

ARTICLE**Oxytocin-Induced Balance of Cortical Inhibition and Excitation Facilitates Behavioural Changes in Pup Retrieval by Mice****June Yue Dong**

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ABSTRACT

Across species, oxytocin (OT) has been identified as a neuromodulator of social interactions and maternal behaviour. The timing, location, and method of how oxytocin alters neural activity, however, are not well characterized. In order to elucidate oxytocin's mechanism of action, Marlin et al. (2015) investigated differences in pup retrieval among maternal female mice (dams), naïve virgin mice, and experienced virgin mice, as well as the role of oxytocin in eliciting or modifying their behaviours. As muscimol inhibition of the left auditory cortex (AI) prevented pup retrieval, so systemic application of oxytocin accelerated maternal reactions to pup vocalizations. Neural responses to vocalizations confirmed lateralization of responses in the left AI of experienced adult mice. Further, Marlin et al. (2015) observed that current-clamp and cell-attached recordings of the left AI in dams and experienced virgins demonstrated precisely timed combinations of excitatory and inhibitory activity as a response to pup calls. Finally, optogenetic oxytocin stimulation paired with pup vocalizations resulted in the balancing of size and time of inhibition with excitation. These results indicate synaptic balance as the mechanism that allows oxytocin to enhance salience of socially relevant auditory information.

INTRODUCTION

For decades, oxytocin has been known to affect behaviour in mammals. Produced in the paraventricular nucleus (PVN) and the supraoptic nucleus of the hypothalamus, oxytocin functions both as a peripheral hormone and neuropeptide (Gimpl et al. 2001). The hormonal functions of OT include triggering lactation and parturition. Its study in neuroscience, however, has generally focused on social behaviours such as bond formation and parenting, as well as its effect on cognition (Churchland et al. 2012).

During the 1980s, social neuroscience studies on oxytocin focused on its part in maternal rodent behaviour. OT injection was discovered to be conducive to nest building (Fahrbach et al. 1984), but maternal behaviours were blocked by OT receptor antagonists (Fahrbach et al. 1985). While OT knockout (KO) mice did not demonstrate abnormalities in maternal behaviour, (Nishimori et al. 1996) OT receptor KO mice showed maternal behaviour deficits (Takayanagi et al. 2005). Parallel to this research were investigations on the effects of auditory cues, specifically the social stimuli emitted by pups, on maternal behaviour. Low frequency pup vocalizations were found to induce maternal nest building (Ehret et al. 1986), and ultrasonic pup vocalizations were found to induce a search and retrieval process (Ehret 2005). Mouse mothers (dams) would retrieve pups even after vocalizations stopped, and would retrieve mice based on audio recordings of ultrasonic vocalizations, in the absence of pup emitted calls (Ehret 2005). Further, virgin females who hear those vocalizations do not exhibit pup retrieval, and have a lower signal-to-noise ratio of neural response in the AI than dams (Liu et al. 2006). Yet, an earlier study by Pedersen et al. in 1982 observed the interesting phenomenon of virgin female rodents participating in pup retrieval pups after being cohoused with a dam and her pups, or if the virgin female was administered oxytocin centrally (Pedersen et al. 1982). In order to elucidate the role of oxytocin, in particular its spatial-temporal profile and mechanism of action, Marlin et al. merged the two existing paths of inquiry on the respective roles of oxytocin and vocalizations on pup retrieval behaviour (Marlin et al. 2015). They assessed experimental female wild-type mice receiving systemic oxytocin injections, wild-type mice receiving saline vehicle injections, and oxytocin-IRES-Cre mice optogenetically stimulated for release of endogenous oxygen for their amount of time to retrieval.

to label oxytocin in the AI of virgins for immunohistochemical analysis. In experienced animals, the GABA agonist muscimol was injected into the left or right AI to inactivate those regions and compare behavioural differences. In naïve animals, oxytocin receptor antagonists or optogenetic AI stimulation were used to evaluate behavioural differences. Finally, Marlin et al. made *in vivo* and *in vitro* voltage-clamp recordings as well as *in vivo* whole-cell recordings in the AI of dams, naïve, and experienced virgins while playing recorded ultrasonic pup vocalizations, as well as during pairing of these vocalizations of with topical oxytocin or optical stimulation of the AI.

RESEARCH OVERVIEW

SUMMARY OF MAJOR RESULTS

Oxytocin Accelerates Acquisition of Pup Retrieval

Systemic oxytocin administration or optogenetic stimulation of virgin C57BL/6 female mice led to more animals exhibiting pup retrieval than saline-injected control animals, within 12 hours of co-housing, as opposed to 2 days. Isolated animals displayed similar results, with more oxytocin receiving virgins retrieving, and retrieving faster than those given saline.

Oxytocin Receptor and Pup Retrieval Auditory Cortex Lateralization

The OXTR-2 antibody, which does not label cells in OT receptor KO animals, was expressed significantly more in the left auditory cortex of dams as well as naïve virgins compared to the right. Muscimol inactivation of the left but not right AI in experienced females impaired retrieval, while oxytocin administration or optogenetic stimulation of the left AI alone in virgins facilitated earlier retrieval than saline-infused animals. However, in animals that were already experienced in pup retrieval, neither the OTA or L-368,899 OT receptor antagonists affected performance.

Cortical Responses to Pup Calls

Experienced animals exhibited precise left AI spiking as evoked by pup calls during whole-cell recordings, and these responses were stronger than those displayed by inexperienced animals.

Neuromodulation by Oxytocin

Voltage-clamp recordings showed co-tuned and precisely timed cortical reactions in the form of excitatory post-synaptic currents (EPSCs) and inhibitory post-synaptic currents (IPSCs). The temporal profiles of EPSCs and IPSCs and neuronal spiking were highly correlated with pup calls in experienced animals. Inexperienced animals, however, showed no correlation of temporal precision or spiking with pup calls. This mismatch was seen as differences in patterns of inhibition and excitation. Optogenetic pairing of oxytocin with pup calls resulted in a reduction of IPSCs evoked by vocalizations, an alteration occurring in seconds and localized to the left AI. On the other hand, EPSCs were increased, and IPSCs were not increased until forty-five minutes later, balancing the excitation with inhibition and allowing for both IPSC and EPSC strength and reliability. Spike timing increased in precision with IPSC and EPSC pattern matching immediately, but matching trial-by-trial did not occur until after an hour.

DISCUSSION AND CONCLUSIONS

Identifying behavioural differences in time to pup retrieval between experienced and inexperienced animals provided a basis for understanding how oxytocin might play a role in changing neural circuitry or synaptic connections to create a robust response to ultrasonic pup vocalizations. Marked

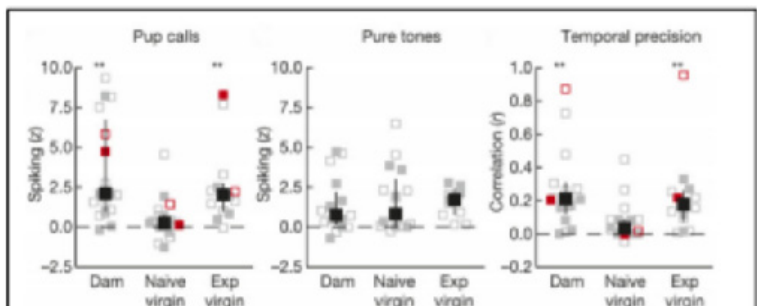


Figure 1. Spiking and Temporal Precision in Experienced Female Left AI. Red indicates analyzed example cells. Current-clamp recorded responses were standardized using z scores for spiking evoked by pup calls and pure tones demonstrating significant differences in spiking from dams ($n = 17$, $P < 0.0004$ vs. $n = 17$, $P > 0.7$), and experienced virgins ($n = 14$, $P < 0.006$ vs. $n = 11$, $P > 0.6$) as compared to naïve virgins ($n = 20$). There were no significant differences between experienced and inexperienced animals listening to pure tones. Trial-by-trial time correlation in dams ($n = 17$, $P < 0.002$) and experienced virgins ($n = 14$, $P < 0.012$) were significantly higher than in naïve virgins ($n = 20$). (Marlin et al. 2015)

similarities between oxytocin mechanisms and those in the rest of the brain, murine or human, provide insight to how neuropeptides can influence the brain. The functional lateralization of pup retrieval to left auditory cortex in mice brains reflects oxytocin receptor expression. This result must be considered in context of human temporal lobe speech processing, which is also lateralized to the left side (Ehret 1987). Similarities in evolutionary use and location of oxytocin neurons in murine and human brains may be important in translational research on oxytocin and its effects. The antagonist-independent performance of experienced animals in pup retrieval suggests that oxytocin receptors are only required for acquisition of retrieval behaviours, but not for maintenance of this behaviour thereafter. Such a mechanism is analogous to the function of NMDA receptors in long-term potentiation induction and not maintenance (Malinow et al. 1989). This is valuable in showing that pup retrieval is a lasting memory, and supports Marlin et al.'s model of oxytocin and cortical plasticity. From the voltage-clamp recordings, and based on the changes in pup retrieval behaviours over time with oxytocin application, they directly demonstrated that the function of oxytocin is to cause changes in brain states, such that

responses evoked by pup calls become robust and temporally precise. The way by which oxytocin effects these changes is first an immediate reduction of AI inhibition in order to increase the salience of pup calls followed by gradual balance of excitation and inhibition for enhanced long-term spiking. Previous research finding that 30-40% of parvalbumin-positive and somatostatin-positive inhibitory interneurons express oxytocin receptors suggested that oxytocin influenced cortical inhibition (Nakajima et al. 2014). The inhibitory balancing effect of oxytocin on pup retrieval is congruent with these findings. Significantly, this suggests that innate social behaviours that can be improved by experience, such as pup retrieval, are governed by a neural circuit which is modulated through cortical inhibition and excitation by neuropeptides such as oxytocin. This addresses a critical question stemming from large bodies of work supporting both possibilities regarding whether OT influences high-order cognitive processes, or influences global states (Churchland et al. 2012). Marlin et al.'s findings seem to indicate that oxytocin has a broad effect on the saliency of sensory signals, rather than directly triggering complex social behaviours per se.

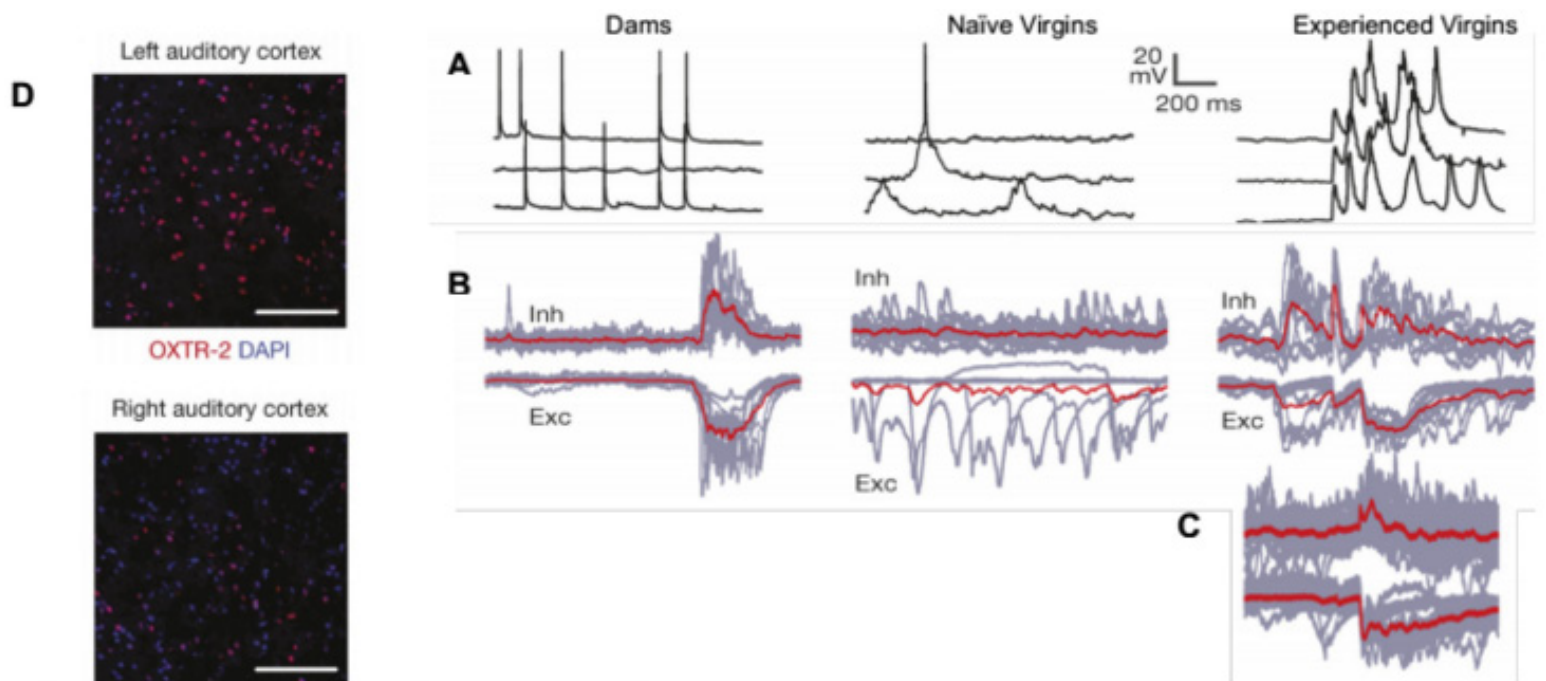


Figure 2. Spiking and Patterns of Excitation and Inhibition in Experienced Female Left AI. (A) Current clamp recordings in dams and experienced virgins demonstrated z-scores > 4.0 and trial-by-trial average correlations of $r > 0.20$, while naïve virgins had a z-score of 0.1 and r of -0.04. (B) Balanced inhibitory and excitatory post-synaptic currents are characteristic of experienced animals and dams. (C) As virgins become experienced at pup retrieval through cohousing, they develop neutrally similar patterns. (D) Changes are lateralized to the left auditory cortex, where oxytocin receptors are expressed. (Marlin et al. 2015)

ANALYSIS AND FUTURE DIRECTIONS

In an experiment examining the effects of a specific actor, negative controls are necessary to show that oxytocin plays a role in pup retrieval at all. Muscimol inactivation is a well-used treatment for inactivation, but is also known to spread (Allen et al. 2008). While the right and left AI of mice are too far apart for cross lateral contamination, it is possible that muscimol spread inactivated other regions of the brain than AI. In order to confirm, fluorophore-conjugated muscimol molecules have been developed to visualize locations of muscimol, and can be used to ascertain the spatial precision of its injection (Allen et al. 2008).

Further, *in vivo* recordings were performed on isoflurane-anaesthetized animals in order to prevent noise from movement, or sudden drops in recordings due to activity of the animal. While isoflurane-anaesthetized *in vivo* recordings are stable, they can dampen signals and are mostly used for sensory perception responses (Margrie et al. 2002). There may be differences between iso-flurane anaesthetized and awake animals especially in cortical changes and neuromodulation based on processing of auditory information, which should be investigated by using awake animals during recording.

Additionally, while the use of optogenetically stimulated AI for oxytocin ensures precise localization, there is individual variability in endogenous oxytocin release (Andari 2015). An interesting future experiment could examine whether there is a correlation between levels of oxytocin release and speed of pup retrieval behaviour acquisition, or even differences in effectiveness and duration. Some other interesting directions for the future include examining the role of olfaction in pup retrieval, and differences in paternal behaviour compared to maternal. While auditory cues are sufficient to trigger pup retrieval, this is also true of olfactory signals (Ehret 2005; Cohen et al. 2011). Olfactory cues may even be enhanced by oxytocin (Wacker et al. 2012). It is likely that both olfactory and auditory signals combine to effect pup retrieval behaviours.

One way to test for this in the future would be to combine Marlin et al.'s study with Kiyokawa et al.'s 2014 study on social olfactory cues of conspecifics. Rather than using a sound-attenuated chamber for the mice to play pup vocalizations and make electrophysiological recordings, the experiment can use pups to odorize the chamber for electrophysiological recordings, and finally combine the odorization and sounds to investigate the dual effects.

While non-conspecific pups placed in the same cage as a dam are cared for, paternal mice often attack stranger pups (Dulac et al. 2014). Notably, some of Marlin's naïve female virgin animals also mauled pups during pre-screening. Considering the increased maternal behaviour caused by oxytocin, as well as the sexually dimorphic effects of oxytocin, it would be interesting to look at how oxytocin may affect paternal or aggressive naïve virgin mouse behaviour (Insel 2010). Oxytocin could potentially have a socializing or calming effect, which is especially useful these naïve outliers were not included in the data analysis.

As OXTR-2 was carefully designed by Marlin et al. an synthesized for low cross-reactivity, and based on the western blot assay and lack of cell labelling in oxytocin knockout animals, this antibody has demonstrated clear specificity for oxytocin receptors. It can be widely used to localize oxytocin receptor expression throughout the brain in mice, in order to map and identify density of oxytocin receptors across wild-type and manipulated animals. It is also possible to adapt OXTR-2 for rats to perform comparative studies. Finally, to validate Marlin et al.'s proposed mechanism of action for oxytocin to enhance pup retrieval, an experiment could be performed which mimics the inhibitory and excitatory patterns of activity through direct stimulation. Not only could this help validate their model, it would also elucidate any other effects of oxytocin by virtue of comparison.

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