Fear-enhancing effects of septal oxytocin receptors

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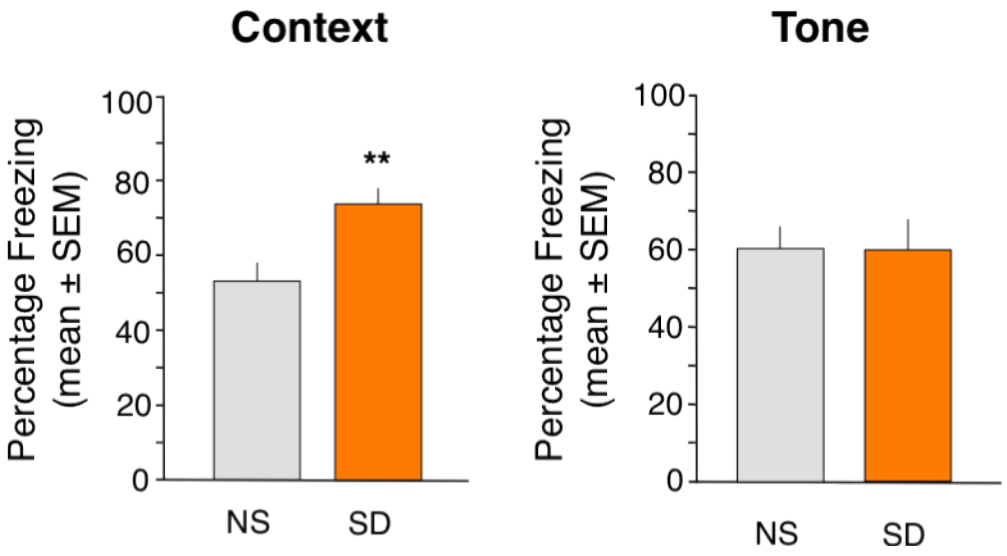
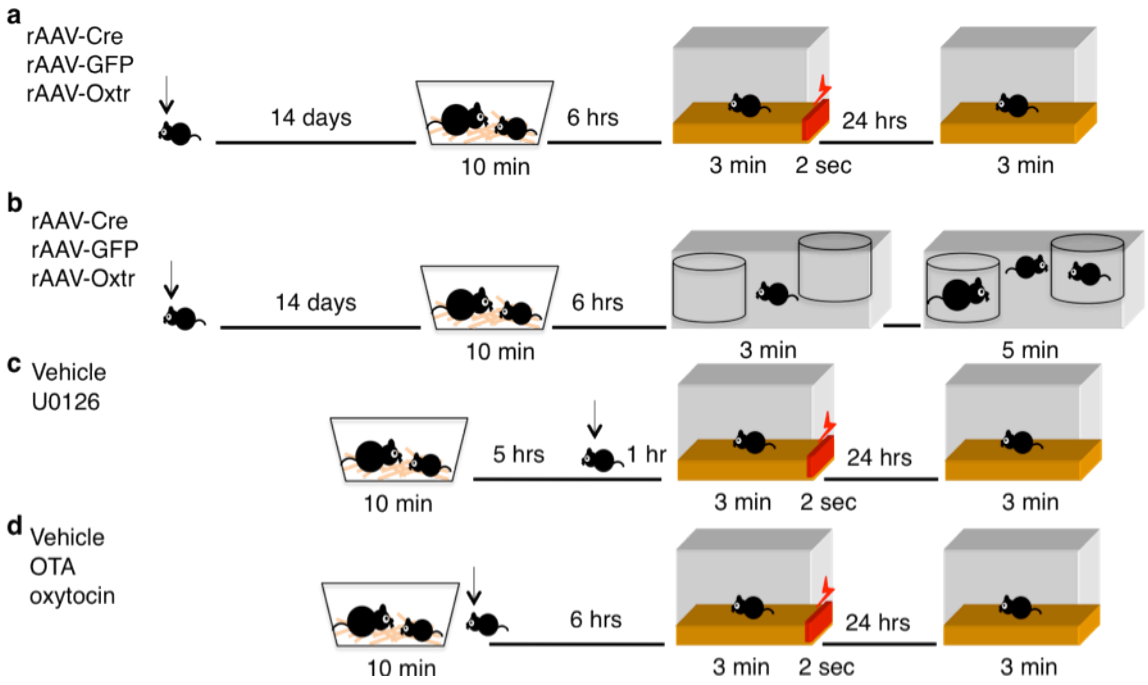
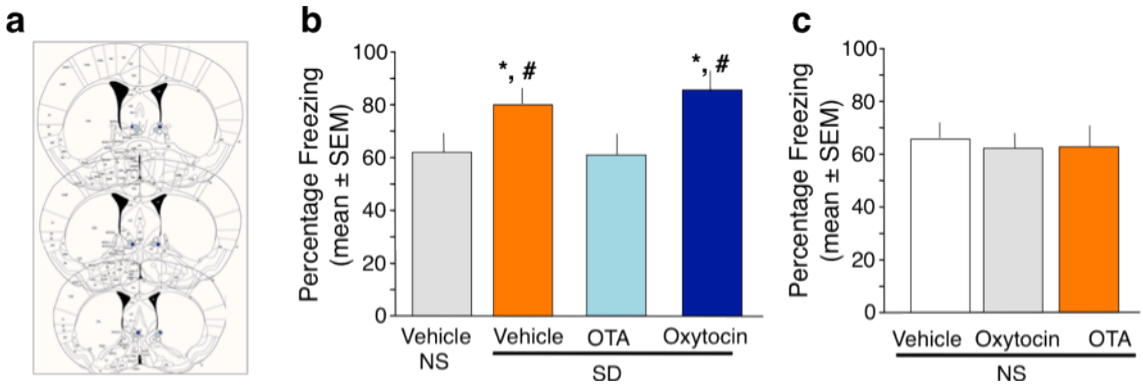


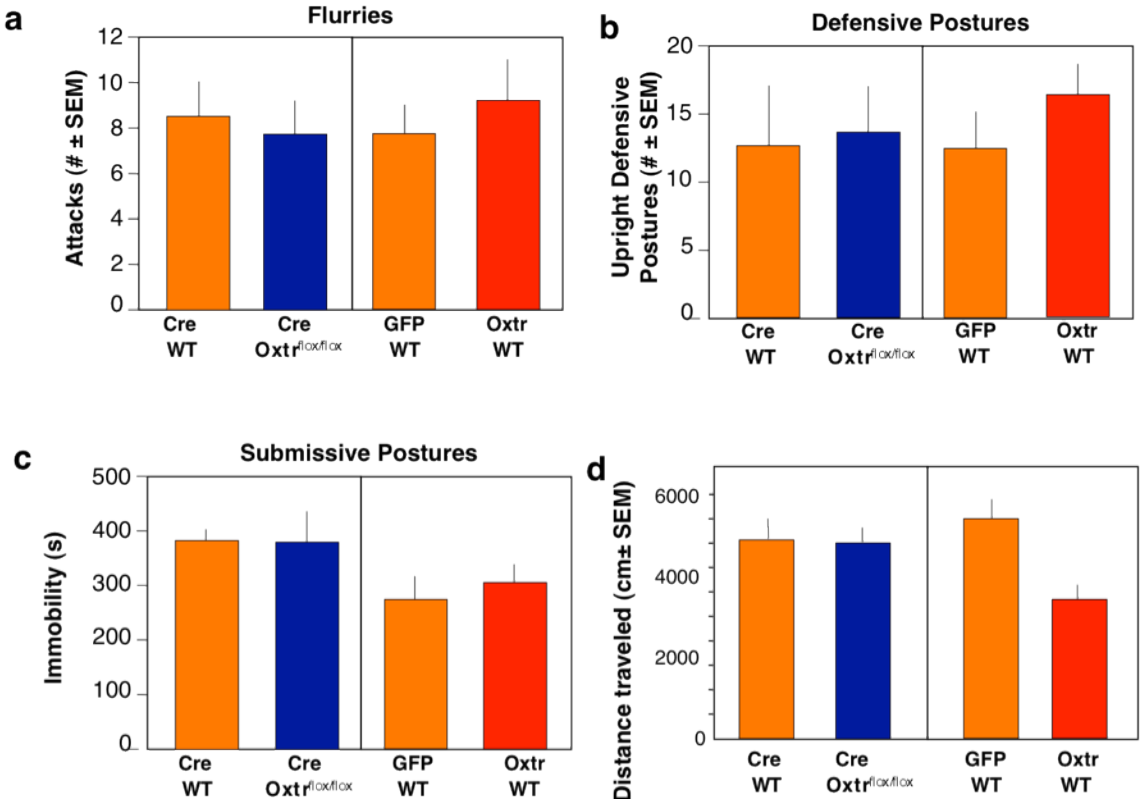
Supplementary Figure 1. Social defeat enhances context- but not tone-dependent fear conditioning. Context-dependent fear was significantly increased when training was performed 6 hr after social defeat [SD (n = 7 mice/group) versus NS (n = 7 mice/group) group (t_{12} = –3.336, P < 0.01)]. There was no difference between NS (n = 7 mice/group) and SD (n = 7 mice/group) mice (t_{12} = –0.000, P = 1) in tone-dependent freezing. The findings indicate higher susceptibility of contextual fear to modulation by social stress. Statistically significant differences: **P < 0.01.



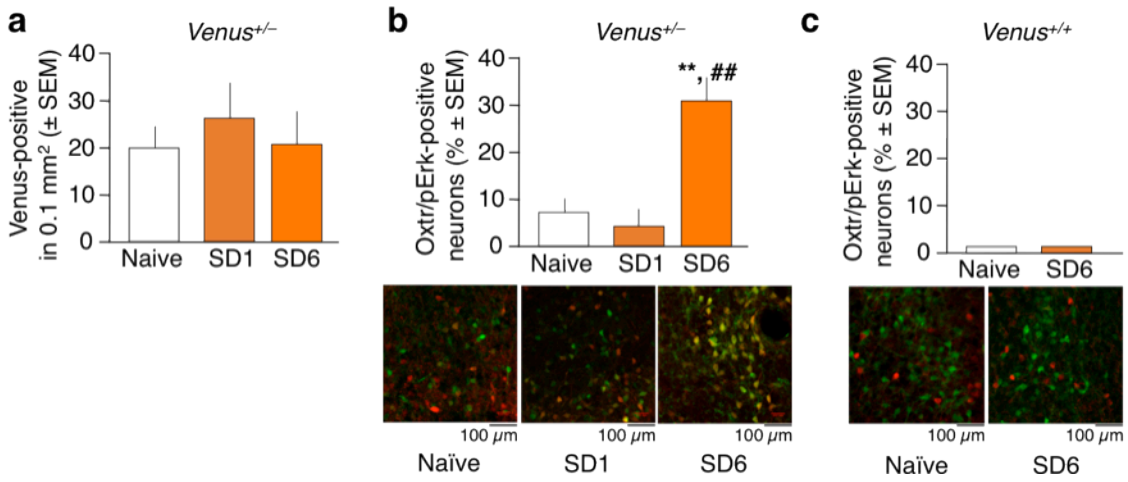
Supplementary Figure 2. Experimental design. **a**, Floxed Oxtr mice were stereotactically injected with rAAV-GFP or rAAV-Cre, whereas wild type mice were injected with rAAV-GFP or rAAV-Oxtr into the lateral septum. After two weeks, the mice were exposed to an aggressive resident for 10 min. Six hours after SD, a time window of high susceptibility to stress[10], the mice were exposed to a context (3 min) followed by footshock (2s, 0.7 mA, constant current). On the following day, the mice were re-exposed to the context for 3 min during which freezing behavior was scored by an observer blind to the experimental condition. **b**, For social recognition, mice were identically injected with viruses as described above, and exposed to SD. Six hours later, mice were first habituated to an empty arena for 3 min, followed by exposure to the aggressor and novel mouse for additional 5 min. The time and activity (distance) spent with each mouse was recorded automatically using the Videomot II tracking system. **c**, The role of Erk-1/2 in SD-enhanced fear was tested by inhibiting U0126 activity 5 hrs after the end of stress (1 hr before fear conditioning) in order to block the post-stress rise of phospho-Erk-1/2. **d**, Oxytocin or the Oxtr antagonist OTA were injected immediately after the end of stress to test their role in SD-enhanced fear.



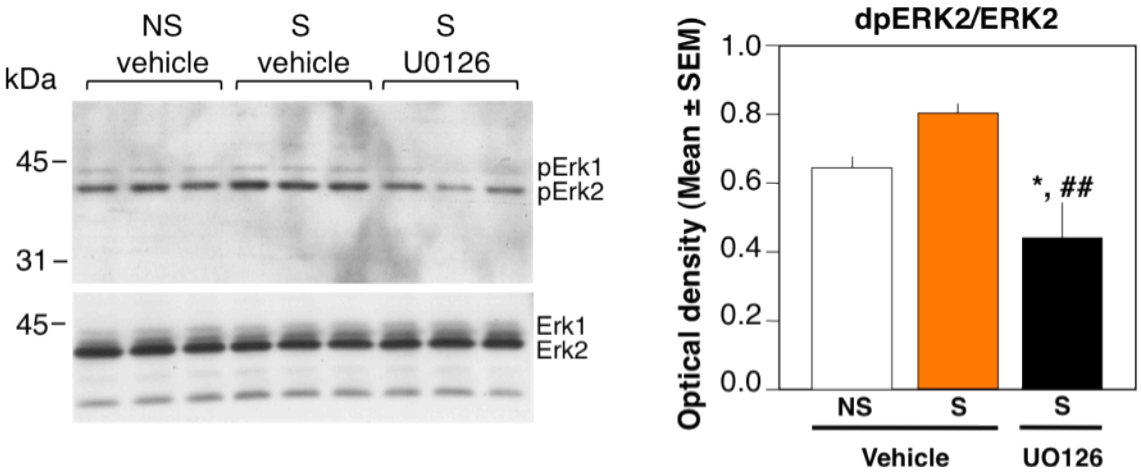
Supplementary Figure 3. Validation of the genetic Oxtr manipulations. **a**, We targeted the viral and pharmacological injections to the antero-posterior coordinates corresponding to the highest number of Oxtr-positive neurons (AP +0.14 to +0.38), as shown by a typical analysis of the injection sites. **b**, Consistent with genetic Oxtr knockdown, pharmacological manipulations of the Oxtr system significantly affected fear ($F_{3,45} = 3.116$, $P < 0.05$). Inhibition of Oxtr using OTA blocked the enhancement of fear by SD ($P < 0.05$, SD vehicle, n = 15 mice/group, vs SD OTA, n = 13 mice/group). Unlike Oxtr overexpression, oxytocin injection did not further increase freezing in response to SD ($P = 0.739$, SD oxytocin, n = 10 mice/group, vs SD vehicle, n = 15 mice/group). This may be due to several factors. First, endogenous release of oxytocin by SD could saturate available Oxtr and prevent further actions of exogenously added peptide. This would not interfere with Oxtr overexpression- on the contrary, more receptor is available to respond to oxytocin. Alternatively, lack of further enhancement may be due to ceiling freezing levels, which we typically see with septal injections performed shortly before or after stress. **c**, As found with genetic manipulations, injections of oxytocin (n = 11 mice/group) or OTA (n = 10 mice/group) did not affect freezing behavior in NS groups ($F_{2,28} = 0.363$, $P = 0.699$ vs NS group, n = 10 mice/group). Statistically significant differences: *$P < 0.01$ vs Vehicle NS, n = 11 mice/group); #$P < 0.01$ vs OTA SD.



Supplementary Figure 4. Oxtr manipulations did not affect behavior in response to social defeat. Defensive and submissive behavior was analyzed in a typical experiment with mice with normal (CRE WT, n = 5 mice/group) and reduced (CRE $Oxtr^{flox/flox}$ n = 4 mice/group) levels of Oxtr and mice with normal (GFP WT, n = 8 mice/group) and increased (Oxtr WT, n = 9 mice/group) levels of Oxtr. Mice with Oxtr knockdown or Oxtr overexpression had similar number of **a,** flurries (t_7 = 0.392, P = 0.71 and t_{15} = 0.352, P = 0.76, respectively), **b,** defensive postures (t_7 = 1.77, P = 0.865 and t_{15} = 1.5, P = 0.155, respectively) and. **c**, submissive postures (t_7 = 0.048, P = 0.963 and t_{15} = 0.727, P = 0.478, respectively). **d**, Oxtr manipulations did not affect activity in the context before shock but after SD, as revealed by similar data in mice showing normal and reduced levels of Oxtr (t_7 = 0.27, P = 0.795) and mice showing normal and increased levels of Oxtr (t_{15} = 1.995, P = 0.065).



Supplementary Figure 5. Social defeat does not affect the activity of the Oxtr promoter but enhances Erk-1/2 signaling in Oxtr-positive neurons. **a**, The number of Venus-positive neurons in the lateral septum did not change 1 (SD1, n = 6 septi/group) or 6 hours (SD6, n = 4 septi/group) after social defeat ($F_{2,12} = 1.594$, $P = 0.243$ vs naïve, n = 5 mice/group). **b**, In heterozygous, *Venus*$^{+/-}$ septal neurons , SD6 (n = 4 septi/group) triggered a significant up-regulation of pErk-1/2 ($F_{2,12} = 6.913$, $P < 0.01$) when compared to the naïve ($^{**}P < 0.01$, n = 5 septi/group) and SD1 ($^{\#\#}P < 0.01$, n = 4 septi/group) groups. **c**, In homozygous, *Venus*$^{+/+}$, Oxtr knockouts, there was no SD-induced up-regulation of pErk-1/2 and there was no co-localization of Oxtr and pErk-1/2 either in naïve (n = 5 septi/group) nor SD6 mice (n = 6 septi/group). Statistically significant differences: $^{**}P < 0.01$ vs Naive NS; $\#\#P < 0.01$ vs SD1.



Supplementary Figure 6. Decreased phosphorylation of pErk-1/2 by U0126. Local micronjection of U0126 (n = 3 samples/group) significantly decreased the levels of pErk-1/2 in the lateral septum ($F_{2,6} = 11.8$, $P < 0.01$) when compared to both NS (*$P < 0.05$, n = 3 samples/group) and S (##$P <$ 0.01, n = 3 samples/group) groups.