the hidden platform was located (d), or in the area where the center of the hidden platform was located (e). For panels c–e, mean frequencies are presented, however data were analyzed using Generalized Linear Models with a poisson distribution and a log link. Males are represented in gray; females are represented by white. The text ns indicates $p > 0.05$.

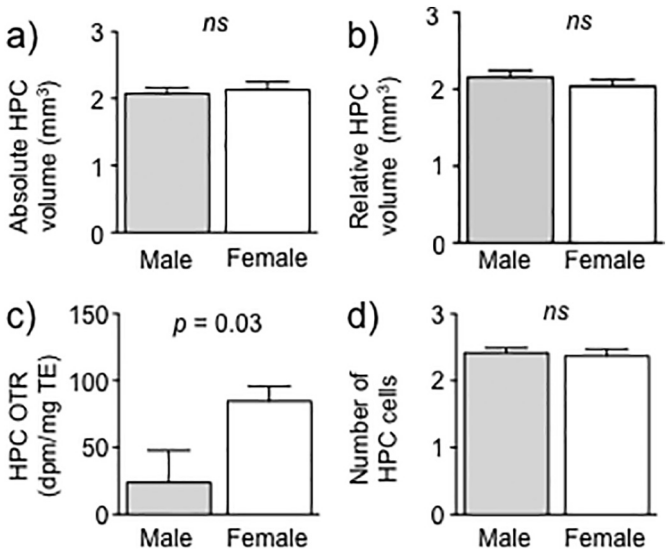

Fig. 5. (a) Mean (± SE) hippocampus (HPC) volume in mm^3. (b) Marginal mean (± SE) relative hippocampus volume (mm^3). (c) Mean (± SE) hippocampus oxytocin receptor (OTR) density measured in disintegrations per minute in tissue equivalence (TE) of 1 mg of rat brain tissue (dpm/mg TE). (d) Mean (± SE) number of cells per area of hippocampus in regions that express OTR. Males are represented in gray; females are represented by white. The text ns indicates $p > 0.05$.

4. Discussion

Our results indicated that male and female prairie voles differ in their capacity for spatial learning and memory. Although many examples across several species have demonstrated that males are often better than females at spatial memory tasks (Gaulin, 1992), most of this work has focused on differences within polygynous species where males' territories or home ranges are larger than females', and where home range size is equated with mate searching. These studies often extend this line of thought to suggest that a difference in male spatial memory boosts reproductive success, because male fitness is theoretically more closely tied to the number of mates they acquire than it is for females (Andersson, 1994; Jacobs, 1995). Indeed, much has been made of this difference, which has been attributed to a purported adaptive function of males to maximize reproductive success through having relatively robust spatial maps. Gaulin and Fitzgerald (1986) used this theory to hypothesize that because monogamous males and females on average have the same number of mates (i.e., one), males and females of monogamous species should show little to no sex differences in spatial ability compared to polygynous species. Of course this ignores the fact that extra pair copulations are common in most monogamous species, including prairie voles (Ophir et al., 2008). Nevertheless, by focusing on species differences in voles (monogamous prairie voles, and polygynous meadow voles), they demonstrated that meadow vole males navigated a sub-set of several mazes better than female meadow voles, and that this difference was not observed in any of the mazes run by prairie vole males and females (Gaulin and Fitzgerald, 1989). When Sawrey et al. (1994) tested male and female prairie voles in the Morris water maze, they detected no sex difference, suggesting that male and female prairie voles do not differ in spatial memory (but see below). However, our data contradict the conclusion that male and female prairie voles are equally good at spatial memory tasks. Furthermore, our data indicate that sex differences in spatial memory need not relate to sex differences in territory size, since male and female prairie voles have home ranges that are similarly sized (Gaines and Johnson, 1982).

4.1. Space use and sex-specific selection

There has been an historical emphasis on the relationship between cognitive spatial ecology and territory size, and its relationship to mating system. Our results show that male and female prairie voles demonstrate a sex difference in the precision of spatial learning and memory despite having similarly sized natural home ranges. We acknowledge that this sex difference could be attributed to a difference in perceptual ability, however our experiment was not designed to test this possibility. In either case, these data encourage a reconsideration of such ideas and indicate that a more nuanced approach to sex differences in cognition is warranted. Evolutionary theory supports the notion that males and females in monomorphic species face convergent selective pressures that account for a lack of differences between the sexes. However, although the selective pressures on males and females are relatively similar for monogamous species, they are not identical for males and females and the conventional pressures associated with sex-specific selection are not completely absent. As such, the door is open for selection to operate on the sexes differently, even in monogamous species, potentially leading to forms of 'cryptic sexual dimorphism' (for example see de Vries, 2008; de Vries and Villalba, 1997). Indeed, our results challenge the notion that the absolute size of a home range indicates the only meaningful differences in space use, and encourage consideration that the specific ways in which animals actually behave in space should be given more attention.

Why might males need better spatial learning and memory relative to females? Although male and female prairie voles typically occupy the same amount of space, the ecological demands and selective pressures to maximize mating success for each sex likely lead to different functional uses of that space. Male prairie voles benefit most from aggressively defending their territory, guarding their mate, and protecting their paternity (Getz and Hofmann, 1986; Getz et al., 1997; Jacquot and Solomon, 2004; McGuire et al., 1990). This adaptive behavior of territoriality has high cognitive demands for effectively processing spatial information. A male presumably needs to recognize the boundaries indicating where his territory and neighboring territories end and begin. For example, a male resident should benefit by identifying conspecifics and processing the spatial (and social) context when encountering individuals near his territory (c.f., Ophir et al., 2012, 2008; Phelps and Ophir, 2009). A familiar neighbor within its proper boundary or an unfamiliar intruder will elicit very different behavioral responses (Rosell et al., 2008) and tentatively carries differential fitness consequences.

In contrast, females have almost none of the aforementioned constraints to ensure reproductive success. Studies on prairie voles show that females increase fitness by mating with multiple males (Wolff and Dunlap, 2002) and confusing paternity (Dewsbury, 1984). Therefore unlike males, females have less need to defend a territory than males of this species (Wolff, 1993). Thus, females might not experience the same selective pressure to improve this form of spatial processing that males experience. Indeed, a substantial body of work has emphasized the importance of the hippocampus for input, storage, and retrieval of information (Nadel, 1991), in the identification of context (Komorowski et al., 2009), and for encoding item-specific responses within a spatial representation (Komorowski et al., 2009). The spatial information that male, but not female, prairie voles use to successfully defend their territory and paternity is surely processed, at least in part, by the hippocampus. Thus, it is not surprising that males might experience more intense selection and cognitive demand for hippocampal dependent tasks compared to females. Despite the fact that prairie voles lack sex differences in outward morphology and territory size, evidence from studies on prairie vole life history provides evolutionary support for the conclusion that males have more ecological demands for spatial cognition (Getz and Hofmann, 1986; Getz et al., 1997; Jacquot and Solomon, 2004; McGuire et al., 1990).

Although our results are consistent with the work derived from life history and field studies, studies on spatial memory in voles conflict with our results and indicated that prairie voles do not demonstrate a sex difference (Gaulin and Fitzgerald, 1989, 1986; Sawrey et al., 1994).

Much of the work by Gaulin and colleagues to this end (Gaulin, 1992; Gaulin and Fitzgerald, 1986, 1989) were conducted on wild-caught animals that were tested in the lab. It is plausible that a difference in spatial experience (like living freely in the wild compared to our lab-reared animals) could impact spatial memory processing and performance, and therefore potentially explain why our results appear to disagree with previous work. However, Gaulin and Wartell (1990) demonstrated that wild caught prairie voles and lab-reared prairie voles, whose spatial experiences differed by over three orders of magnitude, did not differ in spatial performance. Thus, is it unlikely that the major differences in spatial experience of our animals with those from previous studies account for our results.

Sawrey et al. (1994) was the only example of a study that used the Morris water maze to test spatial memory and learning in voles other than ours. Unlike our experiment, however, Sawrey et al. (1994) limited their analyses to consider visits to the correct quadrant containing the platform. In fact, our results show the same effect when we only measured the frequency subjects entered the quadrant (see Fig. 4c). This measure addresses a gross account for remembering the general vicinity of the platform, a task that clearly both sexes can adequately handle. However, on closer examination, males are more precise in their ability to locate the platform than females (Fig. 4b). This indicates that sex differences in prairie voles' spatial memory persist, but that these differences are subtle. Unfortunately, we are unable to determine if the sex differences in spatial memory that we have reported are a result of overt selective pressures that differentially operate on how males and females use space or if these differences are pleiotropic carry-overs from a non-monogamous ancestor. Nevertheless, our results indicate that special focus should be placed on considering how subtle sex differences in spatial cognition might contribute to the expression of sex differences in more observable behaviors that carry heavy fitness consequences, like the expression of mating tactics.

4.2. Neurobiology of spatial processing

Because males and females differed in spatial learning and memory performance, we expected that hippocampal volume would also differ, with males having larger hippocampi than females. This expectation was based on work demonstrating a direct relationship between hippocampal volume and spatial memory ability (Krebs et al., 1989; Maguire et al., 2000). However, we did not find this relationship. Our results show that males perform better at the Morris water maze, a measure of spatial memory and learning, but that this difference in ability did not relate to any measure of hippocampal neuroanatomy we collected. We did, however, find a striking difference between male and female hippocampal OTR density. Importantly, oxytocin activation of OTR is known to interfere with learning and memory (Popik and van Ree, 1998). Although its effects seem to be dose dependent and may be region specific, endogenous levels of oxytocin appear to attenuate memory and learning under many learning-memory contexts (Heinrichs et al., 2004; Ophir et al., 2009; Popik et al., 1992; Viviani and Stoop, 2008). In this case, we interpret a greater density of OTR as indicative of greater sensitivity to OT. If true, animals that have more hippocampal OTR should be worse at spatial learning and memory. Our results showing that females were worse at spatial memory and learning and exhibited greater OTR density than males are consistent with this expectation.

Why might structure size and neuron number be the same while gene expression within neurons differ? Many of the cognitive tasks required for spatial competence are hippocampal dependent (see above). However, modifying or investing in more brain tissue is energetically expensive, especially if it is maintained year round (Roth and Pravosudov, 2009). Our data highlight a correlation between OTR and memory, but do not demonstrate a causal relationship. Nevertheless, other studies have implied OT-OTR in spatial memory (e.g., Tomizawa et al., 2003; Owen et al., 2013). Based on the strong evidence suggesting that OT-OTR binding impacts spatial processing (see Ophir, 2017), we hypothesize that sex-specific selection has operated on male hippocampal OTR to attain similar effects that growing the volume of this structure might achieve, without the considerable energetic costs of tissue maintenance. Although a great many hypotheses are possible about the physiological, behavioral, or cognitive consequences of this OTR difference, the role of hippocampal OTR in spatial memory is consistent with our interpretation that it plays an important role in modulating this behavior. The fact that estrogen regulates OTR transcription provides a putative mechanism for the sex difference (Bale and Dorsa, 1998; Fleming et al., 2006).

While more evidence is needed to substantiate our hypothesis, there is some justification to support it. The adaptive specialization hypothesis (ASH) states that selective pressures imposing cognitive demands on individuals will affect specialization of brain regions responsible for cognitive processing (Sherry et al., 1992). Although the ASH has been specifically used to support the observable differences in hippocampal size between food-caching and non-food-caching birds (Krebs et al., 1989), the same theory could be applied to differential expression of gene products, which are presumably less expensive and potentially produce similar functional outcomes. By modulating central processing of spatial information through the modification of gene products like OTR between the sexes, prairie voles could achieve a simpler and energetically cheaper solution to differential spatial memory processing than by increasing structure volume. Indeed, hippocampal volume may only provide a coarse proxy for finer scale changes in structure that may be influencing behavior that are most observable at the endophenotype (Roth et al., 2010). Such a solution should be even more common when the differences in ability are subtle, transient, or need to be flexible. The sex difference we observed in prairie voles seems to be more in line with the former, but certainly dynamic changes in memory ability of this species could be possible too.

5. Conclusion

Using a comprehensive approach that integrates mating behavior, hippocampal function, and hormonal modulation, we have identified a potential proximate mechanism that might shed light on why sex differences in spatial cognition persist in a momogamous and monomorphic species.

Our results encourage a reconsideration of the idea that sex differences in spatial cogntion are absent in monomorphic species, and we offer our current findings as justification for a greater emphasis on the nature of how space is used. We highlight a particularly interesting potential role for oxytocin in accounting for the observed sex difference, acting as an amnesic to inhibit learning and memory. Our study provides the first evidence that the density of hippocampal OTR might be involved in modulating spatial memory performance. Finally, we suggest that adaptive specialization via a neuropeptide hormone enables modulation of cognitive performance without the relative costly investment in brain mass.

Ethics

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the sponsoring institution.

Authors' contributions

M.A.R. conceptualized the experiment, performed the behavioral testing and brain analyses, analyzed data, and wrote the manuscript. L.E.H. assisted in behavioral trials and behavioral and brain analyses. K.J.W. assisted in neuroanatomical processing and analyses. A.G.O. conceptualized the experiment, developed the experimental design, analyzed data, and wrote the manuscript.

Competing interests

We declare that we have no competing interests.

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