PNAS
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Supplementary Information for

Extra-hypothalamic oxytocin neurons drive stress-induced social vigilance and avoidance

Natalia Duque-Wilckens[1,2], Lisette Y. Torres[1], Sae Yokoyama[1], Vanessa A. Minie[1], Amy M. Tran[1], Stela P. Petkova[3], Rebecca Hao[1], Stephanie Ramos-Maciel[1], Roberto A. Rios[1], Kenneth Jackson[4], Francisco J. Flores-Ramires[5], Israel Garcia-Carachure[5], Patricia A. Pesavento[4], Sergio D. Iñiguez[5], Valery Grinevich[6], Brian C. Trainor[1]

Correspondence: Brian Trainor
Email: bctrainor@ucdavis.edu

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Movie S1

www.pnas.org/cgi/doi/10.1073/pnas.2011890117

Supplementary Information Text

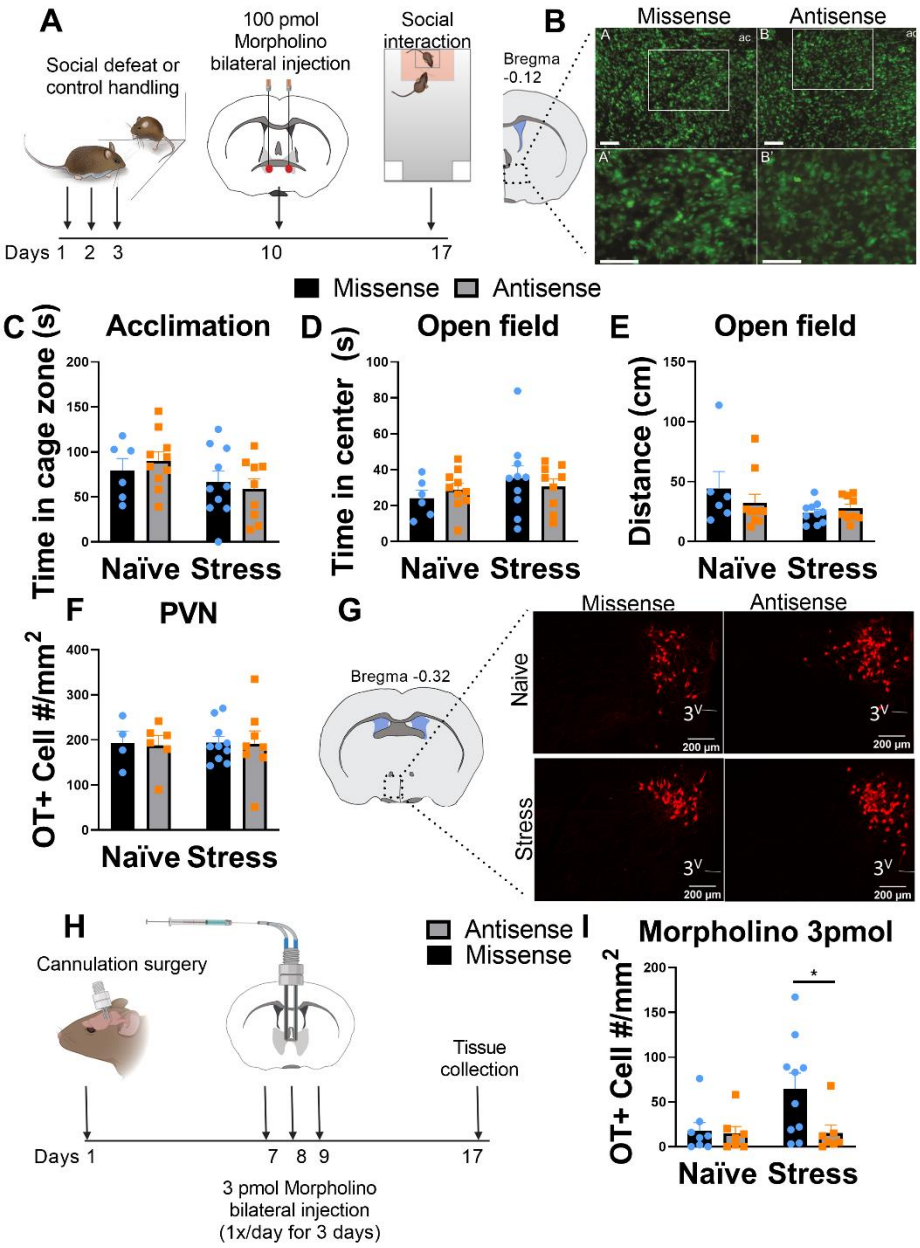
Supplementary Methods

Quantitative Real-Time PCR

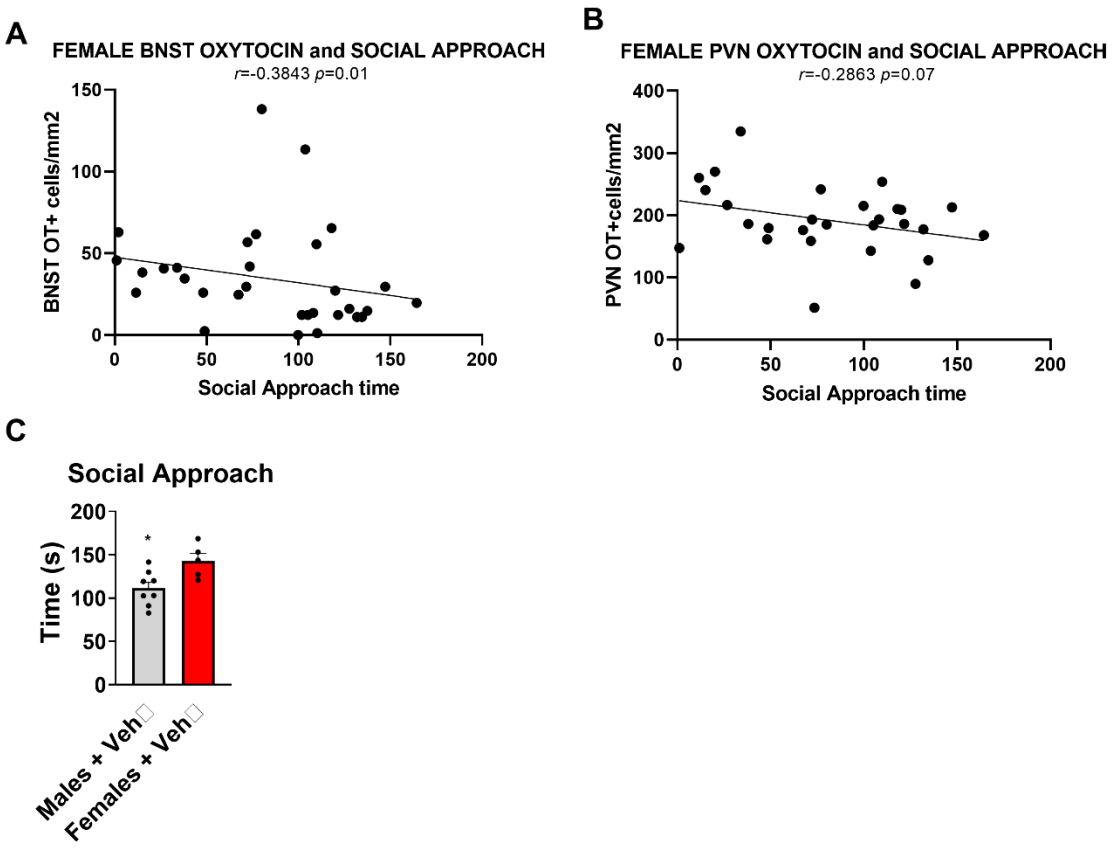
Anterior BNST was dissected from fresh frozen tissue and RNA was extracted using RNAeasy Mini Kits (Qiagen) and QIAzol as a lysis reagent (Qiagen). Reverse transcription was performed using iScript (BioRad) and SYBR green chemistry on an Applied Biosystems ViiA7 instrument was used to sequence amplification. Specific forward and reverse primers for *Oxtr* (Genbank accession: MN265350): and *B2m* (Genbank accession: XM_006995122) mRNA were designed based on California mouse sequence (see Supplementary Table 2). There were no differences in cycle thresholds between groups for *B2m*. *Oxtr* mRNA was normalized to *B2m* expression in each sample.

Fluorescent In-Situ Hybridization

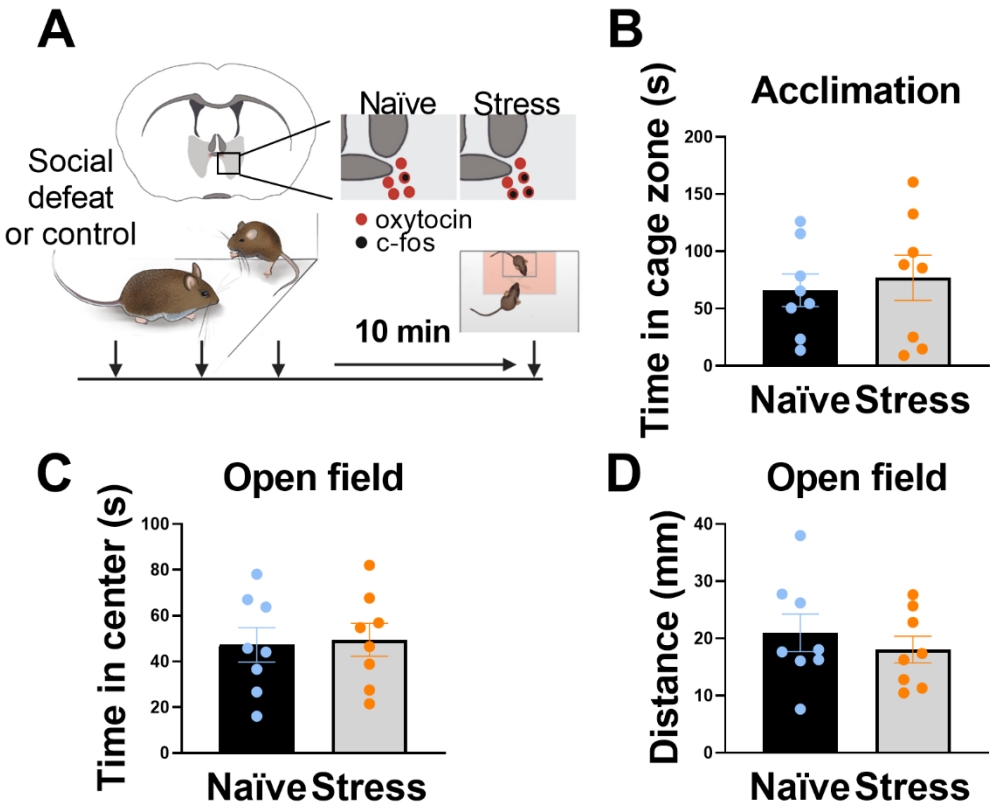
Fluorescent in situ hybridization was performed following ACDBio RNAscope multiplex fluorescence methods. Probes were designed to detect California mouse Oxtr (2017-2961 of GFCW01069365.1), Gad1 (1188-2104 of GFCW01047509.1), and vGlut2 (675-1662 of GFCW01050557.1). Brains from adult male (n=2) and female (n=2) mice were flash frozen and cut at 20 µm. Sections were fixed in cold 10% neutral buffered formalin for 15 min and then dehydrated in a series of ethanol baths (50%, 70%, 100%), after which protease IV was applied to each section for 30 min. Sections where then washed in phosphate buffered saline (PBS). The probes were diluted 1:50 and applied to slides for 2 h at 40° C: one set of sections were probed for *Oxtr*/*Gad1* and the other set for *Oxtr/vGlu2*. Next, slides were rinsed in wash buffer and incubated sequentially in amplification buffer (AMP) 1 for 30 min at 40° C, then rinsed again in wash buffer and incubated in AMP 2 for 30 min at 40° C. Finally, slides were developed in Cy3 (*Oxtr*) for 15 min and then fluorescein (*Gad1 or vGlu2*) for 30 min, and coverslipped in Vectashield with DAPI. Z-stack images were collected with a 20x confocal. For colocalizations analyses, *Oxtr* nuclei were counted and then determined to be *Gad1* or *vGlu2* positive or negative.



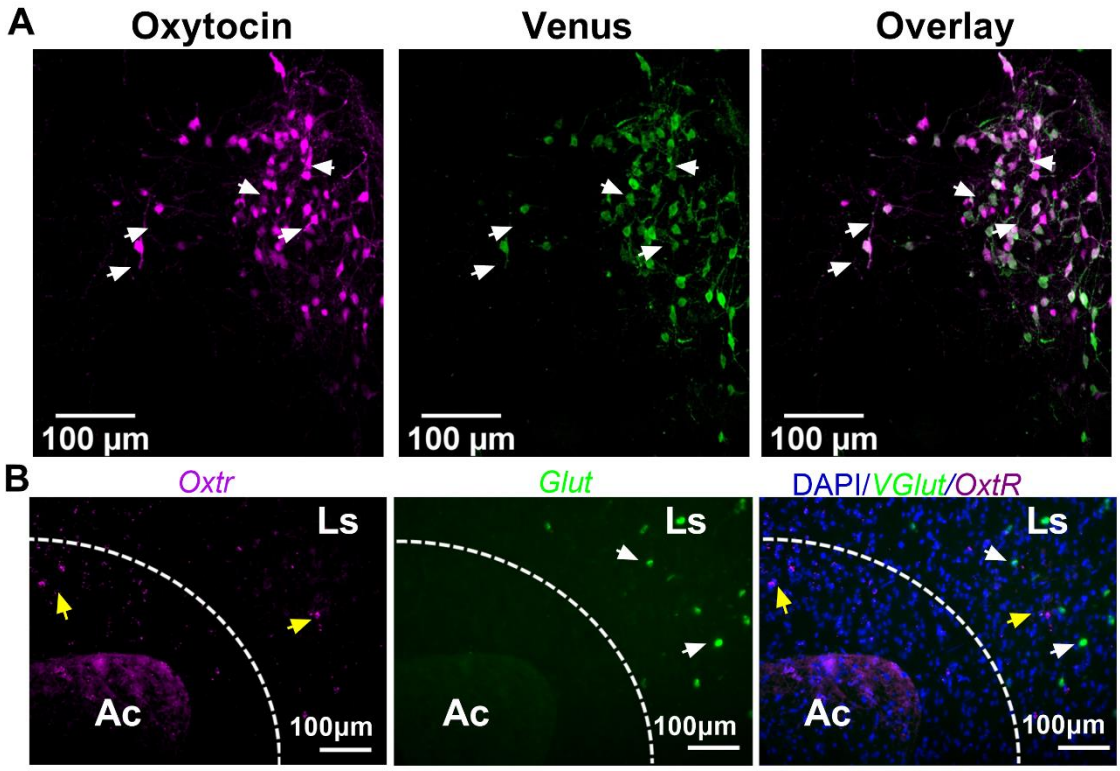
Supplementary figure 1. A. Timeline of experiment assessing effects of one bilateral 100pmol morpholino injections within BNSTmv on oxytocin expression and behavior. **B.** Representative image of Nissl stain used to assess tissue integrity after morpholino injections. **C,D,E.** There were no effects of antisense injections on approach, time spent in center, or distance traveled when no social target was present. **F.** Injections of antisense in the BNSTmv did not affect oxytocin cell numbers in the PVN. **G.** Representative photomicrographs of oxytocin+ cells in the PVN of naïve and stress females receiving morpholino missense and antisense. **H.** Timeline of experiment assessing effects of three daily bilateral injections of 3pmol morpholino injections within BNSTmv on oxytocin expression. **I.** Stress increased BNSTmv oxytocin cell number in animals receiving missense, but this was prevented by injections of morpholino missense.



Supplementary Figure 2: Correlational analyses of social approach and oxytocin cell counts in female California mice. Oxytocin cell counts were negatively correlated with social approach in BNST (A) while there was a non-significant trend for a negative correlation in the PVN (B). Female California mice treated with a vehicle infusion into the BNSTam spent more time in social approach (t_{11}, 2.78, $p < 0.05$) than male California mice receiving the same treatment (C). * $p < 0.05$.



Supplementary figure 3. A. Timeline of experiment assessing the behavior of male California mice 10 min after a third episode of social defeat. **B,C,D**. Social defeat did not affect approach, time in center, or distance traveled when there was no social target present.



Supplementary figure 4. A. Representative photomicrographs of oxytocin, Venus, and colocalizations in the PVN after injection of OTpr AAV. **B**. Representative photomicrographs showing expression of OxtR and Glut. No Glut+ cells were found in the BNSTam, but there were Glut+ cells in the adjacent lateral septum. Ac=anterior commissure, Ls=lateral septum

Supplementary Table 1. Antibodies and reagents in California mouse tissue

Reagent	**Dilution**	**Company (catalog #)**
Mouse anti-oxytocin antibody	1:2000	Sigma-Aldrich (MAB5296)
Chicken anti-GFP antibody	1:1200	Abcam (AB13970)
Goat anti-mouse biotinylated antibody	1:250	Vector (BA9200)
Goat anti-Chicken Alexafluor 488 antibody	1:500	Abcam (AB150169)
Streptavidin Alexa fluor 350 conjugate antibody	1:500	*Thermofisher scientific (S11249)*
NeuroTrace™ 500/525 Green Fluorescent Nissl Stain	1:100	*Thermofisher scientific (N21480)*

Supplementary Table 2. Antibodies and reagents in C57/Balb6 tissue

Reagent	**Dilution**	**Company (catalog #)**
Mouse anti-oxytocin antibody	1:5000	Sigma-Aldrich (MAB5296)
AffiniPure Fab Fragment Goat Anti-Mouse antibody	1:25	Jackson Laboratories (115-007-003)
Goat anti-mouse biotinylated antibody	1:500	Vector (BA9200)
Streptavidin Alexa fluor 555 conjugate antibody	1:500	*Thermofisher scientific (S21381)*

Supplementary Table 3. Sequences for qPCR primers

Gene ID	**Forward Sequence**	**Reverse sequence**
Oxtr	GCCCTTGACGCCTTTCTTCT	TTCCTTGGGCGCATTGAC
B2m	TCTAGTGGGAGGTCCTGTGG	TGCGTTAGACCAGCAGAAGG

Supplementary video 1. Video of social interactions tests representative of stress naïve missense female, stressed missense female, and stressed antisense female.