The Role of Dopaminergic and Oxytocinergic Systems in Social Affiliation and Bonding Behaviors of Convict Cichlids

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Undergraduate Thesis

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Introduction

What drives social bonding and affiliative behaviors in animals? Understanding these mechanisms provides insight into how relationships form, how support systems function, and how empathy develops across species, including their roles in mediating consolation behaviors (Burkett et al., 2016). Social bonding refers to forming close emotional connections between individuals and serves as a key mechanism for consolation (Carter & Porges, 2016). Emotionally bonded individuals are more likely to recognize distress in others and respond with comforting behaviors (Insel, 2010).

Affiliative behaviors are prosocial actions aimed at establishing and maintaining social bonds. These behaviors can include physical touch, proximity, and grooming. Such behaviors are regulated by neurobiological systems, particularly the neuroendocrine system, which links neural activity to hormonal responses (Lema, 2014). Many animals exhibit affiliative behaviors that foster bonding, including comforting distressed individuals—a behavior known as consolation (de Waal, 2008). Exploring these behaviors in non-human species enhances our understanding of the biological processes underlying social connections, potentially informing human social behaviors and mental health (Oliveira et al., 2011).

Convict cichlids (Amatitlania nigrofasciata) are excellent models for studying social bonding and consolation behaviors due to their display of bi-parental care and stable pair bonds. "Bi-parental care" refers to both parents actively participating in the care of offspring (Clutton-Brock, 1991), while "stable pair bonds" involve consistent partnerships maintained between two individuals over an extended period (Black, 1996). Unlike temporary pairings, stable pair bonds imply mutual recognition and continuous cooperation. Due to these social characteristics, convict cichlids offer valuable opportunities to examine neural and hormonal pathways involved in affiliative behaviors (Oldfield & Hofmann, 2011). Studying these processes in cichlids elucidates the fundamental biology of social attachment and emotional support, potentially revealing insights into human social bonding disorders, depression, and anxiety.

When we transitioned to neuroendocrine aspects, it was crucial to understand how hormonal systems regulate these behaviors. The neuroendocrine system connects the brain with hormone-producing glands, allowing neural activity to influence hormonal responses and consequent behaviors like stress response, emotions, and social interactions (Insel, 2010). Oxytocin and dopamine were critical hormones in these processes. Oxytocin, often labeled the "bonding hormone," was associated with social behaviors such as trust, empathy, caregiving, and notably, pair-bonding (Carter, 2014). Oxytocin and its teleost homolog, vasotocin, are known to promote partner bonding and parental cooperation, particularly during the early stages of pair formation. Research across vertebrates has shown that these nonapeptides facilitate social bonding, and their disruption via receptor antagonists typically dampens affiliative tendencies (Oldfield & Hofmann, 2011; O'Connell et al., 2012). During close social interactions, oxytocin reinforced emotional bonds between individuals (Insel, 2010). Dopamine, a neurotransmitter that also functions hormonally, was integral to the brain's reward pathways and motivates social behaviors through pleasure and reinforcement, thereby encouraging the continuation and strengthening of pair bonds (Berridge & Kringelbach, 2015). Thus, these chemicals mediated affiliative behaviors by facilitating emotional connectivity and responsiveness within pairs (Gunaydin et al., 2014).

Additional hormones such as serotonin and GABA also influence bonding and emotional responses in humans. Serotonin regulates mood, emotion, and aggression (Young, 2007), while

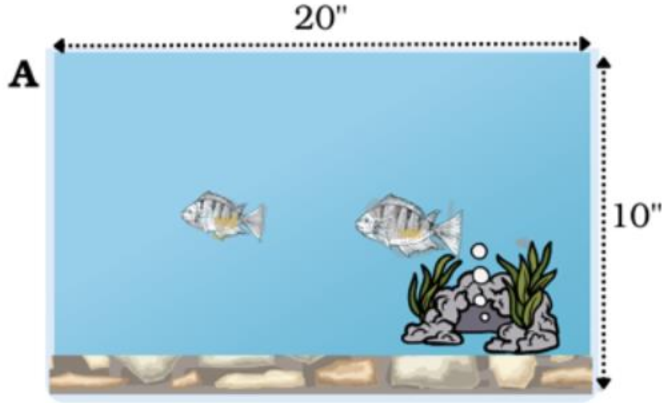
GABA (gamma-aminobutyric acid) modulates neuronal excitability and contributes to reducing anxiety (Möhler, 2006). However, their roles are not as directly linked to affiliative behaviors and consolation as dopamine and oxytocin.

Prior research has highlighted oxytocin's role in consolation behavior. However, emerging evidence indicates dopamine, especially via the dopamine D2 receptor pathway, might also be crucial (Burkett et al., 2016). The dopamine pathway involves the release of dopamine from neurons in areas such as the ventral tegmental area (VTA), projecting to structures like the prefrontal cortex, where dopamine binds to certain receptors, particularly the D2 subtype, modulating reward, and motivational aspects of social interactions (Schultz, 2013). To thoroughly investigate these mechanisms, convict cichlids were administered oxytocin and dopamine D2 antagonists to observe their roles in consolation behavior, as their neuroendocrine systems mirror those found in humans (Lema, 2014).

Based on this framework, we hypothesize dopamine D2 receptor activity significantly mediated affiliative behaviors related to consolation in convict cichlids, predicting pharmacological blockade of these receptors will notably reduce consolatory responses. Conversely, oxytocin receptor blockade is anticipated to produce smaller or negligible effects. Clarifying the contributions of dopamine and oxytocin signaling in this vertebrate model may enhance our understanding of the neuroendocrine basis of social behavior across species.

Methods

To conduct this study, we established dyads of opposite-sex convict cichlids, reflecting natural pair-bonding behavior observed in the wild. Mature adult cichlids were maintained in a controlled laboratory environment with regulated water conditions and lighting designed to closely mimic their natural habitat. Dyads were initially housed in 10-gallon tanks equipped with sedimentary pots as shelters for an acclimatization period of one week. During this period, pair bonding was confirmed by closely observing interactions, and dyads exhibiting excessive aggression were separated immediately to prevent injuries.



Acclimatization procedure

To ensure that observed behaviors were genuine and not influenced by unfamiliarity with the environment, we implemented a structured acclimatization protocol. Each dyad spent a minimum of one week in their initial tanks before the experiments. We closely monitored interactions during this acclimatization phase to confirm pair bonding and to reduce stress-related behavioral changes. This structured process successfully created a stable environment within each tank, enhancing the reliability and consistency of observed behaviors.

Experimental setup

After the initial acclimatization, bonded dyads were transferred to larger 20-gallon experimental tanks for at least one additional week before behavioral testing commenced. This allowed further environmental adjustment, significantly reducing potential stress-induced variations in behavior. Any dyads continuing to exhibit aggression or experiencing injury during this period were removed from the study and transferred to recovery tanks for further observation and care.

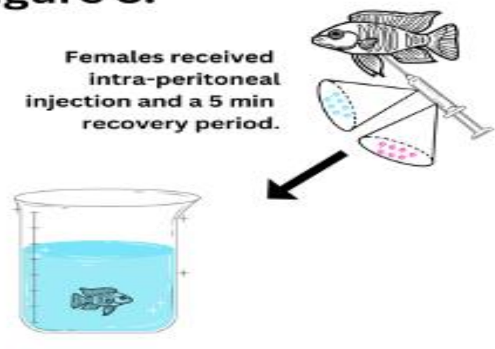
Figure 2.

Drug Treatment Protocol

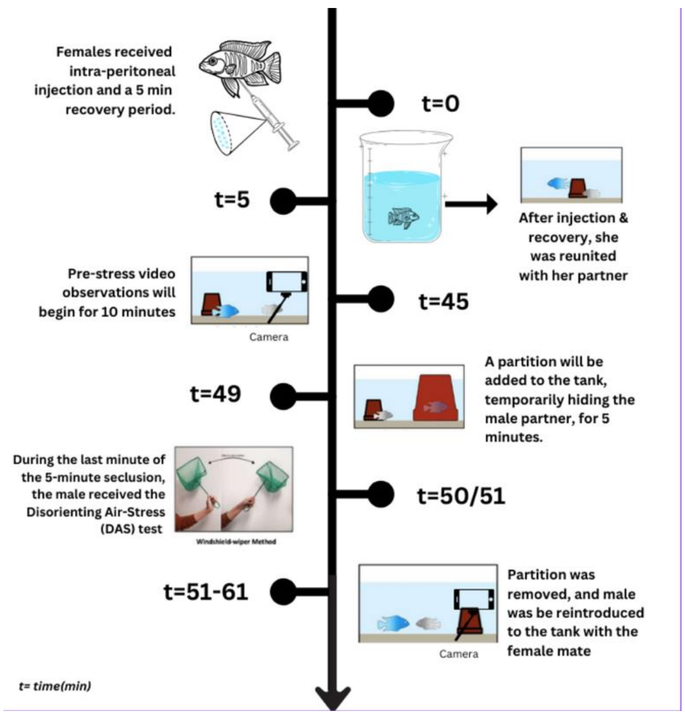
Following established protocols by Oldfield and Hofmann, female convict cichlids were administered intraperitoneal injections consisting of phosphate-buffered saline (PBS) as a control, an oxytocin antagonist (Manning's Compound), or a dopamine D2 antagonist. Each compound was administered at a dosage of 3.2 μg/g fish body weight, prepared in a PBS solution at a concentration of 0.35 μg/μl. These dosages were selected based on previous empirical studies demonstrating consistent physiological effects without adverse health impacts (Oldfield & Hofmann, 2011; Burkett et al., 2016). Each female from the respected dyad received one treatment (5 dyads for both the Control and the Oxytocin antagonist; 8 dyads for the D2 antagonist).

Following injections, females were observed closely for five minutes to ensure health and responsiveness prior to reintroduction into experimental tanks, ensuring methodological rigor and reliability.

Figure 3.



Step by Step



Female Injection Procedure

The female was gently removed from the tank and taken for injection. The injection was administered using a double-blind procedure using a Nanofil syringe. Following the injection, the female was placed in a large beaker to recover for five minutes (t=5).

Observation Period

After the recovery period, the female was reintroduced into the tank with her mate. Visual observations began and continued for 30 minutes (t=35). Ten minutes later, pre-stress video observations began (t=45).

Stress Induction (Males)

To induce stress, a partition was added to the tank, temporarily hiding the male partner for five minutes (t=50). During the last minute of this seclusion, the male underwent the Disorienting Air-Stress (DAS) test (t=50/51)

DAS Procedure

The DAS procedure was conducted for one minute. Once the male is inside the net, the disorienting air-stress was administered by moving the net from side to side in a vertical or upright plane, mimicking a windshield wiper motion. This motion was maintained at a consistent frequency of 1Hz, paced using a metronome. The motion continued for a total of one minute.

Reintroduction to Female

After the DAS procedure, the partition was removed, and the male was reintroduced into the tank with his female mate. Along with the partition being removed. (t=51).

Video Recording.

Behavioral video observations of the female toward the reintroduced male continued for 10 minutes after stress (t=61), concluding the experimental session. The videos will be reviewed later to score the behaviors of the cichlids.

Scoring the Behaviors

Behaviors observed in the video were scored using a scale focused on consolation and displacement behaviors. These include "approached/was approached," "affiliation/received affiliation," and "displacement."

Behavioral Observation and Scoring

Behaviors observed in videos were scored using clear operational definitions to ensure consistent and reliable assessment. Specific behaviors included:

Approach/Was Approached: Defined as one fish moved within less than two body lengths proximity to its partner.

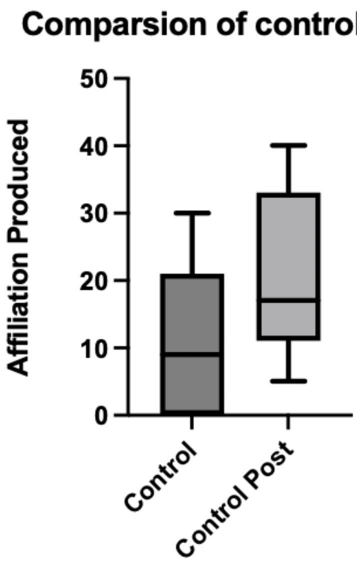
Affiliation/Received Affiliation: Defined by sustained proximity with reciprocal behaviors such as backward swimming or tail wagging.

Displacement: Identified by active avoidance or withdrawal from affiliative interactions.

After conducting the experiments and collecting the data from the video observations. A comparison across pre and post stressor was drawn from the various drug treatments.

Results

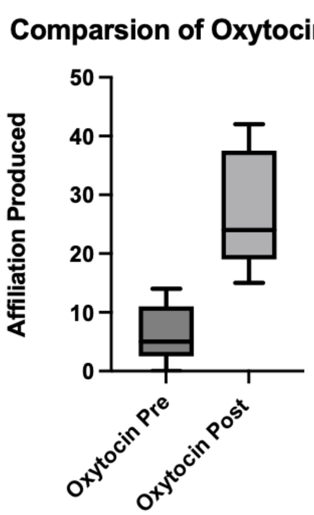
Fig1.



	Control	Control Post
1	0	5
2	0	26
3	9	17
4	30	40
5	12	17

**Comparison of affiliation produced by female convict cichlids when treated with phosphate-buffered saline solution. A total of 5 dyads*

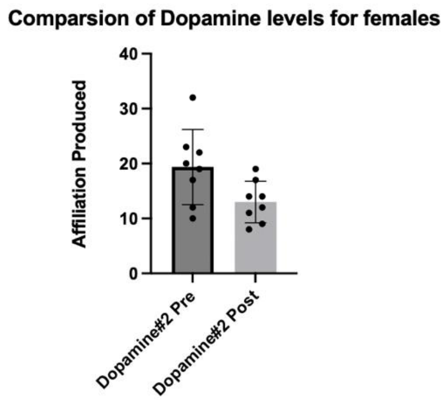
Fig2.



	Oxytocin Pre	Oxytocin Post
1	5	42
2	5	24
3	0	33
4	14	23
5	8	15

**Comparison of affiliation produced by female convict cichlids when treated with oxytocin antagonist. A total of 5 dyads*

Fig3.



	Dopamine#2 Pre	Dopamine#2 Post
1	32	19
2	10	9
3	22	14
4	19	12
5	23	17
6	17	11
7	12	8
8	20	14

* *Comparison of affiliation produced by female convict cichlids when treated with Dopamine2 antagonist. A total of 8 dyads*

Expectations

Previous literature on social bonding in monogamous fish species such as convict cichlids (Amatitlania nigrofasciata) has consistently demonstrated the critical role of oxytocin-family neuropeptides in modulating affiliative behavior. In convict cichlids, administration of oxytocin/vasotocin receptor antagonists has previously been shown to reduce behaviors like synchronized swimming, gentle contact, and frontal displays as key indicators of pair bonding (Oldfield & Hofmann, 2011).

In contrast to this established body of work, our study revealed an unexpected outcome following oxytocin antagonist administration. Five female convict cichlids were injected with an oxytocin receptor antagonist known as Manning's compound. Their affiliative behaviors were measured before and after stressors were placed on the male. The stressor placed on the male has been shown to result in an increase in affiliation produced by the female once the male is stressed in hopes of helping their partner (Reddon et al., 2020).

Based on the literature, we predicted that antagonist treatment would suppress social affiliation. However, our results demonstrated a significant increase in affiliative behaviors from pre- to post-injection. These included elevated rates of physical proximity, partner-directed approaches, and contact gesture behaviors typically associated with social bonding (Fig 2). Control females, who received no antagonist, showed only a slight increase in affiliation during the same period (Fig1). The comparison of box plots clearly illustrates this divergence: post-injection affiliation in the oxytocin antagonist group rose sharply compared to the control group. This outcome rejects the widely accepted view that oxytocin antagonism impairs sociality. Instead, it suggests a more complex or perhaps compensatory neuroendocrine response in female convict cichlids.

Unexpectedly, novel research suggests that dopamine D2 antagonists may mediate the formation and maintenance of prosocial bonds in fish. Previous literature identified that haloperidol, which is a widely prescribed antipsychotic is known to act as a dopamine (D2) antagonist (Oliveri, 2019). 8 female convict cichlids were injected with haloperidol, which was observed for social affiliative behaviors directed towards their male partners. A significant decrease in social affiliative behaviors was identified in female convict cichlids treated with haloperidol (Fig 3), compared to female convict cichlids treated with either Manning's compound (Fig2) or the placebo (Fig 1).

These reductions in affiliative behavior included decreased physical proximity, fewer partner-directed approaches, and reduced contact gestures. These findings align with recent literature, reinforcing the hypothesis that the dopamine D2 pathway could be involved in mediating consolation behaviors (Bshary et al., 2016).

Conclusion

Although this outcome is novel, it complements an expanding body of literature that underlies the nuance of the neuroendocrine system. It is important to recognize that multiple neural pathways likely interact to facilitate the formation of social bonds, and ongoing research into these mechanisms will further enhance our understanding of social bonding processes. Interestingly there is a correlation when female convict cichlids are treated with a D2 (Dopamine 2) antagonist, a drop in affiliative behaviors produced. Notably, the comparison of female convict cichlids treated with Manning's compound (Oxytocin antagonist), or Placebo/Control (phosphate-buffered saline) displayed a similarity of an increase in affiliative behaviors from pre to post stress. The study suggests there is a correlation between a decline in affiliative behaviors if treated with a D2 Antagonist.

The findings are unique and provide a deeper understanding of the neuroendocrine system, which may involve multiple pathways in the formation and maintenance of pair bonds. This research offers insights into how our neuroendocrine system may function in maintaining and forming bonds in humans. By exploring other neuroendocrine pathways, such as serotonin and GABA, and examining the effects of blocking their functions, we can develop a broader understanding of how these systems work. Although it is not ethically feasible to explore the effects of disrupting these pathways in humans, studies using convict cichlids offer valuable insights into what may occur in human bonding.

Despite the compelling findings, one of the study's primary limitations was the challenge of creating pair bonds between dyads. In many instances, dyads failed to form a pair bond, leading to time loss and setbacks. Without the need to account for disbanding and creating new dyads to attempt pair bonding, the study's sample size could have been much larger. Additionally, the surplus workload of disbanding and creating bonds consumed valuable time, further limiting the study's efficiency. During the acclimatization period, if a dyad showed minimal or no signs of bonding, it was disbanded to determine whether maintaining the pair was appropriate. If a dyad displayed aggressive tendencies during the acclimatization period, the removal process was initiated, resulting in the return of both convict cichlids to their respective community tanks. This process allowed for the introduction of a new dyad to replace the previously unsuccessful pair. Developing a more effective technique for efficiently pairing dyads would improve future studies, increasing the sample size available for experimentation. Although the study does not definitively answer the question of what drives social bonding, it suggests that the neuroendocrine system is far more complex than previously understood. The research contributes to our understanding of the fundamental biological mechanisms underlying social bonding and affiliative behaviors. It delves into the complexity of the neuroendocrine system, offering a fresh perspective on the factors at work when bonds are formed and maintained. The findings indicate that the dopaminergic system may be more impactful in this process than previously believed. Insights from this study, using an animal model such as monogamous vertebrates (i.e., convict cichlids), offer a valuable perspective on how human social behaviors and disorders may function. The research also provides a deeper understanding of how certain neurochemical systems play a larger role in maintaining and forming social bonds.

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