Dromard, Borie, Chakraborty et al. "*Disengagement of somatostatin neurons from lateral septum circuitry by oxytocin and vasopressin restores social fear extinction and suppresses aggression outbursts in Prader-Willi syndrome model*"

Supplemental video

Video 1. Typical attack
Optogenetic silencing of OXT neurons from the SON during extinction learning causes aggression.

Supplemental Figures

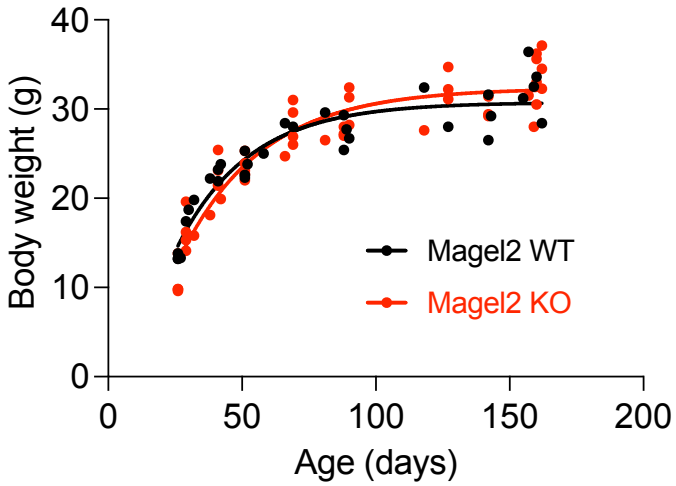

Figure S1. No effect of genotype on body weight.
Lines show the average wet weight and dots the individual measures for N= 8 *Magel2*WT and 12 *Magel2*KO. Two-ANOVA: effect of age $F_{(12,55)}$=34.68 p<0.0001 and effect of genotype $F_{(1,13)}$=0.002 p=0.9.

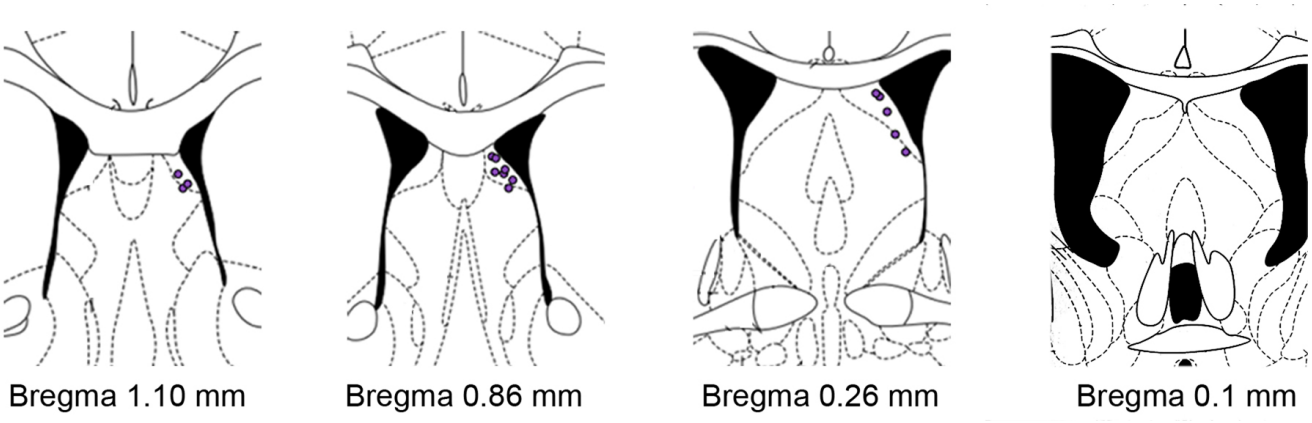

Figure S2. Position of type-1 cells recorded in the LS.

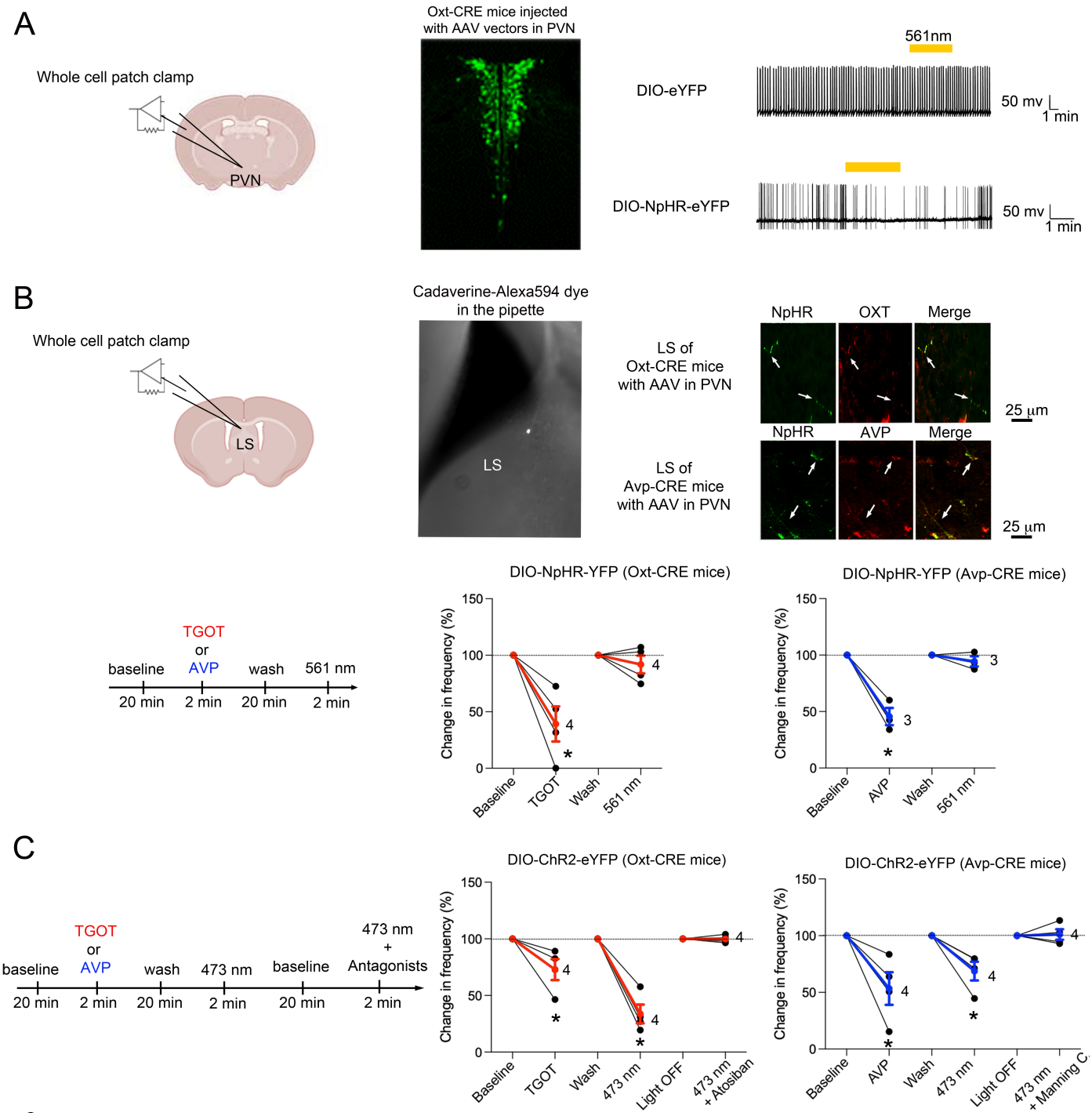

Figure S3. Optogenetic control of OXT and AVP release in the LS.
(A) Patch clamp recordings of action potentials in coronal acute slices of PVN from transgenic mice injected with the AAV virus DIO-NpHR-YFP or DIO-YFP as control. Light stimulation with (561 nm continuous) reduced the firing frequency of action potentials in cells expressing NpHR-YFP contrary to cells expressing YFP alone.
(B) Patch clamp recordings of action potentials in coronal acute slices of LS from the indicated transgenic mice injected with the AAV virus DIO-NpHR-YFP. NpHR is detected in OXT-containing fibers in the LS of *Oxt*-CRE mice as well as in the AVP-containing fibers in LS of *Avp*-CRE mice. The dye cadaverine-Alexa594 was administered via the patch pipette to mark the type-1 cells that respond to 0.1 µM TGOT or 1 µM AVP for 2 min. Responding cells were

selected for exposure with yellow light (561 nm continuous for 2 min). NpHR stimulation in OXT fibers or AVP fibers in the LS did not activate responding cells as suggested with Arch3.0-YFP for other types of neurons by the study from Mahn et al. 2016 (1). This study emphasizes NpHR3.0 is currently the most suitable tool for synaptic terminal silencing. One sample t-test, red lines are means±SEM of N= 4 cells in slices of *Oxt*-CRE mice, and blue lines are means±SEM of N=3 cells in slices of *Avp*-CRE mice.

(C) Peptide release from OXT or AVP fibers in the LS triggered by stimulation of ChR2-YFP in acute slices from *Oxt*-CRE mice or *Avp*-CRE mice injected with the AAV virus DIO-ChR2-YFP in the PVN. Type-1 cells were pre-selected before exposure with blue light (473 nm 30 Hz, 10 ms for OXT fibers or 473 nm 20 Hz, 5 ms for AVP fibers). Antagonists of AVPR (10 nM Manning compound) or OXTR (10 nM Atosiban) were incubated 5 min prior blue light exposure. One sample t-test, red lines are means±SEM of N= 4 cells in slices of *Oxt*-CRE mice and blue lines are means±SEM of N= 4 cells in slices of *Avp*-CRE mice. ChR2 stimulation caused local release of endogenous peptides because the effect was blocked by pre-incubation with the OXTR antagonist in *Oxt*-CRE mice and by the AVPR antagonist in *Avp*-CRE mice.

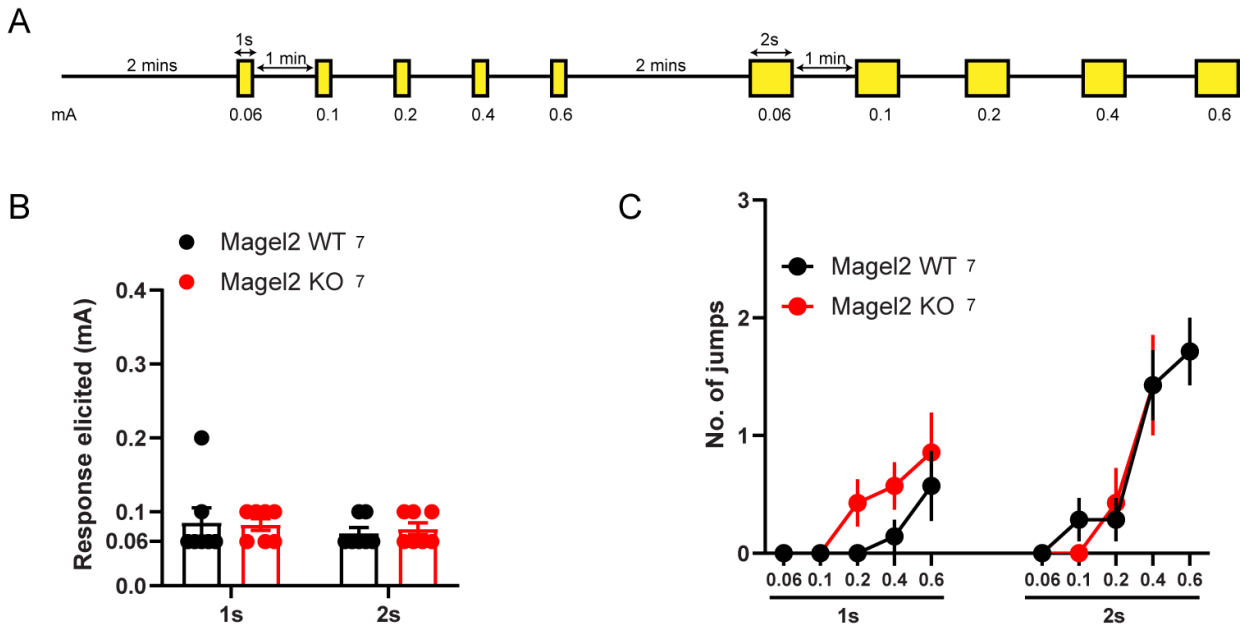

Figure S4. Shock-ramp test in *Magel2*KO mice revealed no effect of genotype on pain sensitivity.

(A) Experimental design. After a 2 minute baseline without any shock, animals received 1s-long electrical shocks of increasing intensity (0.06 – 0.6 mA) at an inter-shock-interval (ISI) of 1 minute. Following another shock-free period of 2 mins, a second electrical ramp was applied of 2s-long electrical shocks of increasing intensity (0.06 – 0.6 mA), at the same ISI. (B) Minimum shock intensity necessary to elicit a behavioral response was comparable between wildtype and *Magel2*KO mice, for both 1s and 2s shocks (Two-way repeated measures ANOVA: Effect of genotype, $F_{(1, 12)}$= 0.01, p=0.92, effect of shock duration, $F_{(1, 12)}$= 1.03, p=0.33, interaction of genotype and shock duration $F_{(1, 12)}$= 0.19, p=0.67). N=7 for each group. (C) Number of jumps during the shock-ramps were also comparable across groups, with only effects of shock intensity and duration, but no effect of genotype (Three-way repeated measures ANOVA: effect of genotype, $F_{(1, 12)}$= 0.41, p=0.54, effect of shock-intensity ($F_{(4, 48)}$= 27.57, *p<0.0001*, effect of shock-duration $F_{(1, 12)}$= 39.84, *p<0.0001*, interaction of genotype x shock-intensity, $F_{(4, 48)}$= 0.71, p=0.59, interaction of genotype and shock-duration, $F_{(1, 12)}$= 2.96, p=0.11, interaction of genotype with shock-intensity and shock-duration, $F_{(4, 48)}$= 0.23, p=0.93).

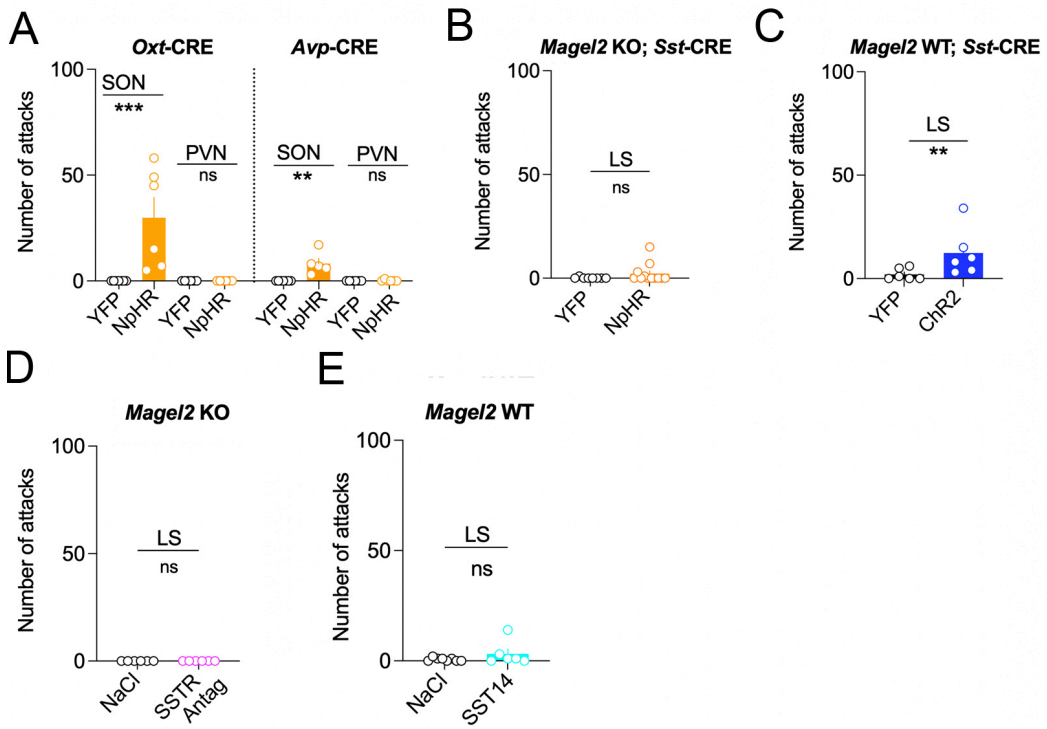

Figure S5. Number of attacks.

(A) Number of attacks on the stimulus mice during SFC+ extinction. *Magel2* WT;*Oxt*-CRE mice injected with YFP compared to NpHR3-YFP in the SON. Mean ±SEM N=8-6, respectively, Mann Whitney test two-tailed p=0.0006. *Magel2* WT;*Oxt*-CRE mice injected with YFP compared to NpHR3-YFP in the PVN. Mean ±SEM N=7-6, respectively, Mann Whitney test two-tailed p>0.9. *Magel2* WT;*Avp*-CRE mice injected with YFP compared to NpHR3-YFP in the SON. Mean ±SEM N=6-7, respectively, Mann Whitney test two-tailed p=0.0013. *Magel2* WT;*Avp*-CRE mice injected with YFP compared to NpHR3-YFP in the PVN. Mean ±SEM N=7-6, respectively, Mann Whitney test two-tailed p>0.9.

(B) Number of attacks on the stimulus mice during SFC+ extinction. *Magel2* KO;*Sst*-CRE mice injected with YFP compared to NpHR3-YFP in the LS. Mean ±SEM N=9, Mann Whitney test two-tailed p=0.16.

(C) Number of attacks on the stimulus mice during SFC+ extinction. *Magel2* WT;*Sst*-CRE mice injected with YFP compared to ChR2-YFP in the LS. Mean ±SEM N=6-8, respectively Mann Whitney test two-tailed p=0.02.

(D) Number of attacks on the stimulus mice during SFC+ extinction. *Magel2* KO mice injected with cyclosomatostatin in the LS. Mean ±SEM N=7/group, Mann Whitney test two-tailed p>0.9.

(E) Number of attacks on the stimulus mice during SFC+ extinction. *Magel2* WT mice injected with SST14 in the LS. Mean ±SEM N=8-7, respectively, Mann Whitney test two-tailed p=0.39.

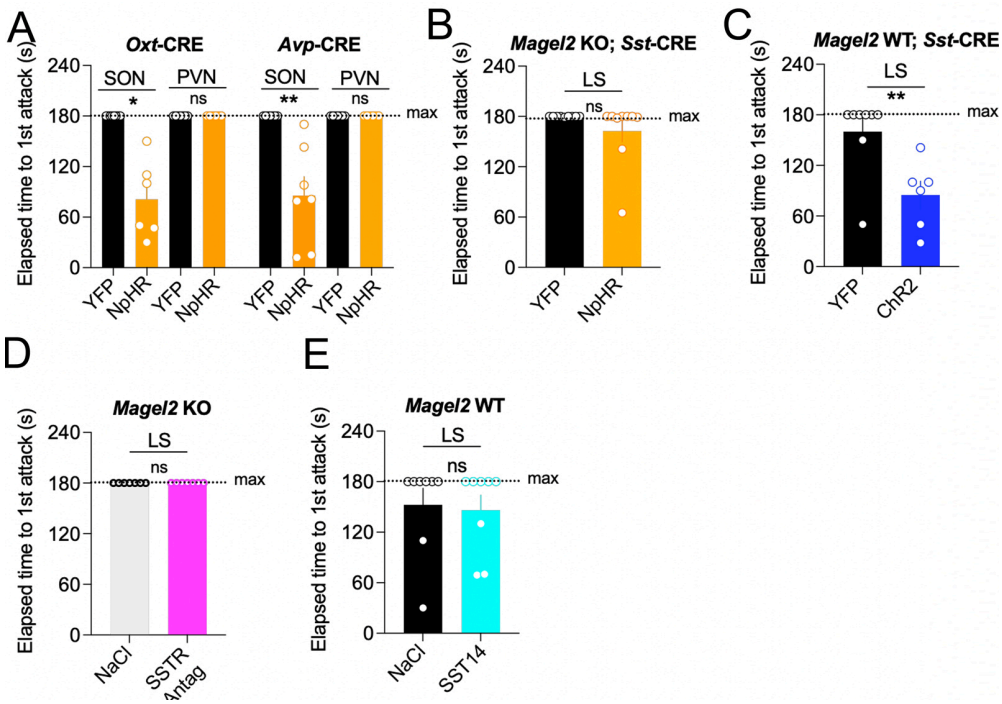

Figure S6. Time to first attack.

(A) Latency to first attack on the stimulus mice during SFC+ extinction. *Magel2* WT;*Oxt*-CRE mice injected with YFP compared to NpHR3-YFP in the SON. Mean ±SEM N=8-6, respectively, Mann Whitney test two-tailed p=0.0003. *Magel2* WT;*Oxt*-CRE mice injected with YFP compared to NpHR3-YFP in the PVN. Mean ±SEM N=7-6, respectively, Mann Whitney test two-tailed p>0.9. *Magel2* WT;*Avp*-CRE mice injected with YFP compared to NpHR3-YFP in the SON. Mean ±SEM N=6-7, respectively, Mann Whitney test two-tailed p=0.0006. *Magel2* WT;*Avp*-CRE mice injected with YFP compared to NpHR3-YFP in the PVN. Mean ±SEM N=7-6, respectively, Mann Whitney test two-tailed p>0.9.

(B) Latency to first attack on the stimulus mice during SFC+ extinction. *Magel2* KO;*Sst*-CRE mice injected with YFP compared to NpHR3-YFP in the LS. Mean ±SEM N=9, Mann Whitney test two-tailed p=0.13.

(C) Latency to first attack on the stimulus mice during SFC+ extinction. *Magel2* WT;*Sst*-CRE mice injected with YFP compared to ChR2-YFP in the LS. Mean ±SEM N=6-8, respectively Mann Whitney test two-tailed p=0.0077.

(D) Latency to first attack on the stimulus mice during SFC+ extinction. *Magel2* KO mice injected with cyclosomatostatin in the LS. Mean ±SEM N=7/group, Mann Whitney test two-tailed p>0.9.

(E) Latency to first attack on the stimulus mice during SFC+ extinction. *Magel2* WT mice injected with SST14 in the LS. Mean ±SEM N=8-7, respectively, Mann Whitney test two-tailed p=0.8.

Supplemental methods

Histology Specific antibodies against c-Fos (Cell Signaling) for double staining with Neurophysin I (OXT), Neurophysin II (AVP) mouse monoclonal antibodies (H. Gainer, NIH) or SST rat polyclonal antibodies (Abcam) were diluted in the blocking buffer (3% normal donkey serum in PBS 0.1% triton X-100. Synthesis of d[Lys(Alexa-647)^8]VP was previously described (2), and its affinity is 54, 205 and 2796 times higher for OXTR than for AVPR1B, AVPR1A and AVPR2, respectively. Drugs were injected intra-LS with cannulas. To reveal AVPR, d[Lys(Alexa-647)^8]VP was used with a competitive OXTR ligand (TGOT) as previously described (2). To reveal OXTR, d[Lys(Alexa-647)^8]VP was used with a competitive AVPR ligand (Manning compound). Counterstainings were done in PFA-fixed tissue and images acquired with a fluorescent microscope (Zeiss, AxioImager Z1). Cells were counted in dorso-rostral parts of LS acquired images, normalized to the surface of the region and averaged between groups, guided by Paxinos Mouse Brain atlas (Figure S2).

Intracerebral infusions. 0.9% NaCl, 1 nM SST14 or 1 µM cyclosomatostatin (Sigma Aldrich, 500 nL/hemisphere, 100 nL/min) were infused in the LS (AP +0.3 mm, ML +/-0.3 mm, DV -2.5 mm) using bilateral injectors through guide cannulas (Phymep, 26G) and diffusion perimeter was estimated by injection of Alexa-594-cadaverine (Life Technology, 50 µM). Injections started 5 min before the indicated trial.

Average number of shocks received per group (means±SEM). *Magel2* WT: 2.1±0.4 (NpHR3.0, AVP PVN→LS), 2±0.25 (NpHR3.0, AVP SON→LS), 2±0.37 (NpHR3.0, OXT PVN→LS), 2.16±0.47 (NpHR3.0, OXT SON→LS), 2.5±0.34 (ChR2, AVP PVN→LS), 2.33±0.42 (ChR2, OXT PVN→LS), 2.1±0.3 (ChR2, SST cells), 2.4±0.6 (SST14, LS) 2±0.3 (NaCl, LS). For *Magel2* KO: 2.7±0.6 (eYFP, SST cells), 3.2±0.5 (NpHR3.0, SST cells), 2±0.3 (NaCl, LS), 2±0.3 (cyclosomatostatin, LS).

Opto-stimulation protocols details. Following optogenetic stimulations were performed at least 3 weeks post-surgery – NpHR3.0, 561 nm: continuous stimulation; ChR2, 473 nm: AVP neurons: 20 Hz, 5 ms, OXT neurons: 30 Hz, 10 ms, SST neurons: 15 Hz, 20 ms (3-5).

Recombinant expression of transgenes. Recombination of halorhodopsin DIO-NpHR3-eYFP (or DIO-eYFP as control) was robust in PVN (AVP-neurons: 71%, OXT-neurons: 62%, Figure 3B) and SON (AVP neurons: 78%, OXT neurons: 81%, Figure 3E). In SST neurons in the LS, DIO-NpHR3-eYFP: 88% recombination, DIO-ChR2-eYFP: 71% recombination.

Functional verification of opsins. The AVPR antagonist Manning compound (10 nM) blocked the electrophysiological responses in LS upon ChR2 stimulation (20 Hz, 5 ms) in the vasopressinergic PVN→LS pathway. The OXTR antagonist Atosiban (10 nM) blocked the electrophysiological responses in LS upon ChR2 stimulation (30 Hz, 10 ms) in the oxytocinergic PVN→LS pathway. Stimulation of NpHR for 2 min in fibers from the vasopressinergic or oxytocinergic PVN→LS pathways did not result in counterintuitive activation in the responding cells of LS, although recordings of somatic activity in the PVN resulted in sustained inhibition (Figure S3).

Shock ramp test. Wildtype and *Magel2*KO mice were subjected to a modified version of the shock ramp test, which has been previously used in a rat model of autism (6). Each animal was placed on an electrical shock-grid kept inside a sound-proof chamber, and allowed to explore

the context for 2 mins in the absence of any shock. Then, electrical shocks of 1s duration each (0.06, 0.1, 0.2, 0.4, 0.6, 1 mA) were delivered in succession, with a 1 minute inter-shock-interval (ISI). Following another no-shock period of 2 mins, a second ramp of electrical shocks was delivered, wherein each shock was of 2s duration (0.06, 0.1, 0.2, 0.4, 0.6, 1 mA) at the same ISI. Behavior was recorded live and scored offline. Lowest threshold of shock that elicited a response – defined as the shock intensity at which they showed typical response(s) to shock-delivery (backpedaling/withdrawing of paw, darting/running, freezing, jumping) - was recorded for each mouse. Additionally, the number of jumps was also scored for both genotypes.

Supplemental references

1. Mahn M, Prigge M, Ron S, Levy R, Yizhar O. Biophysical constraints of optogenetic inhibition at presynaptic terminals. Nat Neurosci. 2016 Apr;19(4):554-6.
2. Borie AM, Dromard Y, Guillon G, Olma A, Manning M, Muscatelli F, Desarménien MG, Jeanneteau F. Correction of vasopressin deficit in the lateral septum ameliorates social deficits of mouse autism model. J Clin Invest. 2021 Jan 19;131(2):e144450
3. Knobloch HS, Charlet A, Hoffmann LC, Eliava M, Khrulev S, Cetin AH, et al. Evoked Axonal Oxytocin Release in the Central Amygdala Attenuates Fear Response. Neuron. 2012 Feb;73(3):553–66.
4. Raam T, McAvoy KM, Besnard A, Veenema AH, Sahay A. Hippocampal oxytocin receptors are necessary for discrimination of social stimuli. Nat Commun. 2017 Dec 8;8(1):2001.
5. Besnard A, Gao Y, TaeWoo Kim M, Twarkowski H, Reed AK, Langberg T, et al. Dorsolateral septum somatostatin interneurons gate mobility to calibrate context-specific behavioral fear responses. Nat Neurosci. 2019 Mar;22(3):436–46.
6. Anstey NJ, Kapgal V, Tiwari S, Watson TC, Toft AK, Dando OR, Inkpen FH, Baxter PS, Kozić Z, Jackson AD, He X. Imbalance of flight–freeze responses and their cellular correlates in the Nlgn3−/y rat model of autism. Molecular Autism. 2022 Jul 18;13(1):34