Title: Sniffing and Oxytocin: Effects on Olfactory Memories.

Authors: Stoop R

Journal: Neuron

Year: 2016 May 4

Issue: 90

Volume: 3

Pages: 431-3

DOI: 10.1016/j.neuron.2016.04.033

Sniffing and Oxytocin: effects on olfactory memories

Ron Stoop
Center for Psychiatric Neuroscience
Department of Psychiatry
Lausanne University Hospital
rstoop@unil.ch

In this issue of Neuron, Oettl et al. (2016) show how oxytocin can boost processing of olfactory information in female rats by a top-down regulation from the anterior olfactory nucleus onto the main olfactory bulb. As a result interactions with juvenile conspecifics receive more attention and are longer memorized.

Oxytocin (OT) appears unstoppable on its way up the "social ladder" of effects exerted in higher brain regions. Isolated and synthesized in the 1950's it became known first and foremost for its peripheral effects on lactation and delivery. Over the last decades, however, OT has gained increasing attention for its role in brain structures important for social behavior. Although its neuromodulatory effects were initially reported in the limbic system, more and more findings are revealing OT effects in different telencephalic areas.

As part of this movement, Oettl et al., report in this issue of Neuron how, in the anterior olfactory nucleus (AON), oxytocin can boost the processing of socially relevant olfactory information. The olfactory system in rodents plays an important role in processing of social cues and, although less so in humans, may serve as a suitable model how oxytocin affects processing in other sensory systems. In addition, since oxytocin in humans is often intranasally administrated, any information about its effects in this system may be important to keep in mind.

The approach, that Oettl et al. took, starts with a series of behavioral observations, followed by an in vitro electrophysiological analysis in slices before combining odor exposure with electrophysiology in a head-fixed rat preparation. Oxytocin effects were in all three approaches tested by pharmacological application of a specific OT agonist or by optogenetical stimulation of OT producing neurons in the paraventricular nucleus (PVN) of the hypothalamus (Knobloch et al., 2012).

In the behavioral experiments an adult female Wistar rat was exposed to a younger female and anogenital exploration time was measured as an indicator of social interest. During a second exposure to both the previous juvenile female and a not yet encountered juvenile, preference for the latter was used as an indicator of social memory, and in rats does not last beyond one hour. Optogenetic stimulation of OTergic neurons in the PVN, however, not only increased anogenital exploration upon the first encounter, but also maintained preference for the new juvenile for at least two hours . These findings show that OT can both boost interest for a social stimulus, and concomitantly increase social memory.

These changes in anogenital exploration may suggest OTergic modulation in the olfactory system, and the anterior olfactory nucleus (AON) indeed expresses high levels of OT receptors (OTRs). However, OTergic neurons in the PVN project to many brain regions (Knobloch et al., 2012) and their stimulation might evoke equally well OT release

in other regions that affect social motivation (Dolen et al. 2013). To address this important point, the authors turned to a special mouse model that allowed specific OTR ablation in the AON. Whereas control mice (in this case males) retained memory for exposure to juvenile male mice for more than 30 minutes, conditional OTR KO mice did not exhibit such memory, thereby confirming an involvement of OT signaling in the AON for social memory. Somewhat unexpectedly, the OTR KO mice showed an increased exploration of juveniles upon first contact, further suggesting that recognition memory is not necessarily related to initial exploration time.

To explore the circuitry through which OT may affect olfactory signaling, the authors made use of an in vitro slice preparation of the olfactory bulb that included the AON. Whole cell patch clamp recordings in the AON revealed pyramidally-shaped neurons that, upon depolarizing current injections, produced action potentials with regular firing patterns. Perfusion of the specific OT receptor agonist Thr^4Gly^7-OT (TGOT) increased the frequency of excitatory postsynaptic currents (EPSCs) more than four-fold in more than 50% of the neurons and of inhibitory postsynaptic currents (IPSCs) in nearly 20 %. In slices prepared from rats that had been infected with ChR2 virus in the PVN, these effects could be efficiently mimicked by blue light exposure. The effects were blocked by incubation with an OTR antagonist. Furthermore, when excitatory and inhibitory currents were pharmacologically blocked, TGOT was still able to increase the number of action potentials evoked by depolarizing current injections. While these findings show a functional release of OT by hypothalamic projections onto the AON, they also raise the question which targets are affected downstream of the AON.

The main olfactory bulb is a primary target of AON neurons and the authors show that retrograde labeling in the main olfactory bulb (MOB) could indeed efficiently label AON neurons. Importantly, all of the retrogradely labeled AON neurons also stained positive by an antibody specifically raised against the OTR (Mitre et al., 2016). Further recordings specifically revealed granule cells (GCs) in the MOB as downstream OT targets, as these cells also showed increased EPSC frequencies after bath perfusion of TGOT or after blue light stimulation. Furthermore, these TGOT effects disappeared after severing connections between AON and the MOB. GCs are known to send inhibitory projections to mitral cells (MCs), and, consistently, TGOT also increased IPSCs in MCs. Taken together, these patch clamp recordings are consistent with a top-down regulation by the AON through glutamatergic projections onto GCs neurons and GABAergic projections onto MCs. OTR activation increased inhibition of MC neurons, the main inputs from the olfactory pathway, by exciting pyramidal neurons in the AON:

The increased inhibition by OT of MCs is reminiscent of recent findings in the hippocampus where OT increases spontaneous IPSCs, leading to increased signal to noise ratio for excitatory inputs (Owen et al., 2013). Interestingly, Oettl et al. here report an application of this filtering effect in an in vivo situation. To study the processing of olfactory input in vivo, Oettl et al. head-fixed anaesthesized rats in a stereotaxic frame and lowered extracellular recording electrodes in the MOB, searching for MC by their responses to the puffing of different odors onto the nose. An additional cannula targeted the AON for simultaneous injection of TGOT and assessing its neuromodulatory effects.

In this manner, the authors could show for 20 odor-neuron pairs that TGOT caused an increase in odor-evoked responses and a simultaneous decrease in spontaneous baseline activity. Although TOT enhanced existing sensitivity to odors in this way, it never induced responses to odors in cells that were initially not responding. This seems an elegant demonstration of an OTR based filtering mechanism that boosts excitatory responses of MC neurons to odors, without turning previously non responding neurons into responders.

Taken together, through this combination of behavioral and electrophysiological approaches in vitro and in vivo, Oettl et al reveal how OT, by boosting novel signals in favor of reducing familiar ones, can filter socially relevant odors processing through the olfactory bulb. Besides OT, the olfactory bulb also expresses vasopressin receptors for which a social recognition role has been reported in male rats (Tobin et al, 2010). Based on these findings, Wacker and Ludwig (2012) predicted that, "upon smelling the familiar juvenile, vasopressin could inhibit the passage of information from glomeruli to higher processing centers by either directly or indirectly filtering out mitral cell output representing that juvenile's odor profile, while mitral cells representing the odor of the novel juvenile would not be inhibited. Higher brain regions, including the cortical and medial amygdala would still be activated by the novel juvenile's odor profile. Thus, the social salience of the novel juvenile would supersede that of the familiar one, and the focal male would engage in more olfactory investigation of the novel versus familiar social stimulus" (Wacker and Ludwig, 2012).

Receptors for vasopressin and OT are often expressed in adjacent regions and their functions have been suggested to be complementary as well as gender dependent (Stoop et al., 2015). The recent development of a high quality antibody against the OTR now allows to specifically determine its expression at the cellular level (Mitre et al., 2016). An antibody of similar quality for the vasopressin receptor could be a great asset to provide valuable additional information not only about the precise function of this peptide in this system but also about its potential interactions with the OTR.

Besides filtering effects, the present findings also suggest that OT in the AON may boost short-term memory. Using the current preparation for further electrophysiological experiments, could it be possible to take the current findings one step further and discover whether and how such memory may be related to changes in synaptic plasticity? Upstream, in the accessory olfactory bulb, OT has already been shown to induce long-term potentiation in excitatory input from mitral cells onto granule cells (Fang et al., 2008). Recent findings have also shown a role for OT in memory formation downstream of the AON, in the olfactory piriform cortex, where it plays a role in assigning social relevance to initially neutral olfactory stimuli (Choé et al., 2015). Maybe a coordinated release of OT from hypothalamic projections onto successive brain regions involved in the processing of olfactory information provides a basis for the creation of a coherent pattern of activation that sustainably affects memory across regions important for social interactions (Stoop, 2014).

Recent findings in the auditory cortex (Marlin et al. 2015) have shown that OT supplements increase responsiveness to auditory calls from young pups in virgin rats,

thereby inducing maternal behavior similar to that displayed by the original mother. The story emerges that OT can convey social relevance in different sensory modalities and boost transmission of sensory signals at different steps along their processing pathways. Such effects of OT may be closely intertwined with its function in development when sensory experiences cross-modally regulate development of all sensory cortices (Zheng et al., 2014). Filtering of relevant social stimuli by OT may thus not be restricted to adult life but already play a role at earlier stages in development. No doubt this provides a rich scenario for further exploration of the beneficial effects of OT in the treatment of impaired social behaviors at different ages.

REFERENCES

Choe, H.K., Reed, M.D., Benavidez, N., Montgomery, D., Soares, N., Yim, Y.S., and Choi, G.B. (2015). Neuron *87,* 152-163.

Fang, L.Y., Quan, R.D., and Kaba, H. (2008). Neurosci Lett. *438*, 133-137.

Knobloch, H.S., Charlet, A., Hoffmann, L.C., Eliava, M., Khrulev, S., Cetin, A.H., Osten, P., Schwarz, M.K., Seeburg, P.H., Stoop, R., and Grinevich, V. (2012). Neuron *73,* 553-566.

Marlin, B.J., Mitre, M., D'amour, J.A., Chao, M.V., and Froemke, R.C. (2015). Nature *520*, 499-504.

Mitre, M., Marlin, B.J., Schiavo, J.K., Morina, E., Norden, S.E., Hackett, T.A., Aoki, C.J., Chao, M.V., and Froemke, R.C. (2016) J Neurosci *36*:2517-2535.

Lars-Lennart Oettl, L.L, Ravi, N. Schneider, M. Scheller, M.F., Schneider, P., Mitre, M. da Silva Gouveia, M., Froemke, R.C., Chao, M.V., Young, W.S., Meyer-Lindenberg, A., Grinevich, V., Shusterman, R., and Kelsch, W. (2016). Neuron ….

Owen, S.F., Tuncdemir, S.N., Bader, P.L., Tirko, N.N., Fishell, G., and Tsien, R.W. (2013) Nature *500*, 458-462.

Stoop, R., Hegoburu, C., and van den Burg, E. (2015) Annu Rev Neurosci. *38*, 369-388.

Stoop, R. (2014). Curr Opin Neurobiol. *29*: 187-193.

Stoop, R. (2012) Neuron. *76,* 142-159.

Tobin, V.A., Hashimoto, H., Wacker, D.W., Takayanagi, Y., Langnaese, K., Caquineau, C., Noack, J., Landgraf, R., Onaka, T., Leng, G., Meddle, S.L., Engelmann, M., and Ludwig, M. (2010). Nature *464*, 413-417.

Wacker, D.W., and Ludwig, M. (2012). Horm Behav. *61*, 259-265.

Zheng, J.J., Li, S.J., Zhang, X.D., Miao, W.Y., Zhang, D., Yao, H., and Yu, X. (2014) Nat Neurosci. *17,* 391-399.