Oxytocin and Vasopressin Receptors in the Pouched Rat

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Abstract

This is the abstract. It consists of two paragraphs.

Introduction

The neuropeptides, oxytocin (OT) and vasopressin (VP), have receptor distributions in the brain that can modulate a variety of social behaviors such as parental care, affiliation, and aggression, among other behaviors (Caldwell and Albers, 2015). The densities of their associated receptors, oxytocin (OTR) and vasopressin (V1aR and V1bR) receptors, are often species- and sex-dependent.

- What do we know about the distribution and relative densities of receptors (i.e. what can it tell us, what's been done on behavior?)

-How might life history differences play into patterning of central distribution of these receptors?

-What tends to be conserved?

-How does comparative analysis help? (Kelly and Ophir, 2015)

-Why did we do this study? What were we examining?

-We wanted to describe the presence and relative density of OTR and V1aR in pouched rat brains in males and females, to see if there were differences in presence and density between sexes

-We wanted to explore how the patterning of these receptors might differ from other rodents and see if it further supports the ideas found in the recent metaanalysis (Freeman et al., 2020)(see where pouched rats fall in this framework)

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Methods

-Brain removal

-Statement of animal welfare/approvals

Ten male and ten female brains were stored at -80^{o}C temperature. Brains were split into two hemispheres and coronally sectioned at 20µm thick. Due to the large size of the pouched rat brains, only the left hemisphere was sectioned if it was in good formation, and each section kept was followed by two discarded sections.

-Thus we mounted every... third section,

> -The cerebellum was removed and the olfactory bulbs placed in centrifuge tubes. 4 - Brain sections were placed onto six sets of Superfrost Plus Plexiglas Slides (Fisher Scientific, Pittsburg, PA USA). Slides were stored in the -80^{o}C freezer until they were needed for autoradiography labeling to visualize receptor density. 125I radioligands were used to label oxytocin receptor (ornithine vasopressin analog, 125I-OVTA); NEX 254, PerkinElmer; Waltham, MA) or vasopressin 1a receptor (vasopressin (Linear), V-1A antagonist (Phenylacetyl1, 0-Me-D-Tyr2 [125I-Arg6]-); NEX 310, PerkinElmer), as described by (Ophir et al. 2013).

- Change this.

The radiolabeled slides were left stored in film cassettes and later exposed to phosphoreimaging screens (Fujifilm, Tokyo, Japan) for 23 hours. Under dark light, the screens were removed from the cassettes and placed in a Typhoon FLA 7000 laser scanner (GE Healthcare, Marlborough, MA, USA). The screens were then analyzed using ImageJ Version ? . Three sequential slices were measured for density by encircling the regions of interest using the ImageJ program. The program automatically calculated mean values and area for regions of interest and background. 125I-labeled radiographic microscales were used to produce decay formulas in order to transform average intensity measures into standardized disintegrations per minute (dpm) values.

Transformed values from the cortex taken as background from the same sequential (every 3rd) slices at each region of interest were subtracted from the standardized values of these regions of interest to calculate the final mea4n receptor density value (units are dpm/mg tissue equivalence (TE); for 1 mg in rat brain).

-cresyl violet

-use of atlas

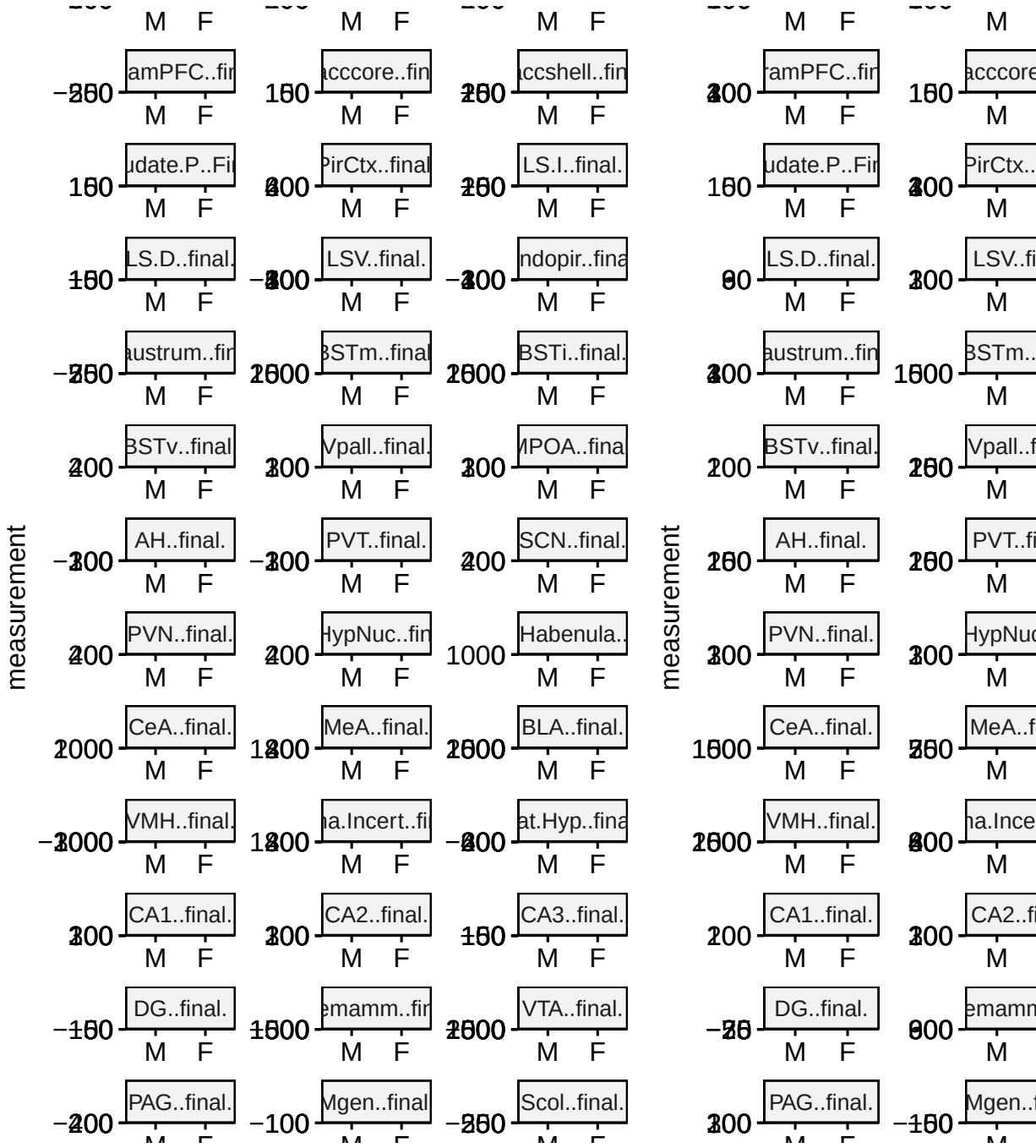
OTR was measured in the olfactory bulb (OB), anterior olfactory nucleus (AON), prefrontal cortex (PFC), piriform cortex (Pir), nucleus accumbens (NAcc), lateral septum (LS), endopiriform (Den), claustrum (VCL), lateral bed nucleus of the stria terminalis (BSTl), medial bed nucleus of the stria terminalis (BSTm), ventral bed nucleus of the stria terminalis (BSTv), ventral pallidum (VPall), medial preoptic area (MPOA), anterior hypothalamus (AH), paraventricular thalamus (PVT), suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), magnocellular hypothalamic nucleus (MCPO), medial habenula (MHb), central amygdala (CeA), medial amygdala (MeA), basolateral amygdala (BLA), ventromedial hypothalamus (VMH), zona incerta (ZIR), lateral hypothalamus (PrFLH), hippocampal CA1, hippocampal CA2, hippocampal CA3, dentate gyrus (DG), premammilary ventral nucleus (PMV), ventral tegmental area (VTA), periaqueductal gray (PAG), medial geniculate (MG), superior colliculus (SuG), and the ventral CA3. V1aR was measured in the same regions except for the MCPO and the MHb.

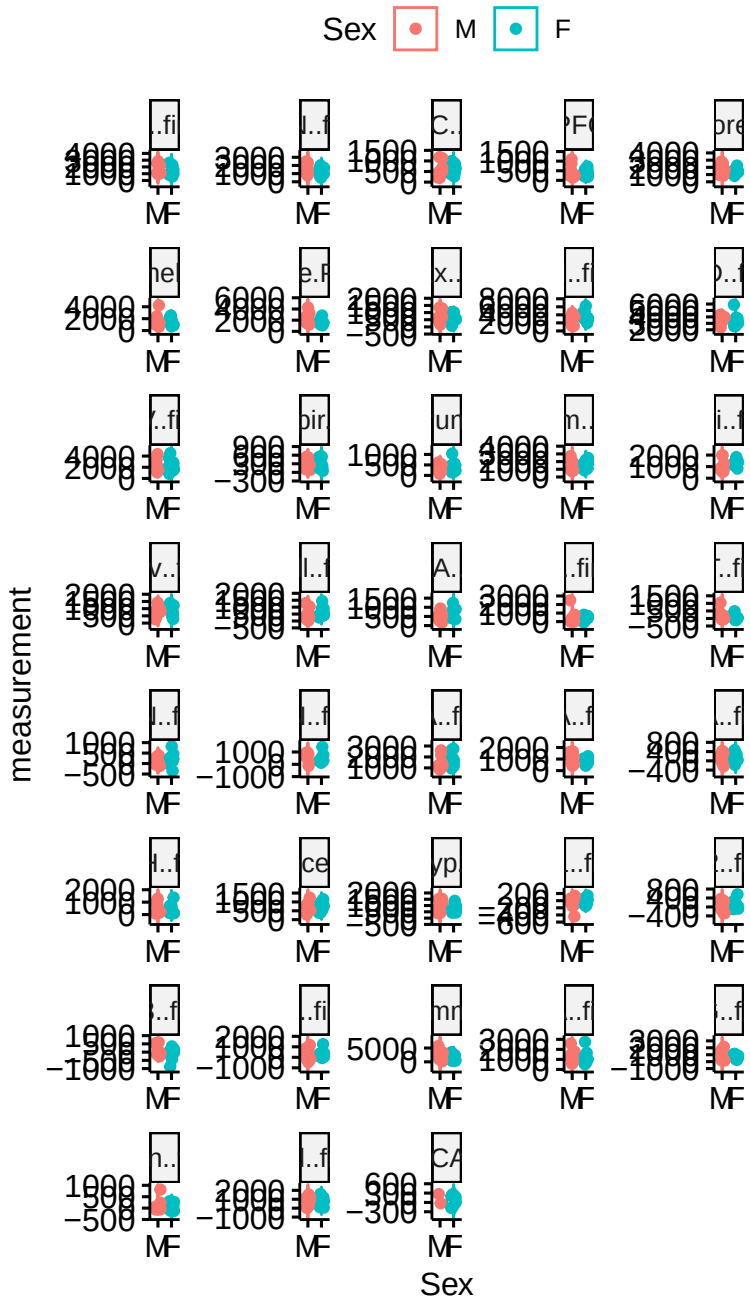
-statistical methods

Results

After comparing male and female densities in the measured regions, most regions showed no differences between sexes. The superior colliculus had higher densities of OTR in females compared to males (Female mean: 199.35, Male mean 51.46, $t_{(13.58)} = 2.79$, $p = 0.01$), however, this was no longer significant after corrections for multiple comparisons.

There was a significant effect of father condition (F(r aov2[[3]][1],r aov2[[4]][1]) = r aov2[[5]][1], p = r aov2[[6]][1]), a significant effect of postnatal day (F(r aov2[[3]][3],r aov2[[4]][3]) = r aov2[[5]][3], p = r aov2[[6]][3])


measurement
M F
amPFC..fin
acccore..fin
accshell..fin
LS.I..final.
LS.D..final.
LSV..final.
ndopir..fina
BSTi..final.
BSTv..final.
Vpall..final.
MPOA..fina
AH..final.
PVT..final.
SCN..final.
PVN..final.
HypNuc..fin
Habenula..
CeA..final.
MeA..final.
BLA..final.
VMH..final.
a.Incert..fi
at.Hyp..fina
CA1..final.
CA2..final.
CA3..final.
DG..final.
emamm..fin
VTA..final.
PAG..final.
Mgen..final
Scol..final.



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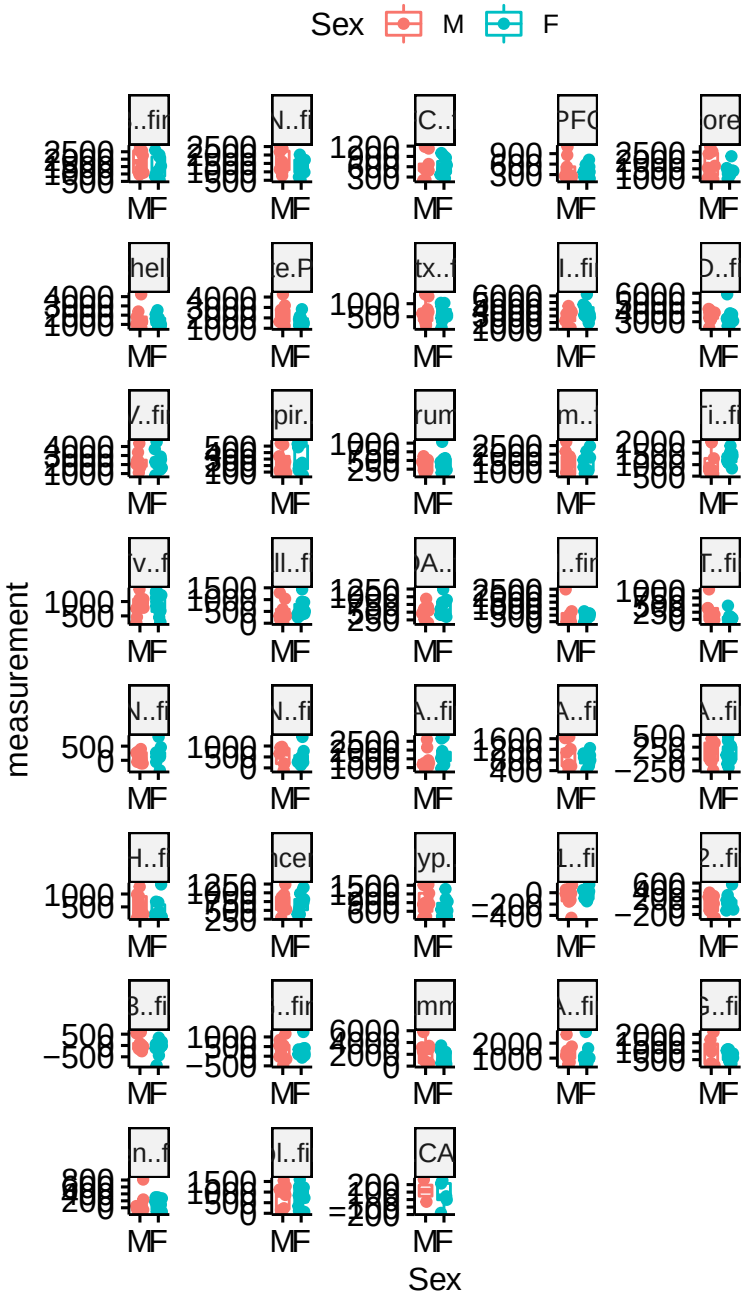


-Where did we see binding for OTR

-Where did we see binding for V1aR

-Sex differences

-Predict with PCA

Discussion

-We found OTR in … V1aR in….

-Sex differences in densities/presence and absence

-We found that overall patterns were similar to…..

-Caveats

-Unknown age

-Unknown reproductive status

-Different experiences possible

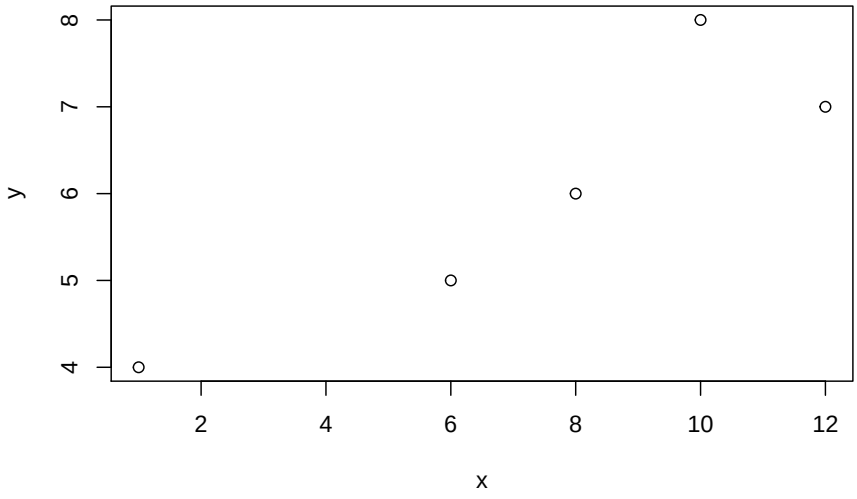
-What this means, similarities to other species

-Relevance for behavior or life history

What still needs to be known?

```
x <- c(1,6,8,12,10)
y <- c(4,5,6,7,8)

plot(x,y)
```



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