Molecular Mechanisms of Oxytocin Receptor Activation on Social Behavior

Keywords: oxytocin receptor, G-protein, intracellular signaling, hormones, social behavior

Introduction: Within a species, the same hormone can have opposing effects on social behavior depending on sex, seasonal variation, and environmental context[1]. Since oxytocin (OT) is known to modulate social behavior, it is an ideal system to study the molecular mechanisms regulating this versatile action. I will thus investigate the opposing effects of OT on social behavior by characterizing oxytocin receptor (OTR) activation of distinct intracellular signaling pathways. *My integrative approach will span multiple levels of biological organization—molecular to the whole organism—and have two major implications: 1) as the behavioral effects of the same hormones often vary, my results will provide a plausible mechanism by which this occurs; and 2) OTR is conserved across taxa, so my project will contribute to our understanding of hormonal basis of social behavior in many species.*

Background/rationale: Oxytocin (OT) is a neuropeptide that is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus. OT regulates species typical social behavior primarily by activating oxytocin receptors (OTR) in specific brain regions. Activation of OTR in the nucleus accumbens (NAcc), for example, promotes pair bonding behavior in the socially monogamous prairie vole[2]. In contrast, less OTR is expressed and presumably activated in the NAcc of the polygynous montane vole[2]. Intranasal administration of OT in mice has also been shown to increase social behavior in males but decreased the same behavior in females[3]. While OTR activation is thought to modulate these behaviors, the evidence consists of manipulations that may not be specific for OTR (e.g., non-selective OTR antagonists) or indirect measure (e.g., OTR mRNA) that do not directly assess OTR activity.

The actions of OT depend on G-protein signaling. The OTR is a G-protein-coupled receptor, which transduces receptor binding to intracellular messengers. OTR couples to two different G-proteins (i.e., G_q and G_i). *Signaling through G_q increases cellular activity whereas G_i signaling decreases cellular activity, thus providing a potential mechanism for opposing actions*[4]. The G_q pathway is reliant on phospholipase C (PLC) and activation (phosphorylation) of protein kinase C (PKC). Additionally, G_q is associated with the extracellular signal-regulated kinase (ERK) pathway. In contrast, G_i signaling is not dependent on PLC and inhibits cellular activity by reducing cyclic adenosine monophosphate[5]. In rats, inhibition of the ERK pathway has been found to reduce the anxiolytic effects of OT during open field testing[5]. However, no study has directly determined which G-protein pathways are responsible for the effects of OTR on social behavior.

Hypothesis: Prairie voles exhibit long-lasting pair bonds that are mediated by the OT system. Specifically, pair bonding behavior in female prairie voles depends on OTR activation within the NAcc[2]. **Here, I will examine the effects of G-protein activated signaling pathways on pair bonding in female prairie voles.** *I hypothesize that inhibiting the G_q and G_i pathways will allow me to determine which promotes pair bonding behavior and whether they have opposing effects. Based on G_q increasing cellular activity, I predict that inhibition of this pathway will reduce pair ponding behavior and levels of phosphorylated proteins. In addition, I will determine whether the pathways have opposing effects by statistically comparing the G_q and G_i inhibition groups.*

Aim 1: The effects of G-protein signaling inhibition on pair bonding behavior

By simultaneously activating OTR and inhibiting downstream targets of G_q and G_i during initial cohabitation with a male, will allow me to determine the effects of each pathway on pair bonding in females. I will administer direct infusions of a selective OTR agonist (TGOT; Thr(4) Gly(7)-

OT) and pathway inhibitors into the NAcc shell of sexually naïve females: control (only TGOT) and three experimental groups (TGOT+ inhibitor). Following a six-hour cohabitation period, a time know to be sufficient to form a bond, female subjects will perform a partner preference test (PPT).

Experimental Design

Groups (n = 15 each)	Aim 1	Aim 2
TGOT	6-hr cohab.	6-hr cohab.
TGOT+PTX	↓	↓
TGOT+U-73122		
TGOT+PTX+U-73122	PPT	IHC/WB

Note: Treatment will be given 30 minutes prior to cohab.

G-protein pathway inhibitors:
G_i pathway inhibitor: Pertussis toxin (PTX)
G_q pathway inhibitor: U-73122
PTX targets G_i subunits, while U-73122 inhibits PLC activity of the G_q pathway.

Partner preference test: Following cohabitation, I will place subjects into a three chamber apparatus (3-hrs) in which they can spend time with their partner or a stranger of the opposite sex. More time spent with the partner than stranger is indicative of pair bond formation.

Predictions: Since G_q increases cellular activity, I predict that the G_q inhibition group (TGOT+U-73122) will demonstrate significant deficits in partner preference compared to the control group. In addition, I further expect a significant difference between the G_q and G_i inhibition (TGOT+PTX) groups, thus providing evidence for the pathways having opposing effects on the same behavior.

Aim 2: Examining the role of G-protein inhibition on downstream signaling targets

To provide additional evidence for which G-protein promotes pair bonding behavior, I will inhibit each pathway and quantify downstream proteins. Following 6-hour cohabitation, subjects (same manipulations as Aim 1) will be sacrificed, brains flash frozen, tissue prepared for immunohistochemistry (IHC) and western blot (WB) analysis, and then downstream targets measured. If my prediction of G_q was supported, I would expect decreased levels of activated (phosphor-proteins) proteins such as pERK immunoreactivity (IR) and pPKC (assess via immunoblotting) in the NAcc of the G_q inhibition group (TGOT+U-73122).

Future directions: This project, for the first time, will examine the role of G-protein signaling pathways on social behavior and will serve as a foundation from which to build my dissertation. Building on these results, I will investigate activation of these signaling pathways on male pair bonding, in response to varying social contexts, and on gene expression. Alternatively, since transactivation of the epidermal growth hormone receptor has been shown to mediate OTR signaling *in vitro*[5], I will explore this possibility if my predictions were not supported.

Intellectual Merit/ Broader Impacts: My graduate advisor, Dr. Karen Bales, is an expert in oxytocin and pair bonding in prairie voles. Dr. Brian Trainor, who has expertise in quantification of phosphor-proteins and stereotaxic microinjections, will provide further mentorship and serve on my dissertation committee. We thus have excellent intellectual and physical resources to execute this proposal. As a member of an underrepresented group, I will actively recruit underrepresented students for these experiments. By working on this project, they will gain the experience and tools necessary to become successful graduate school applicants. As such, this project will contribute to increasing diversity in academia. My assistants and I will present the results at local high schools in underserved communities, academic conferences, and submit manuscripts to journals. All research plans, raw data, and R code for statistical analyses will be publicly available at my Open Science Framework account (https://osf.io/y8qx7/).

References: [1]Adkins-Regan, E. (2005). *Hormones and animal social behavior.*; [2]Ross, H.E., et al. (2009). *Journal of Neuroscience,* 29(5), 1312-1318.; [3]Steinman, M. Q., et al. (2015). *Biological Psychiatry*, 80(5), 406-414.; [4]Gimple, G. & Fahrenholz, F. (2001). *Physiological Reviews*, 80(2), 629-683.; [5]van den Burg, E. H., & Neumann, I. D. (2011). *Journal of Molecular Neuroscience*, *43*(2), 200-208.