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Is oxytocin receptor signaling really dispensable for social attachment?

In an article in press at *Neuron*, Berendzen et al. used CRISPR-Cas9 to genetically modify the oxytocin receptor gene (*Oxtr*) in prairie voles [1]. The authors claim to have generated *Oxtr* null alleles based on results that indicate modified binding of an analog of oxytocin. No data are reported that show null results of RNA or protein expression or loss of downstream signaling, at least one of which would be necessary to claim that mutant alleles have no (or "null") function. The authors show that prairie voles carrying these alleles display typical social behaviors including pair bonding, pup-directed parental behaviors, and nursing and, contrary to decades of work, conclude that "the oxytocin receptor is not required for social attachment in prairie voles." We have concerns about the major conclusions of the paper and worry that many will misinterpret the findings. For example, a popular press article reviewing the paper in the Atlantic [2] states "Now researchers are finding that oxytocin may be not only insufficient for forging strong bonds, but also unnecessary." At best, we suggest the authors have knocked down function of the major transcript but not ablated total gene function. This is an important distinction because it is critical for proper interpretation of the data and misinterpretation of these results has many downstream consequences for the field and for our understanding of ourselves. Genes are tricky beasts and without thorough molecular examination of mutants, one can easily assume that they have the full story when they do not.

1. Are the reported Oxtr mutants true null alleles?

Berendzen et al. generated three mutant alleles, none of which removes the entire gene sequence from the genome. The authors analyzed the genomic sequence of their mutants and predicted that the first two mutant alleles (*Oxtr*^{1} and *Oxtr*^{4}) would result in premature stop codons and the third mutant allele (*Oxtr*^{5}) would generate a 1.7 kb deletion encompassing some of the coding region of the gene and would not make a transcript. No data are provided at the RNA transcript or protein level to molecularly confirm these gene products. Instead, assumptions are made about the transcriptional potential of each mutant and subsequent downstream consequences. Importantly, a 2018 study examining *Oxtr* in mouse hippocampus indicates that the canonical transcript is not the only transcript of *Oxtr* in the brain. Towers et al. identified several versions of *Oxtr* which originate from alternative transcription start sites, alternative splicing of exons, or both [3]. They find a total of eight versions of *Oxtr* (isoforms). We have previously confirmed that at least one of these isoforms is present (*Oxtr*-H) in prairie vole brain tissue [4] and importantly, it would not be impacted by any of the three mutants presented in Berendzen et al. A thorough examination of *Oxtr* isoforms and their downstream function is necessary to make claims about OXTR functional nulls. Furthermore, there is precedent in the G-protein coupled receptor family for alternative isoforms to be translated and to impact downstream signaling (see literature on mu opioid receptor [5]).

2. How might different forms of Oxtr impact oxytocin binding?

OXTR is a Class A G-protein coupled receptor containing seven highly conserved transmembrane domains which form a binding pocket for oxytocin [6]. Ligand-bound OXTR can bind several different G proteins, both excitatory and inhibitory, depending on the context [7]. OXTR can homodimerize via interactions between transmembrane helices 1 and 2 [8] and can heterodimerize with vasopressin V1a and V2 receptors [9], dopamine D2-type receptors [10], β_2-adrenergic receptors [11], and serotonin receptors $5HTR_{2A}$ and $5HTR_{2C}$ [12,13]. Ligand-bound OXTR can be internalized in a β-Arrestin dependent manner via interactions between the C terminus of OXTR and β-Arrestin [14]. We share these studies to emphasize that different portions of the full-length OXTR protein are involved in specific biochemical interactions, some of which may be preserved in the mutant forms of *Oxtr* described in Berendzen et al. Specifically, it remains possible that alternative forms of OXTR can modulate activity of other neurotransmitter systems in the absence of optimal oxytocin binding.

3. Is oxytocin signaling abolished in Oxtr mutants?

The crux of the argument presented by Berendzen et al. rests on the claim that the described *Oxtr* mutants cannot bind oxytocin ligand. The evidence provided is a lack of binding of [125I]-OVTA, a competitive antagonist of OXTR at a single timepoint. To our knowledge, a pharmacological study determining the binding affinity of oxytocin and OVTA for OXTR in voles has never been published. Lacking this information, we don't know if the [125I]-OVTA binds to OXTR with similar affinity as oxytocin. We also do not know if [125I]-OVTA is selective for OXTR in voles at the concentration used. Critically, the authors do not provide any direct evidence for lack of oxytocin ligand binding in these assays. Binding capacity is likely reduced in these mutants, but with the data presented it cannot be said to be abolished. Oxytocin signaling capacity of these mutant alleles is not directly assayed. For example, a different group used CRISPR-Cas9 to generate a mutant *Oxtr* allele in prairie voles and directly showed lack of oxytocin signaling using a TGFα shedding assay [15]. Evidence for lack of oxytocin signaling could also be shown with pharmacological manipulations. For example, we would expect that oxytocin receptor antagonists would prevent pair bond formation in wild type animals, as shown in previous research [16–19], but would have no effect in animals with a true *Oxtr* null allele (though see this review for a more detailed discussion of limitations of these pharmacological experiments [20]). As this evidence is not provided, we cannot be sure that these mutant alleles fully prevent oxytocin

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signaling.

4. Summary

We believe there is insufficient evidence for the main conclusion made by Berendzen et al. specifically that oxytocin receptor is not genetically required for a pair bond. First, they have not created a true "knockout" that prevents the full transcriptional ability of *Oxtr*. Two of their mutant alleles could still make a number of potentially functional transcripts, including a portion of the protein containing the first full transmembrane domain, a potentially functional unit which has been shown to modulate GPCR activity in other systems [21]. Second, there was no biochemical or pharmacological evidence indicating that oxytocin signaling was truly abolished, which would alleviate the first concern. Finally, the lack of a behavioral phenotype is worrisome. We acknowledge that there are many genes involved in this complex behavior and that it is possible that OXTR may not be a major driver of pair bonding. Even so, the animals produced by the genetic manipulations described here continued to not only show selective social behaviors that characterize pair bonding in prairie voles [22], but also have the capacity to rear offspring. Although not specifically described this would require milk ejection, a function shown in many studies to necessitate a functional oxytocin receptor (reviewed elsewhere [20]). We applaud Berendzen et al. for providing the field with *Oxtr* mutants for future work, but we urge these authors to follow up their work with careful and thorough experiments that provide conclusive evidence for their claims.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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