

THE EFFECT OF SOCIAL EXPERIENCE ON THE SYNERGISTIC NEUROMODULATION OF MOTOR CIRCUITS

By
Katie N. Clements
July 2022

Director of Dissertation: Fadi A. Issa
Major Department: Biology

ABSTRACT

Forming social groups is a critical adaptive strategy for the survival of many animal species, where conflict between members can result in dominance relationships. Dominance relationships are often formed through aggressive interactions and influence the cognitive and physiological properties of individuals in a status-dependent manner. The aim of this dissertation is to discern how zebrafish utilize aggression and visual cues to reinforce a stable dominance relationship, and what effect stable dominance has on the neurobiological processes underlying motor behavior, with a focus on dopamine signaling. Dopamine has been implicated in aggression, social regulation, and modulation of motor circuits, making it a central point of study regarding the relationship between social dominance and motor behavior. Using behavioral, pharmacological, and genetic approaches, I have found that (1) in addition to physical aggression, zebrafish alter their stripe coloration intensity to reinforce dominance, (2) stable dominance alters dopamine signaling through decreased expression of the dopamine transporter and the dopamine receptor type-1b, (3) changes in dopamine signaling manifest in altered escape and swimming locomotor behavior, and (4) dopamine acts synergistically with other neurotransmitters to regulate status-dependent motor circuit activation. Results from this dissertation have provided evidence for how stable dominance impacts modulation of motor circuits, revealing how changes in relative excitability of multiple neuromodulatory inputs provide a mechanism for the nervous system to

adapt to changes in social conditions and allow animals to select a socially appropriate behavioral response.

THE EFFECT OF SOCIAL EXPERIENCE ON THE SYNERGISTIC NEUROMODULATION
OF MOTOR CIRCUITS

A Dissertation

Presented To the Faculty of the Interdisciplinary Program in Biology, Biomedicine, and

Chemistry, Harriot College of Arts and Sciences

East Carolina University

Submitted in Partial Fulfillment of the Requirements for the Degree

Doctor of Philosophy in the Interdisciplinary Program in Biology, Biomedicine, and Chemistry

by

Katie N. Clements

July, 2022

© Katie N. Clements, 2022

The Effect of Social Experience on Synergistic Neuromodulation of Motor Circuits
By
Katie N. Clements

APPROVED BY:

Director of Dissertation _____
Fadi A. Issa, PhD

Committee Member _____
Eric Anderson, PhD

Committee Member _____
Timothy Erickson, PhD

Committee Member _____
Kurt Marsden, PhD

Committee Member _____
Edmund Stellwag, PhD

Chair of the Department of Biology _____
David Chalcraft, PhD

Dean of the Graduate School _____
Kathleen T. Cox, PhD

ACKNOWLEDGEMENTS

I would like to thank: My parents, Carolyn and Buddy Clements, for always being my biggest supporters. I could not be here without the sacrifices you both have made to ensure I had every opportunity to get to this point, and I'm forever grateful. My partner, Christopher Knuckles, for continuously inspiring me, challenging me, and making sure I feel supported and loved every day. My fur-baby, Miles, for simply existing. You make all my days better. My friends, Alexandra Venuto, Skyler Carrell, Danielle Finger, Christopher Anderson, and Albert Tucci, for being my graduate school family and making the tough days more manageable. It's been a crazy ride and I'm so thankful to have made friends for life.

I specifically want to thank Alexandra Venuto for being the person who I could go to for help with everything for my dissertation. Whether it be with knowledge about molecular tools for my research, giving me feedback on all my writing, or sitting with me for hours to work through code in RStudio for statistics and graphing, you are one of the biggest reasons this project is complete, and I can't put into words how much you mean to me.

In addition, I would like to thank my advisor and committee members: Drs. Fadi Issa, Eric Anderson, Tim Erickson, Kurt Marsden, and Edmund Stellwag, for being a large part of my intellectual journey at ECU. I've grown tremendously since becoming a scientist in 2015 and each of you has played a large role in that growth.

Lastly, I would like to thank the Biology Department, and all those who work within the front office. Each one of you has worked tirelessly to ensure the students are supported, and that work does not go unnoticed. Thank you.

TABLE OF CONTENTS

LIST OF FIGURES	vi
LIST OF ABBREVIATIONS.....	viii
CHAPTER 1: FOUNDATION OF KNOWLEDGE	1
Introduction	1
Social Dominance Formation – The Winner Effect	1
Physiological Correlates to Social Rank.....	2
The Social Brain	4
DA Signaling and Location	6
Reciprocal Relationship of Social Dominance and DA Signaling	10
Zebrafish Social and Motor Behavior.....	12
Directions for Dissertation.....	17
CHAPTER 2: SOCIAL STATUS RELATED DIFFERENCES IN MOTOR ACTIVITY BETWEEN WILD TYPE AND MUTANT ZEBRAFISH	20
Introduction	20
Materials and Methods.....	24
Results.....	28
Discussion	37
CHAPTER 3: THE ROLE OF THE <i>slc6a3</i> AND <i>drd1b</i> IN THE ESTABLISHMENT OF SOCIAL DOMINANCE RELATIONSHIPS AND LOCOMOTOR BEHAVIORS.....	42
Introduction	42
Materials and Methods.....	43
Results	53
Discussion	65
CHAPTER 4: SOCIALLY MEDIATED SHIFT IN NEURAL CIRCUIT ACTIVATION REGULATED BY SYNERGISTIC MODULATORY SIGNALING.....	68
Introduction	68
Methods	70
Results	73
Discussion	84
CHAPTER 5: REFLECTIONS AND FUTURE DIRECTIONS	87
Zebrafish use stripe intensity to enforce stable social dominance relationships	87
Decreased <i>slc6a3</i> and <i>drd1b</i> expression do not influence stable social dominance..	89
Status-dependent changes in neuromodulatory input onto motor circuits.....	90
Conclusions and future experiments.....	96
REFERENCES	102
APPENDIX A: ANIMAL USE PROTOCOL APPROVAL LETTER	125
APPENDIX B: ANIMAL CARE AND USE COMMITTEE APPROVAL LETTER	126
APPENDIX C: SUPPLEMENTAL FIGURE – LDOPA	127
APPENDIX D: SUPPLEMENTAL FIGURE – ANTAGONIST SWIMMING.....	128
APPENDIX E: SUPPLEMENTAL FIGURE – DAT ANTAGONIST.....	129
APPENDIX F: SUPPLEMENTAL FIGURE – M-CELL AND DRD1 COEXPRESSION .	130
APPENDIX G: SUPPLEMENTAL FIGURE – GLUTAMATE AND DOPAMINE COEXPRESSION	131

LIST OF FIGURES

Figure 1: Schematic illustration of cross-species brain comparison

Figure 2: The dopamine signaling pathway

Figure 3: The Mauthner-mediated escape circuit

Figure 4: Body color patterns of AB and TLN zebrafish and experimental setup

Figure 5. Visual social cues are important for stability of social relationships

Figure 6. Visual social cues affect swimming and territorial displays

Figure 7. Improper visual social cues eliminate social differences in probability of C-start escape response

Figure 8. Social status-dependent regulation of body color intensity in zebrafish

Figure 9. Experimental setup for social dominance and escape testing

Figure 10. *slc6a3* and *drd1b* gene expression are decreased in subordinates compared to dominants

Figure 11. Establishment of *slc6a3* mutant

Figure 12. Social dominance formation comparison between *slc6a3*^(-/-), *drd1b*^(-/-), and WT siblings

Figure 13. Comparison of place preference in tanks between *slc6a3*^(-/-), *drd1b*^(-/-), and WT siblings.

Figure 14. Escape and swimming behavior for *slc6a3*^(-/-) and *slc6a3*^(+/+) siblings

Figure 15. Escape and swimming behavior for *drd1b*^(-/-), *drd1b*^(+/+) siblings, and *drd1b*^(+/-) siblings

Figure 16. Dopaminergic modulation of the escape and swim circuits is socially regulated

Figure 17. GABAergic modulation of the escape and swim circuits is socially regulated

Figure 18. Glycinergic modulation of the escape and swim circuits is socially regulated

Figure 19. Hindbrain glycinergic neurons show status dependent Drd1 co-expression

Figure 20. Socially induced morphological plasticity of hypothalamic DA neurons

Figure 21. Working model for status-dependent signaling changes from DAergic A11 region to the M-cell motor circuit

Figure 22. The effect of L-DOPA on startle escape

Figure 23. Effects of Drd1, GABA_A, and glycine receptor antagonists on swimming

Figure 24. Dat antagonist decreases escape in WT group-housed fish

Figure 25. No expression of Drd1 on M-cells.

Figure 26. No evidence for Glutamate-Dopamine co-expression in PTar

LIST OF ABBREVIATIONS

5-HT	Serotonin
AC	Adenylyl Cyclase
<i>actb2</i>	<i>actin, beta 2</i>
BamHI	Bacillus amyloliquefaciens HI
BNST	Bed Nucleus of the Stria Terminalis
CaMKII	Calcium/Calmodulin Dependent Protein Kinase II
cAMP	Cyclic AMP
cDNA	complementary DNA
COMT	Catechol – O – methyltransferase
CRISPR	Clustered Randomly Interspersed Short Palindromic Repeats
Ct	Cycle threshold
DA	Dopamine
DARRP-32	Dopamine- and cAMP-regulated Phosphoprotein, 32 kDa
Dat/DAT	Dopamine Transporter (zebrafish protein)/ (mouse protein)
DNA	Deoxyribonucleic Acid
DRD1	Dopamine Type-1 Receptor (mouse protein)
Drd1b	Dopamine Type-1 Receptor b (zebrafish protein)
Fps	Frames-Per-Second
gDNA	guide Deoxyribonucleic Acid
GPCR	G-Protein Coupled Receptor
LS	Lateral Septum
M-cell	Mauthner Neuron
MAO-A	Monoamine oxidase – A
MAO-B	Monoamine oxidase – B
MeA	Medial Nucleus of the Amygdala
<i>mitfa</i>	<i>microphthalmia-associated transcription factor a</i>
MLR	Mesencephalic Locomotor Region
mPFC	medial Prefrontal Cortex
mRNA	messenger Ribonucleic Acid
PBS	Phosphate Buffered Solution
PCR	Polymerase Chain Reaction
PKA	Protein Kinase A
PVN	Paraventricular Nucleus
qPCR	quantitative polymerase chain reaction
RNA	Ribonucleic Acid
ROI	Region of Interest
sgRNA	single guide RNA
<i>slc6a3</i>	<i>solute carrier family 6 member 3</i>
SNC	Substantia Nigra Pars Compacta
TL	Tüpfel Longfin
TLN	Tüpfel Longfin Nacre
VIIIth Nerve	Vestibulocochlear Sensory Nerve
VMAT2	Vesicular Monoamine Transporter 2
VMPFC	Ventromedial Prefrontal Cortex

VSSC	voltage sensitive sodium channel
VTA	Ventral Tegmental Area
WT	Wild Type

CHAPTER 1: FOUNDATION OF KNOWLEDGE

Introduction

Forming social groups is a critical adaptive strategy for the survival of many animal species. Sociability allows for increased group fitness through aggregation against predation and for allocation of resources, such as access to food, shelter, and mates (Wilson, 1975). Although forming social groups drives success of the overall community, living in groups requires a balance of cooperation and conflict among members. How are social dominance relationships formed through conflict? What are the neurophysiological consequences of social dominance relationships? How might dominance alter the neural basis of behavior in a status-dependent manner? These fundamental questions drive the research for my dissertation work.

Social dominance as a mechanism for stable communities is evolutionarily confluent across species. Social dominance has been described in invertebrates (Moore et al., 1988; see Lord et al., 2021), vertebrates, and spans developmental periods (Bernstein, 1976; Benton et al., 1980; Pagel & Dawkins, 1997; Sapolsky, 2006; Horová et al., 2015; Drea et al., 2021; Knoch et al., 2022). A recent study has shown that children can process social dominance and infer social rank from observing social interactions as early as 15-months of age, supporting that the ability to infer social rank emerges early in development (Mascaro & Csibra, 2012). The early presentation and broad implementation of social dominance across the evolutionary spectrum motivates the examination of how it is formed, the physiological correlates to social rank, and the underlying neurological components of the social brain.

Social Dominance Formation – The Winner Effect

Social dominance formation can be attributed to the “Winner Effect”, where winning an aggressive encounter increases the probability of future victories (Fuxjager & Marler, 2010).

Irrespective of animal species, basic behavioral parameters characterize agonistic interactions. Aggressive agonistic behaviors include chasing and attacking, while submissive behaviors include freezing and retreating (Balph, 1979; Francis, 1984; Kikkawa et al., 1986; Kroes et al., 2006). During aggressive encounters, the more aggressive individual normally becomes dominant among its conspecifics (Blanchard et al., 1988). For instance, among male rats that were housed in groups where one rat displayed either high, moderate, or low levels of aggression, the rat with the highest aggression usually became the dominant animal (Blanchard et al., 1988).

In addition to the use of tactile interactions to convey aggression, many species rely on other sensory modalities to emphasize dominance status after winning a conflict. This can include the display of specific body color patterns (Greenberg & Crews, 1990; Senar et al., 2006; Beltrao et al., 2021; John et al., 2021), size (Chen & Fernald, 2011), and scent or pheromones (Krames et al., 1969; Beauchamp, 1974; Banner & Shamas-Tsoory, 2018). Conversely, the losing animal will be relegated to a subordinate role. As an animal becomes subordinate, it decreases aggressive displays in favor of submissive behaviors (Herberholz et al., 2001; Issa & Edwards, 2006) and may alter body coloration to reduce aggression from the dominant animal (Hoglund et al., 2000; Miyai et al., 2011). The totality of physical aggression, and visual and olfactory displays help to reinforce social dominance and suggests status-dependent physiological plasticity reflecting the animals' social ranks.

Physiological Correlates to Social Rank

Social dominants

There exists a variety of physiological correlates to dominance status including higher testosterone levels (Eberhart et al., 1980; Wang et al., 2012; Maguire et al., 2021) and elevated

glucocorticoid production and excretion (Muller & Wrangham, 2003); suggesting an increase in both reproductive and anti-inflammatory effects in the body. However, other reports have suggested that elevated glucocorticoid levels are short-term (Øverli et al., 1999), and negative effects of circulating stress hormones during agonistic interactions subside in dominant individuals. Social dominance can also induce reproductive success by changes in the appearance (Greenberg et al., 2022) and function (Bayram et al., 2022) of reproductive organs. In addition to hormonal and reproductive changes, social dominance is associated with altered neurotransmitter activity (Narvaes et al., 2014; Watanabe & Yamamoto, 2015), where signaling from serotonergic, dopaminergic, and GABAergic signaling systems are altered in specific brain nuclei associated with social regulation. For example, the habenula regulates many brain functions including its involvement in stress response, aggression, reward processing and depression (Andres et al., 1999; Matsumoto & Hikosaka, 2007). Chou and colleagues (2016) demonstrated that silencing the medial dorsal habenula increases the likelihood of winning, highlighting the physiological correlate of the winner/loser effect. Taken together, social dominance leads to a variety of altered physiological states that can be beneficial to the individual and help to reinforce social dominance.

Social subordinates

Becoming a subordinate also alters the physiology of an individual. Subordinate individuals are at risk of adrenal and spleen enlargement as well as thymus reduction (Blanchard et al., 1993); suggesting decreased endocrine health. Subordinates show altered abilities to regulate hormone signaling due to a hypoactive limbic-hypothalamic-pituitary-adrenal axis (Michopoulos et al., 2012). Social subordination can lead to hyperphagia, or the increased need for constant food consumption, and can lead to eating disorders (Razzoli et al., 2015).

Additionally, subordinates show altered monoamine activity. Studies report subordinates exhibit altered dopamine (DA) (Shively, 1998; Nader et al., 2012) and serotonergic (5-HT) activity in multiple brain regions (Dhingra et al., 1996; Winberg & Lepage, 1998; Xu et al., 2020). For example, Cho et al (2016) also showed that silencing of the lateral dorsal habenula in zebrafish reduces the likelihood of winning agonistic interactions. In addition, increased neural activity in both the hypothalamus and telencephalon were a function of increased submissive behaviors (Kasper et al., 2018). Although there is a variety of different physiological correlates to social rank, we are interested in understanding the neural basis of social dominance and how social rank can influence neural circuit activation.

The Social Brain

Brain regions associated with establishing social hierarchies

Social dominance relationships require interactions between multiple brain regions known as the social behavior network (O'Connell & Hoffman, 2011) (Figure 1). One region, the amygdala, has been studied extensively in the establishment of social dominance in humans (Zink, 2008), monkeys (Rosvold et al., 1954; Emery, 2001), birds (Ikebuchi et al., 2009), and rodents (Bunnell, 1966; Sullivan et al., 2003). Teleost fish have an amygdaloid complex, which is a series of regions that are homologous to the mammalian amygdala (Ganz et al., 2014; Porter & Mueller, 2020), that is active during social encounters (Ganz et al., 2014; Rodriguez-Santiago, et al., 2022) A second region, the hippocampus, receives input from the amygdala and allows for memory consolidation during and after dominance formation (Eichenbaum et al., 1992; Richter-Levin & Akirav, 2000; Montagrin et al., 2017; AlSubaie et al., 2021). Other regions involved in the social network are the striatum and medial prefrontal cortex (mPFC). These regions play an important role in social behavior through coding social action and reward (Baez & Schultz,

2013; Bicks et al., 2015; Qu et al., 2017; Ligneul & Dreher, 2017) and have a reciprocal signaling relationship with the amygdala, allowing for continuous processing of salient social information (Phelps et al., 2004; Freese & Amaral, 2009). Additionally, the lateral septum (LS), plays a key role in regulating social behavior by acting as a relay center for incoming social information from the environment to executive brain regions for processing in humans (Sheehan et al., 2004), birds (Goodson et al., 1999; Corrales Parada et al., 2021), rodents (Bielsky et al., 2005; Menon et al., 2021), and teleosts (Goodson & Kingsbury, 2013; Stednitz et al., 2018). Together, the functional and morphological organization of brain nuclei implicated in social behavior are conserved across vertebrate species and form a plastic “social network” that integrates social information to allow animals to adapt their decisions as a function of their social rank.

Although social relationships are formed and maintained through activation of specific neurobiological pathways, properties within these neurobiological pathways can also be altered by status-dependent neuromodulation. One important neurochemical signaling cascade that is heavily involved in the reciprocal relationship of sociability and neural connectivity is DA (Shively, 1998; Martinez et al., 2010; Nader et al., 2012; Gunaydin et al., 2014; Yamaguchi et al., 2017; Sukhodolskaya et al., 2018; Xing et al., 2022). The following section describes DA signaling pathways and what is known about the role of DA’s modulation of motor behavior.

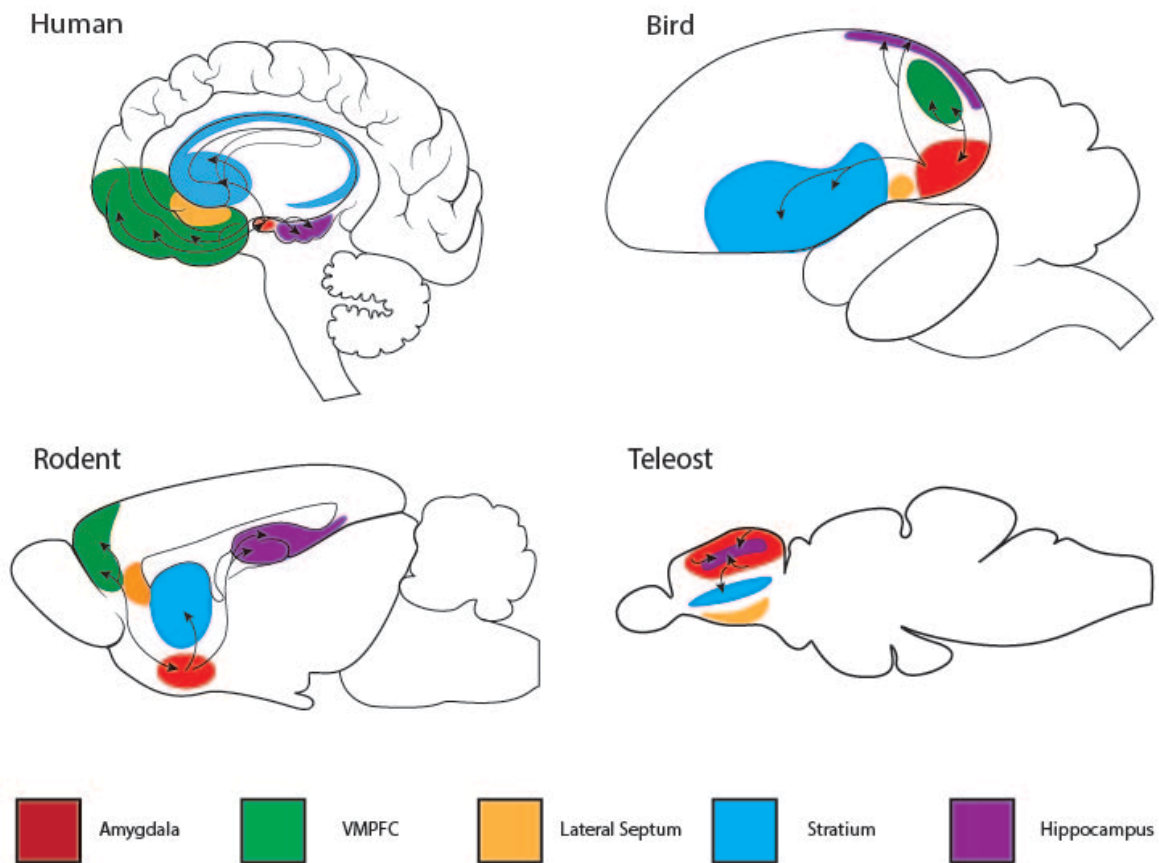


Figure 1. Cross-species comparison of the social brain, emphasizing connected brain regions associated with the establishment and maintenance of social dominance.

DA Signaling and Locomotion

DA signaling

DA is one of the most versatile neurochemicals in the brain. Since its discovery within the nervous system in 1957 (Carlsson et al., 1957, Carlsson, 1993), DA transmission has been implicated in reward and addiction (Wise, 2008; Owesson et al., 2009), learning and memory (Jay TM, 2003), metabolism and feeding behaviors (Feldmen et al., 1971; Quickel et al., 1971; Kholy et al., 2021), obesity (see. Rubi & Maechler, 2010), hormonal regulation (Saltiel &

Thomopoulos, 1981; Sowers et al., 1984;), and regulation of the female reproductive system (Hartwig et al., 1980).

DA is derived from the precursor tyrosine, which is synthesized into L-DOPA by tyrosine hydroxylase, and then into DA by DOPA-decarboxylase (Elsworth & Roth, 1997). DA can either be packaged into synaptic vesicles via vesicular monoamine transporter 2 (VMAT2) or be degraded through monoamine oxidase-A (MAO-A) in the presynaptic terminal (Elsworth & Roth, 1997). Like other neurotransmitters, sufficient excitation of the presynaptic DA neuron can induce DA-packed vesicle fusion at the pre-synaptic terminal membrane, facilitating its release into the synaptic cleft and binding to DA receptors (Sampio et al., 2015; Liu & Kaeser, 2019). DA neurons can exhibit distinct signaling properties. Many dopamine neurons possess en-passant vesicle boutons, known as varicosities, that allow for near- or far-volume transmission of DA (Rice & Cragg, 2008). Additionally, DAergic neurons show non-vesicular release mechanisms, such as reversal of the dopamine transporter (DAT) (Falkenburger et al., 2001).

DA receptors belong to the G-coupled receptor (GPCR) family and consist of seven transmembrane segments (Trumpf-Kallmeyer et al., 1992). There are two types of DA receptors, excitatory D1-like, consisting of D1 and D5, and inhibitory D2-like, with D2, D3 and D4 receptor (Elsworth & Roth, 1997). D1-like (D1, D5) receptors are classified as excitatory in that they increase the likelihood of depolarization of the post-synaptic cell (Civelli et al., 1993). D1-like receptors are associated with the heterotrimeric G protein $G_{\alpha s}/G_{\alpha olf}$, where stimulation of these receptors leads to increased enzymatic activity of adenylyl cyclase (AC), increased cyclic AMP (cAMP), which further activates cAMP-dependent protein kinase A (PKA) (Klein et al., 2019). Increased PKA activity can lead to elevation in synaptic plasticity via modulation of voltage gated sodium channels (Gold et al., 1998) and increased dopamine synthesis (Dagda &

Banerjee, 2017). Conversely, D2-like (D2, D3, D4) receptors are inhibitory, thus, decrease activation of target cells (Civelli et al., 1993). D2-like receptors are associated with the $G_{\alpha i}/G_o$ protein, where activation of these receptor subtypes leads to decreased AC, decreased cAMP, and an inactive PKA (Klein et al., 2019). These decreased levels of AC, cAMP, and active PKA result in decreased dopamine- and cAMP-regulated phosphoprotein, 32 kDa (DARRP-32), and further decreased post-synaptic signaling and synthesis (Svenningsson et al., 2004). Long-term decreased PKA activity can lead to degeneration of dendrites and medium spiny neurons, influencing the signaling properties of these neurons and resulting in diseased states (Dagda & Banerjee, 2017). These DA receptor sub-types are located throughout the central and peripheral nervous systems and work to modulate cellular excitability.

The energetic costs associated in the synthesis and release of DA makes its recycling essential to maintain sufficient levels for proper neurotransmission. DA is recycled into the presynaptic terminal via Na^+ -dependent DA transporter (DAT) or is taken up by neighboring

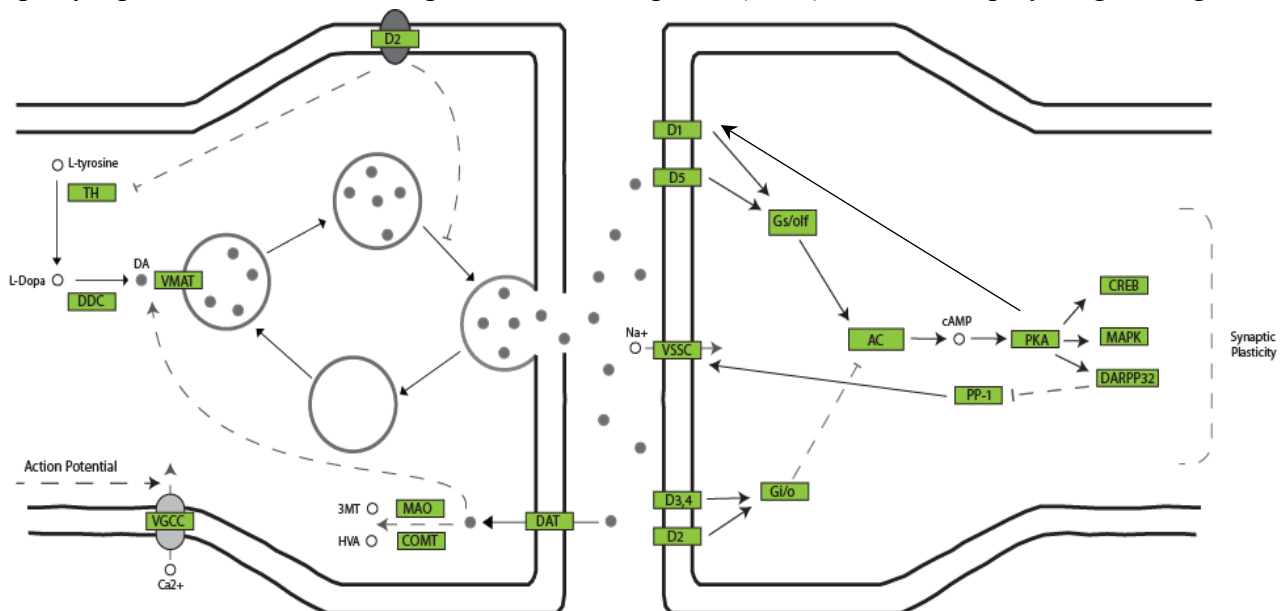


Figure 2. The Dopamine Signaling Pathway. This illustration depicts the synthesis, packaging, release, and downstream signaling cascade of the dopamine pathway. Beginning with synthesis and packaging in the pre-synaptic terminal and resulting in downstream changes in post-synaptic activation based on receptor sub-type.

astrocytes through VMAT2 and is degraded by monoamine oxidase-B (MAO-B) and catechol-O-methyltransferase (COMT) (Elsworth & Roth, 1997; Petrelli et al., 2020). Disruptions in either of these mechanisms leads to physiological and psychological diseased states (Sampio et al., 2015) and makes DA signaling an important research area to examine the social status-dependent disruptions in DA signaling.

Role of DA neuromodulation in physiological processes

DA is important for the development and function of sensory tissues in several organisms including *Drosophila* (Neckameyer et al., 2001), zebrafish (Toro et al., 2015; Haehnel-Taguchi et al., 2018), and humans (Gjedde et al., 2010; Norbury & Husain, 2015). It has been shown to influence chronic pain states through trigeminal pain modulation (Abdallah, 2015; Li et al., 2019), migraines (Fanciullacci et al., 2000; Marmura, 2012) and neuropathic spinal pain and restless leg syndrome (Keeler et al., 2012; Kim et al., 2015).

In addition to its role in regulating sensory information, a large body of work has demonstrated the importance of DA signaling in regulating motor and premotor brain centers. DA controls spinal circuit activity by modulating the firing patterns of the Mesencephalic Locomotor Region (MLR), which is important for locomotion in many species including fish (Bernau, 1991; Sirota et al., 2000), amphibians (Cabelguen et al., 2003), birds (Sholomenko et al., 1991), and mammals (Eidelberg et al., 1981; Garcia-Rill et al., 1987; Marlinsky & Vointenko, 1991; Musienko et al., 2008; Roseberry et al., 2016). The MLR is heavily modulated by DA input from the substantia nigra pars compacta (SNc) (Ryczko, 2013), where firing frequency of DA neurons is an indicator of function. Low frequency tonic firing is associated with habitual motor programs while high frequency phasic bursts are related to reward seeking behavior (Rice, 2011). In addition to firing frequency, decreased DAT activity is shown to lead

to hyperactive motor behaviors (Leo et al., 2018). Importantly, the hypothalamic DA nucleus (PTar) innervates the hindbrain and spinal cord and is the sole source of DA neuromodulation of spinal motor circuits (Mu et al., 2012; Reinig et al., 2017), making it a target region that can be influenced by social factors to regulate motor activity. Taken together, the importance of DA signaling in both the activation of motor circuits and of social behavior provides an opportunity to examine how DA is involved in the reciprocal relationship between social dominance and status-dependent motor circuit activation.

Reciprocal relationship of social dominance and DA signaling

DA signaling in aggression and anxiety

DA is heavily implicated in the regulation of reward, depression, anxiety, motivation, and various aspects of social behavior including aggression and dominance formation. For instance, DA levels in the mesolimbic pathway, an important region for reward and motivation (Day & Carelli, 2007), are elevated in socially dominant rats (van Erp & Miczack, 2000; Lozano-Montes et al., 2019). This causal relationship between DA signaling from the mesolimbic pathway, the nucleus accumbens, and social dominance formation was recently supported in lesion studies (Shan et al., 2022). In addition, dopaminergic projections from the Ventral Tegmental Area (VTA) to the LS have shown to remove inhibitory activity through D2-receptor activation on presynaptic inhibitory neurons, leading to increased aggression (Mahadevia et al., 2021). This described D2 activation has been a point of clinical study for decades, where D2 receptor antagonists are used for treating aggressive behavior (Nikulina & Kapralova, 1992; Schwartzer & Melloni Jr, 2010; Morrison et al., 2015). Together, these findings support the notion that DA signaling is necessary for social dominance formation.

In addition to its role in regulating aggression, DA's function in anxiety is well documented. Decreased DAT expression in *Drosophila* (Pizzo et al., 2013), rats (Cinque et al., 2018), and zebrafish (Kacprzak et al., 2017) leads to an anxiety-like phenotype (Nieuwenhuys & Oudejans, 2012). Like aggression, D2 receptor activation is also implicated in anxiolytic behavior in mice (Casey et al., 2022). Taken together, DA signaling plays an important role in regulating numerous aspects of social behaviors; however, DA signaling can also be shaped by social regulation, as described below.

How social factors affect DA signaling

Although DA can regulate social behavior, DA signaling is, in turn, influenced by external social factors. Rats that undergo social stress show increased DA transporter (DAT) function in the medial prefrontal cortex (mPFC) (Novick et al., 2015), whereas social subordination in monkeys has been shown to decrease DAT expression (Nader et al., 2012). DA levels are reported to be higher in dominants (Okada et al., 2015; Sasaki & Harada, 2020) and social stress can decrease DA receptor type-1 (DRD1) activation in the brain of mammals (Barrot et al., 2005; Shinohara et al., 2018; Zhao et al., 2021). Social dominance can prompt morphological remodeling of specific DAergic brain regions. A study by White & Fernald (1993) showed that soma sizes of cell populations in the hypothalamus reflect social rank, where dominants have increased somas compared to subordinates. Further, cells within the paraventricular nucleus (PVN) of the hypothalamus, the bed nucleus of the stria terminalis (BNST), and the medial nucleus of the amygdala (meA) are significantly decreased in subordinates (Holmes et al., 2007). Changes in cell size can directly affect firing properties in accordance with The Henneman Size Principle (Henneman, 1957), where due to higher input resistance, the firing threshold of a smaller neuron will reach threshold more quickly than a

larger neuron (Stein et al., 2005). These firing threshold changes can result in altered circuit excitability and modified behavior. In addition to morphological changes, social dominance can alter the capability of cells to undergo neurogenesis in regions that receive dopaminergic input (Kozorovitskiy & Gould, 2004; Du et al., 2016). Taken together, social dominance contributes to a variety of DA plasticity at the cellular and network level.

Although we have a good understanding of the neural bases of social behavior and the role DA plays in regulating aggression, important questions remain. First, what role does social dominance play in altering DA modulation of specific motor behavior? Secondly, how does DA signaling work synergistically with other neurotransmitter systems to produce adaptive behavior in a status-dependent manner? Thus, a key question that will be addressed in chapter four is how social dominance regulates the interdependent activities of multiple neuromodulatory pathways to regulate motor behavior in a socially adaptive manner. To address these questions, I used zebrafish as a model organism because of the numerous biological advantages it provides as I describe below.

Zebrafish Social and Motor Behavior

Zebrafish are social animals and exhibit a variety of behaviors such as shoaling (Buske & Gerlai, 2011), courtship (Zempo et al., 2021), and aggression (Teles & Oliveira, 2016). Of equal importance, the structural and functional organization of the zebrafish brain are like mammalian brains (Kalueff et al., 2014), making them advantageous for studying the relationship between social dominance and neural circuit activation. Below, I describe what we currently know about zebrafish social dominance relationships, how social rank can influence motor behavior, and the gaps in our understanding that drive the questions and hypotheses of this dissertation.

Zebrafish and social dominance

Social aggression has been investigated in zebrafish through cognitive, physiological, and neurobiological lenses. Although a naturally shoaling species, when paired zebrafish are aggressive toward conspecifics and will readily form a stable dominance relationship (Larson et al., 2005; Teles & Oliviera, 2016; Miller et al., 2017). In studying zebrafish social dominance behavior, we have learned that these animals have the capacity for long-term social recognition (Madeira & Oliviera, 2017), recognize conspecific agonistic interactions and can alter their behavior towards each conspecific (Abril-de-Abreu et al., 2015), and show altered activation of the social decision-making network based on social phenotype (Teles et al., 2016a). Further, zebrafish of different social rank exhibit altered hormone (Tea et al., 2019) and nonapeptide levels (Teles et al., 2016b). In addition to these cognitive and physiological correlates to social dominance, there has been investigation regarding social dominance and DA signaling in zebrafish.

There is evidence that social dominance alters the DA pathway in zebrafish, where social dominants show an upregulation of DA synthesis (Pavlidis et al., 2011) and higher DA activity in the telencephalon (Teles et al., 2013). Reports in zebrafish show that altered DA signaling, such as a lack of DAT, results in anxiety-like behavior characterized by bottom dwelling (Kacprzak et al., 2017). However, the mechanisms of how social experience affects dopaminergic modulation of motor circuits is not well understood.

Mauthner motor circuit and the escape response

When threatened, zebrafish execute a startle escape behavior response whereby they quickly flex their body away from the perceived threat. This behavior is mediated by a well-defined neural circuit centered around the Mauthner neuron (M-cell) (Figure 3). (Eaton et al., 1977; Canfield & Rose, 1996). The M-cells have previously been described as “command

neurons” in that their firing is “necessary and sufficient” to evoke a startle escape response (Eaton et al., 1991, Di Domenico & Eaton, 1988), although several additional studies have suggested their activation is not necessary for startle escape and place equal importance for activation of other structures such as spiral fibers or the M-cell homologs, MiD2cm and MiD2cm (Liu & Fetcho, 1999; Lorent et al., 2001; McLean & Fetcho, 2004; Lacoste et al., 2015; Hale et al., 2016). However, an established difference between M-cell and non-M-cell startle escape is determined by latency of response from stimulus onset. M-cell startle escape is known as a short-latency response and occurs in less than 15ms, whereas non-M-cell startle is long-latency and occurs between 16-40ms (Eaton et al., 2001; Kohashi and Oda, 2008; Issa et al., 2011). Considering these differences, the relative simplicity of the neural circuit underlying the M-cell escape response has been useful to study sensory-motor integration over the last half of a century (Korn & Faber, 2005).

Morphological studies have uncovered the structural features of the Mauthner cells (Bodian, 1937; see Zottoli, 1978; Cioni et al., 1989). Structurally, the M-cells consist of two large neurons in the hindbrain in rhombomere 4, just behind the ears. M-cell axonal projections cross the midline and descend to the spinal cord, where they unilaterally innervate spinal motor neurons in each body somite (Figure 3). Functionally, the M-cells are responsible for command over lower pattern-like generator and motor neurons as well as participates in a higher brainstem escape network (Eaton et al., 2001). These large neurons have two distinct dendrites, the lateral and ventral dendrite, that are comprised of mixed synapses and receive both neuromodulatory and sensory inputs (Korn & Faber, 2005).

The M-cells receive multiple types of sensory input that influence the two main dendrites. The lateral dendrite receives input from the vestibulocochlear sensory nerve (VIIIth) nerve from

the ear and the lateral line network (Nakayama & Oda, 2004; Zottoli & Faber, 1979; Pereda et al., 2005; Korn & Faber, 2005), while the ventral dendrite receives visual input from the optic tectum and spinal cord (Zottoli et al., 1987). M-cells receive other modulatory input such as cortisol (Speedie & Gerlai, 2007; Bronson & Preuss, 2017) and dopaminergic neuromodulation via the hypothalamus (Mu et al., 2012; Reinig et al., 2017) to allow for integration of salient sensory signals to activate the M-cells appropriately. Together, the input from sensory systems and neurochemical input from modulatory brain regions allow for precise regulation of M-cell excitability.

M-cell activity is modulated by various neurochemical and electrical input. A map of the neurotransmitter make-up of the M-cell has been identified using histological (Sur & Triller, 1994), pharmacological (Wolszon et al., 1997), and molecular techniques (Diamond and Huxley, 1968). These studies identified excitatory glutamatergic inputs, inhibitory glycinergic and GABAergic inputs, and additional neuromodulatory inputs from serotonin (5-HT) and DA neurons (Diamond & Roper, 1973; Lee & Eaton, 1993; see. Korn & Faber, 2005). In addition to neurochemical input, M-cells contain gap junctions that enhance coupling potential of neural input onto the M-cells and afford fast depolarizing currents to activate the cells (Pereda et al., 1995). These inputs allow for regulation of excitability based on incoming sensory information onto the M-cells.

M-cell excitability is synergistically influenced by sensory input and neuromodulatory signaling. Repeated depolarizing stimuli to the M-cells can induce habituation of the startle escape response, as first described by Rogers and colleagues (1963). Although underlying

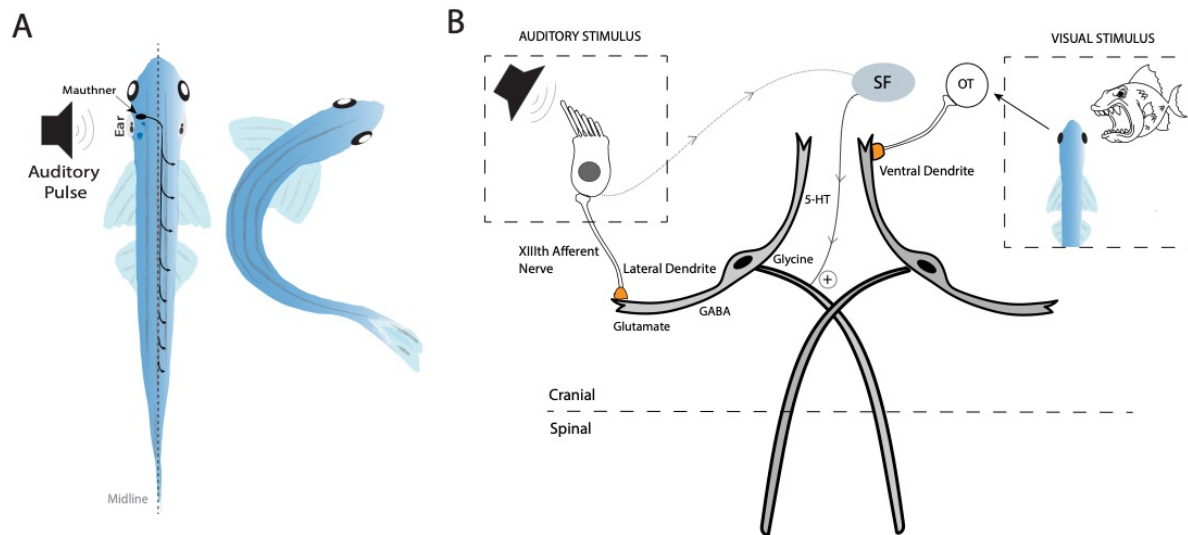


Figure 3. Schematic of the Mauthner-mediated escape response. (A) Representation of the bend away from the stimulus. (B) Illustration of some mechanisms responsible for M-cell activation and excitability modulation. M-cells receive input from auditory and visual stimuli onto the lateral and ventral dendrite, respectively. There is an abundance of glutamate and GABA receptors on the lateral dendrite, Glycine on the soma, and 5-HT on the ventral dendrite. Spiral fibers (SF) are activated by contralateral auditory afferents and provide excitation to commissural M-cells.

habituation of startle behavior in mammals has been a key point of study over the past few decades (Davis & Sheard, 1974; Hughes, 1984; Geyer & Tapson, 1988; Yeomans et al., 2002), the synergistic relationship between sensory input and neuromodulation of M-cell habituation has been more recently investigated, from both molecular (Wolman et al., 2015) and neurochemical lenses (Roberts et al., 2016; Batora et al., 2021). Interestingly, M-cell habituation has been shown to be a result of feedforward inhibition via glycinergic receptors (Marsden & Granato, 2015), where M-cell desensitization to acoustic stimuli is a direct result of CaMKII-mediated phosphorylation of the scaffolding protein gephyrin and subsequent synaptic

accumulation of glycine receptors (Ogino, 2019). Our knowledge of the functional and structural organization of the M-cell circuit coupled with the availability of genetic tools to genetically target the mutation of specific cell populations and signaling pathways provide an opportunity to address our hypotheses of how social dominance alter activation of this specific motor circuit.

Although status-dependent regulation of escape circuits has previously been investigated, our knowledge has been limited mostly to invertebrate systems (Yeh et al., 1996; Krasne et al., 1997; Dyakonova et al., 1999; Edwards & Spitzer, 2006; Fujimoto et al., 2011; Araki et al., 2013): while the investigation of social status-dependent neurophysiological changes in M-cell excitability in teleosts is cursory. Early studies in cichlids show status-dependent changes M-cell excitability (Neumeister et al., 2010). These findings were expanded to show that this startle circuit sensitivity is governed by serotonergic modulation of the M-cells (Whitaker et al., 2011). Further, there is a lack of in-depth investigation of status-dependent neuromodulation of M-cell circuit sensitivity in zebrafish. To date it has been established that, like cichlids, social dominance in zebrafish leads to altered M-cell circuit excitability (Miller et al., 2017). Additionally, it is known that habituation rate of the M-cell to auditory pulses is socially regulated, whereby subordinates are less likely to habituate to repeated auditory stimuli compared to dominants (Park et al., 2018). The neurobiological bases underlying this status-dependent regulation of startle sensitivity and habituation rate is controlled, in part, by the endocannabinoid system (Orr et al., 2021). However, given that the M-cell receives descending dopaminergic, glycinergic and GABAergic inputs, it is likely that the neuromodulatory effects they induce on M-cell excitability may be socially regulated as well. This significant gap in knowledge is a focus of this dissertation.

Directions for this Dissertation

I am interested in investigating the neuromodulatory changes between an animals' social rank and their motor circuit activation. We know that both aggression and visual displays are important for the establishment and maintenance of social dominance relationships, and that the escape response is affected in a status-dependent manner. Further, DAergic neuromodulation is important in both the establishment of social dominance and the excitability of the escape response. Thus, I am interested in discerning (1) how zebrafish utilize visual cues to establish and maintain social dominance, (2) the relationship between dopamine signaling and social dominance formation, and (3) how social dominance regulates synergistic interaction of multiple neuromodulatory inputs to control spinal motor circuits in a socially adaptive manner.

First, chapter two tests the hypothesis that zebrafish use visual cues, in the form of changes in stripe color intensity, to establish and maintain a stable dominance relationship. I will provide evidence that social status influences coloration through stripe intensity along the zebrafish body wall, and disruption of social visual cues destabilizes social dominance relationships. Finally, I will show that social dominance leads to status-dependent differences in motor activity and M-cell mediated startle sensitivity. Disruption of social visual cues leads to unstable social dominance and a loss of status-dependent differences in motor activity.

Chapter three tests the hypothesis that the dopaminergic signaling pathway is socially regulated. I will provide evidence showing that, although Dat and the Drd1b are not necessary for the establishment and maintenance of social dominance, Dat influences place preference in the pairing arena and swimming behavior while Drd1b regulates the startle circuit in a status-dependent manner.

Chapter four expands on what we have found regarding Drd1b receptor activation and its influence on escape behavior. In chapter four, I test the hypothesis that DA modulates M-cell

excitability through activation of glycinergic inhibitory neurons and results in status-dependent expression of *Drd1b* in hindbrain glycinergic nuclei that have been reported to inhibit M-cell excitability. I provide evidence that DA indirectly modulates the M-cell escape circuit through glycinergic input. I present evidence suggesting that blocking the *Drd1* activity can shift dominant M-cell excitability to reflect a subordinate motor phenotype.

Lastly, chapter five will synthesize the results to provide a new framework of how dominance and dopaminergic signaling underly the status-dependent shift in M-cell circuit excitability as an adaptation to social rise and fall. Overall, this dissertation work improves our understanding of the neural bases of social aggression and how modulation of hindbrain networks regulate motor activity as the animals' social environment evolves.

CHAPTER 2: EFFECTS OF VISUAL CUES ON DOMINANCE HIERARCHY FORMATION AND ACTIVATION PATTERN OF ESCAPE AND SWIM BEHAVIORS IN ZEBRAFISH

Published:

Clements, K. N., Miller, T. H., Keever, J. M., Hall, A. M., & Issa, F. A. (2018). Social Status-Related Differences in Motor Activity Between Wild-Type and Mutant Zebrafish. *The Biological bulletin*, 235(2), 71–82. <https://doi.org/10.1086/699514>

Introduction

One essential element of social dominance is the ability of individuals to properly communicate relevant information of their social status (Höjesjö et al., 2000; Grether, 2011). The display of social cues is crucial to stabilize social hierarchies and reduce conflict for resources within a social group (Huntingford et al., 1987). Although many fish species employ various types of social signals to convey their social rank to conspecifics, visual signaling is of particular importance (Korzan et al., 2008). In Teleost fish, the role of visual cues in the formation of social hierarchies has been examined in rainbow trout (*Oncorhynchus mykiss*) (Johnsson, 1997; Höjesjö et al., 2000), cichlid fish (*Astatotilapia burtoni*) (Korzan et al., 2008), and arctic charr (*Salvelinus alpinus*) (Höglund et al., 2000). Highlighting the importance of coloration in social regulation, socially dominant cichlids are conspicuously brighter in coloration with distinct patterns that denote their status compared to subordinates (Korzan et al., 2008).

Zebrafish are brightly colored with longitudinal golden and blue stripes whose color intensity can be regulated during mating and shoaling behaviors through a process called physiological color change (Fujii, 2000; Snekser et al., 2006; Hutter et al., 2012; Singh and Nüsslein-Volhard, 2015). Physiological color change of melanophores can also be regulated based on color of background environment (Logan et al., 2006). These changes in melanophore color intensity are not transient; rather plasticity in color intensity can be improved by cyclical training suggesting a learning component and a capacity for long-term adaptation of body

coloration to environmental changes (Hatamoto K, Shingyoji, 2008). Although these results demonstrate the ability of zebrafish to regulate the intensity of their body color to ethological cues, little information is available of the importance of coloration during dominance hierarchy formation and its effects on zebrafish nervous system function (Pavlidis et al., 2011).

As with many animals, visual information is first integrated in the optic tectum then relayed to various brain regions involved in decision-making and motivational processes (Lambert et al., 2012; Chou et al., 2016; Pantoja et al., 2016). For example, it was shown in zebrafish that visual information from the optic tectum converge on hypothalamic dopaminergic neurons involved in regulating the excitability of spinal motor circuits involved in mediating startle escape and swim behaviors (Mu et al., 2012). This presents an exciting opportunity to test how changes in visual social cues can alter decision-making processes and consequently the activation of spinal motor circuits, one of which is the startle escape response.

The startle escape response and its underlying neural circuit are well-characterized behaviorally and physiologically (Eaton et al., 2001; Korn and Faber, 2005; Medan & Preuss, 2014). It is centered on a pair of reticulospinal neurons called the Mauthner neurons (M-cells) whose activation is necessary to mediate the C-start escape response that propels the animal away from threats by rapid flexion of the body (Figure 5C). Visual information modulates the excitability of the M-cells via descending dopaminergic input and is contingent on the quality of the visual stimulus. Non-threatening visual stimuli increase the activity of the hypothalamic dopaminergic neurons that lead to suppression in synaptic transmission from the visual center to the escape circuit (Mu et al., 2012). Conversely, threatening visual stimuli inactivate some of these neurons, resulting in dis-inhibition of the visuomotor transformation and escape generation. (Yao et al., 2016). In addition, neurons in the medial longitudinal fasciculus, which are crucial

for locomotor output activate during light-evoked swimming suggesting that they are potentially modulated by visual stimuli as well (Sankrithi & O'Malley, 2010). Taken together, these results strongly suggest that modulation of the escape and swim circuits can be tuned by visual cues to facilitate appropriate behavioral responses. Despite this knowledge little is known of how visually mediated social signals influence social interactions, dominance formation and their effects on the activation pattern of the escape and swim behaviors. In this study, we addressed this issue by taking advantage of the genetically modified TLN zebrafish strain that lacks the blue striped pigmentation characteristic of zebrafish to ask the question of how improper display of visual signals affect the dynamics of social interactions, dominance formation, and consequently the activation of the escape and swim behaviors.

The TLN line was produced in Herwig Baier's lab by causing two homozygous mutations in a Tübingen background (Zfin.org, ZDB-GENO-080307-1, 2002). First, a homozygous recessive mutation in the connexin 41.8 (cx41.8) gene causes spotting of pigment cells in adult zebrafish resulting in the Tüpfel long fin (TL) phenotype; second, homozygous mutations were introduced into the *microphthalmia-associated transcription factor a* (*mitfa*) gene which is essential in melanophore development (Lister et al., 1999), resulting in the TLN fish line with a complete lack in dark pigmentation (Figure 4B). The optical clarity of TLN fish due to the loss of melanophore formation is particularly advantageous tool because it extends the period during which embryonic development and functional analysis of brain function can be examined (Lacoste et al., 2015; Förster et al., 2017). However, despite their wide use, the effects of proper body coloration on social behavior and nervous system function are poorly understood.

Here we tested the hypothesis that a lack of proper visual information affects dominance hierarchy formation. Given that visual input plays an important role in modulating the escape and swim circuits, we also tested the notion that a lack of proper visual social displays may lead to abnormal regulation of the escape and swim behaviors. We show that TLN fish form stable social hierarchies,

albeit with a higher prevalence of social dominance instability. Secondly, we show that TLN fish swimming behavior significantly differs compared to AB fish, showing no social status-dependent place

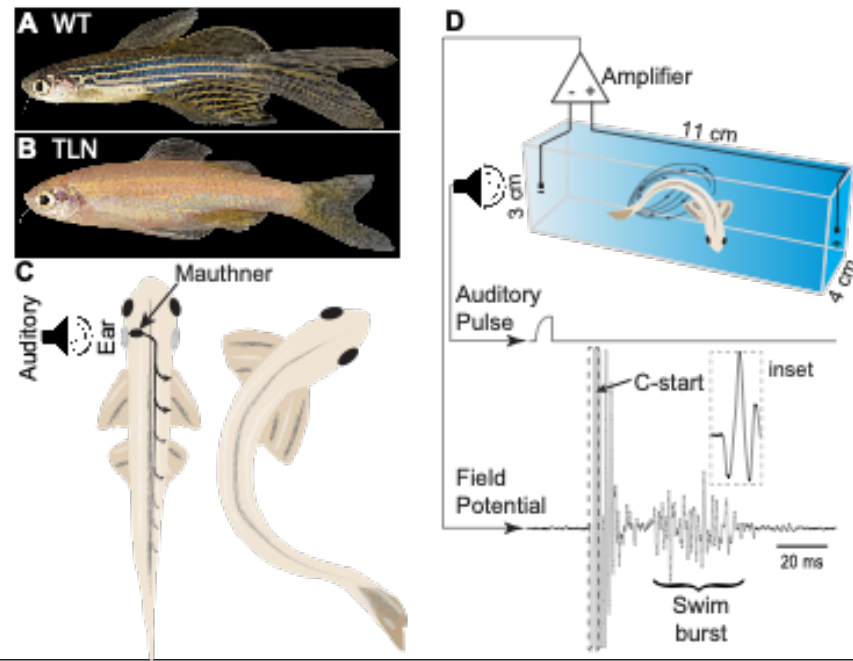


Figure 4. Body color patterns of AB and TLN zebrafish and experimental setup. (A) AB zebrafish are brightly adorned with blue stripes. (B) TLN zebrafish lack body color pigmentation. Insets in A and B are zoomed in images taken from medial part of the animals. (C) Schematic of the C-start escape response mediated by the M-cell. M-cell receives ipsilateral auditory sensory input from the VIII sensory nerve. Stimulation of the M-cell activates contralateral fast motor neurons that innervate the musculature. (D) C-start escape can be initiated by a sudden auditory pulse. Rapid contraction of the musculature during C-start escape generates a far-field potential (C-start: dashed box) that can be recorded using bipolar bath electrodes placed on either side of the testing chamber. The field potential of the C-start escape is characterized by large amplitude and is brief (inset). Escapes are typically followed by swims whose field potentials can be detected (Swim burst) and are significantly smaller and longer in duration. Computer generated auditory pulses and field potentials are digitized and time locked.

preference or differences in overall swimming activity. Thirdly, we show that unlike their AB counterparts, TLN fish do not display social status-dependent differences in the sensitivity of the C-start escape response. Finally, we illustrate that coloration is socially regulated and that lighter coloration denotes social subordination. Our results highlight the importance of proper social visual cues in establishing stable social relationships and regulation of motor behaviors that are essential in regulating socially appropriate behaviors.

Methods

Animal care and use

Experiments were carried out according to the guidelines and approval of East Carolina University's Institutional Animal Care and Use Committee (Animal Use Protocol D320). Adult strain AB zebrafish (*Danio rerio*) and TLN mutant zebrafish were maintained group-housed at 28 °C, pH 7.6, and fed pellet and live brine shrimp food twice daily.

Pairings and behavioral observations

Adult males (age 6-12 months) were initially socially isolated for one week in individual tanks (23 x 13 x 6 cm). Afterwards, animals of equal age and size were randomly paired continuously for two weeks in a novel tank of equal dimensions. Social interactions were observed daily for 2 minutes between 10am-2pm. To assess social rank, we counted the total number of aggressive behaviors. Attacks consisted of bites and chase of an opponent and submissive defensive behavior consisted primarily of retreats. Social rank was assigned based on the ratio of the total number of attacks divided by the number of retreats. Animals with higher index were considered dominants.

Measuring swimming activity

Pairs were filmed daily (early afternoons) for one-minute to monitor changes in swimming activity using a Canon Camcorder (Digital video ZR500). For the group-housed control condition, six males of equal size and age were selected from group-housed tanks and grouped together, and their swimming behavior was video recorded and tracked. Videos were digitized and movement (distance traveled over one-minute periods) of each fish was analyzed using NIH ImageJ software Manual Tracking plugin. Instances when animals were interacting with one another resulted in the exclusion of those video frames from analysis. Total tracked distance was normalized by the number of remaining video frames.

Place preference analysis

The movement of animals was tracked using the Manual Tracking plugin for ImageJ to extract XY coordinates within the housing tank. Videos were down sampled to three frames/second and coordinates for dominants and subordinates were loaded into R software using a custom script. XY coordinates encompassing periods of social interactions were removed from the analysis. Filled contour plots combining data of all dominant and subordinate animals were produced using 2D kernel density estimations generated by the `kde2d` function of the MASS package (CRAN repository) (Venables and Ripley, 2002). The algorithm used disperses the mass of the empirical distribution function over a regular grid of 512 points and uses the fast Fourier transform to convolve this approximation with a discretized version of the kernel followed by linear approximation to evaluate the density at the specified points. Density data was converted into a heat-map probability plot to facilitate illustration of the data set for both social phenotypes.

Measurement of field potentials

Experimental setup, measurement of field potentials, and testing of the sensitivity of the C-start escape response were conducted as described previously (Prugh et al., 1982; Issa et al., 2011). In brief, animals were placed in a testing chamber (dimensions: 11 x 4 x 3 cm) filled with reverse osmosis water with a resistance of ~15 M Ω -cm and temperature of 25°C. The fish were allowed to acclimate for 30 minutes prior to testing (Figure 5D). M-cell mediated escape electric field potentials were recorded using a pair of conductive electrodes (1 mm bare thickness, 3-5 mm metal exposure) placed on either side of the testing chamber (Figure 5D). Signals were amplified 1000-fold and were low-pass filtered at 300 Hz and high-pass filtered at 1 KHz using an AC differential amplifier (AM-Systems model 1700). Signals were digitized then stored (Digidata1550, Axoscope Software, Molecular Devices, Inc., Sunnyvale, CA, USA).

Auditory pulses (1 ms in duration) were computer generated using Audacity software (audacityteam.org), and calibration of decibels was performed using a decibel meter (Sinometer, MS6700). The animals' auditory sensitivity and threshold of the escape response was measured by delivering pulses at a randomized dB at mid-range levels (repeated 3-5 times at each level) with a minimum of 2-minute intervals between trials. Pulse decibel amplitude was randomized and ranged between 70-100 dB re 20 μ Pa with 5 dB increments. Trials were averaged, and the data was curved-fitted with a nonlinear regression analysis using the Boltzmann sigmoidal equation: $Y = Bottom + (Top - Bottom) / (1 + \exp((V50 - X) / Slope))$.

Field potential data analysis

The latency of the escape response was measured using the field potential signals recorded by measuring the time between stimulus onset and beginning of field potential response. The zebrafish escape response is categorized into two main types of escapes. Activation of the M-cell generates a short-latency escape response with a time onset ranging

between 2-15 ms (Figure 3B). Zebrafish also produce long-latency non-Mauthner mediated escapes with a time onset ranging between 15-40 ms (Eaton et al., 2001; Kohashi and Oda, 2008). Each type of escape generates a distinct electric signal that permits the reliable identification of escape based on both potential signature and its latency from stimulus onset (Issa et al., 2011). The latency of the electrical signal was defined as the time difference between the onset of the auditory stimulus and the beginning of the large phasic field potential generated during the short-latency M-cell mediated escape response. Data was tabulated into Microsoft Excel and analyzed using Prism (GraphPad software Inc., San Diego, USA). All values are provided as mean and $\pm$ SEM unless otherwise stated.

Acquisition and analysis of Pigment color intensity

Prior to imaging, the fish were briefly anesthetized with 0.02% MS-222 diluted in system fish water. The fish were placed onto a petri-dish and imaged using an Olympus SZX29 stereo microscope equipped with a CCD computer-controlled camera (ToupTek Inc. Ltd, ToupView software). Images (2048x1536) were taken at ambient light while minimizing reflective lighting by using a light diffuser. Multiple images were taken at the same magnification at the anterior and medial parts of the animals. Once images were taken the animals were returned to their isolation tanks to recover for 30 minutes followed by the onset of pairing for two weeks.

Using NIH ImageJ, images were converted to gray-scale. This process prevented discrimination among the three different types of chromatophores known to contribute to the overall color pattern of zebrafish (Singh and Nüsslein-Volhard, 2015). Nevertheless, this approach allowed the quantification in the overall change in color intensity. For each image, one region of interest (ROI) was selected for each of the medial colored stripes that extend the length of the animals (stripe 1 & 2 in Figure 5A). Plot profiles of the average gray-scale intensity values

were generated for each of the selected ROIs. Gray-scale ranged from 0-255, whereby 0 equaled absolute black and 255 equaled absolute white. Data was averaged across the anterior and medial images. The same process of image acquisition and analysis was repeated after two weeks of pairing (Day 14). To circumvent potential bias, behavioral observations, image acquisition, and data analysis were conducted blind by three different investigators. One individual oversaw the behavioral observations. The fish were handed down to a second investigator who obtained the images. Images were then provided to a third investigator who analyzed the data. Neither one of the investigators who acquired and analyzed the data was aware of the social status of the fish until the data was fully analyzed.

Results

Proper visual signals are important to maintain stable dominance relationships

To determine the importance of visual signals in regulating social interactions and formation of social dominance we paired and observed the aggressive interactions of TLN zebrafish for two weeks and compared their behavior to AB pairs. We measured the level of aggression between the two groups by recording the number of aggressive (attacks) and submissive (retreats) behaviors of each pair (Figure 5). We found that AB pairs formed stable dominance relationships within 2 days of pairing. By the third day, dominants performed most of the aggressive chasing and biting behavior (Figure 5A, left panel), while subordinates mainly retreated from interactions (Figure 5A, right panel). The differences in the average number of attacks and retreats significantly differed between the two social phenotypes (Avg. attacks:

Dom= 5.46 ± 0.59 ; Sub= 0.72 ± 0.29 . Avg. retreats: Dom= 0.57 ± 0.25 ; Sub= 5.19 ± 0.61 .

Wilcoxon signed rank test, two-tailed, $P=0.0001$). In addition, once dominance was established it remained stable for the remainder of two weeks. TLN pairs displayed similar patterns of aggressive activity and level of aggression did not significantly differ from AB pairs (Figure

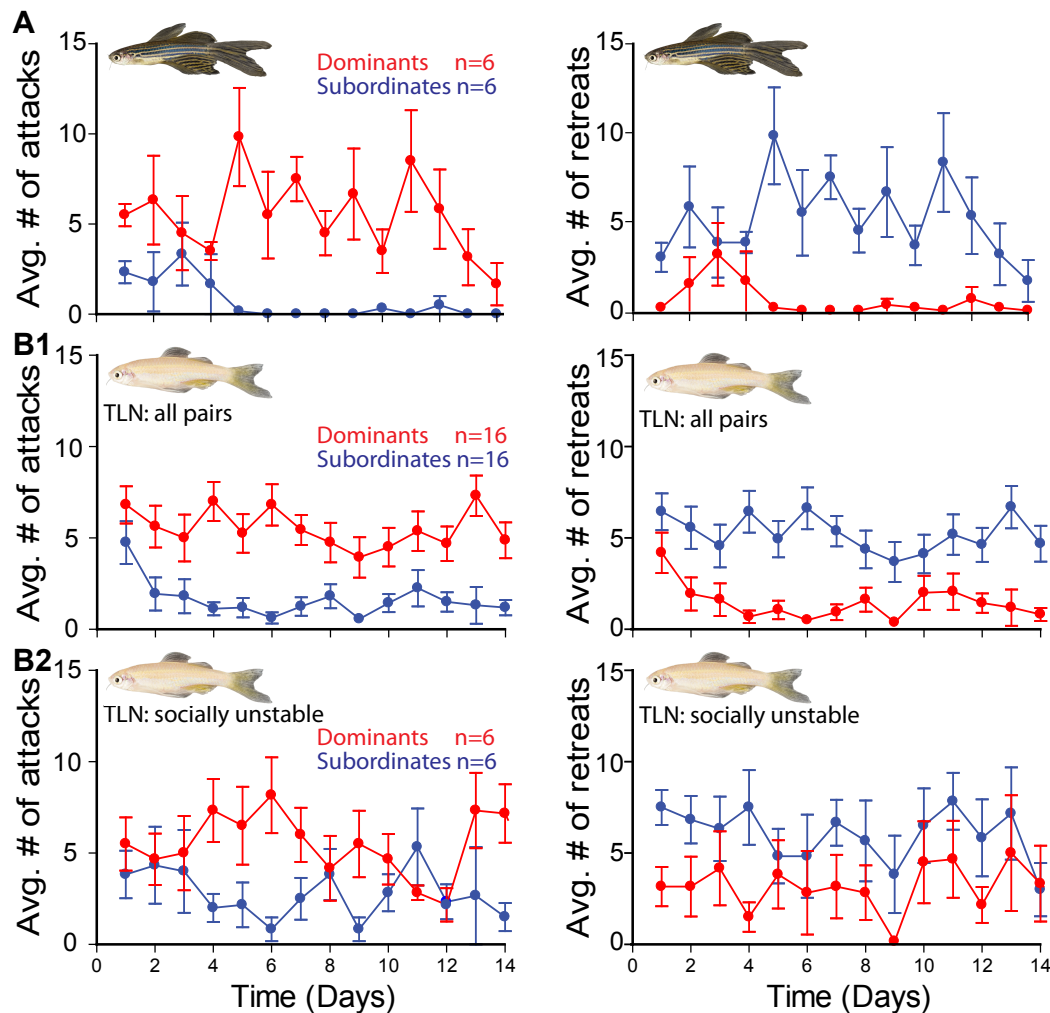


Figure 5. Visual social cues are important for stability of social relationships. Both AB and TLN zebrafish color morphs engage in agonistic interactions (attacks and retreats). (A) Average number of attacks and retreats between AB dominants and subordinates ($n=6$ pairs). (B) Average number of attacks and retreats between TLN dominants and subordinates of all TLN pairs regardless of social stability ($n=16$ pairs). (C) Average number of attacks and retreats between TLN dominants and subordinates of pairs that experienced social instability ($n=6$ out of 16 TLN pairs).

5B1, Avg. attacks for TLN Dom= 5.45 ± 0.28 ; TLN Sub= 1.58 ± 0.26 . n= 6 AB, n= 16 TLN, Mann Whitney Two-tailed test, P= 0.8362). Although the behavior patterns of TLN pairs appeared like those of AB, closer examination showed that more than a third of TLN pairs switched their social status at least once during the pairing period (Figure 5B2; n= 6/16 pairs, 37.5%). These frequent reversals in social status were not observed in AB pairs, and reversals were likely a result of an increased frequency of attacks by the TLN subordinates. We compared the aggression level between AB and TLN subordinates and found a significant difference in the average number of attacks over the observation period (WT Sub: 0.72 ± 0.29 ; TLN Sub= 1.58 ± 0.26 Mann Whitney Two-tailed test, P = 0.0137). This suggests that proper visual social communication is important in regulating social interactions and stability of dominance relationships.

Proper visual social signals are important to maintain territoriality

To observe how visual signals affect swimming activity and patterns of spatial distribution of the animals we measured the average distance swam every day during a 1-minute interval for TLN and AB pairs. Furthermore, we generated density plots of swimming activity to determine the spatial probability distribution of all animals observed over 1 min of filming sampled at three frames/sec (Figure 6). We found that as social dominance was established in AB pairs, dominants maintained high levels of swimming activity while subordinates significantly decreased their swimming behavior compared to dominants and group-housed control fish (Figure 6A. Kruskal-Wallis test One-way ANOVA, Dunn's Multiple Comparison Test, $P < 0.0001$, [difference in rank sum: group-housed vs. Dom = -14.13, group-housed vs. Sub = 13.53, Dom vs. Sub = 27.67]; group-housed n= 12, Dom n= 7, Sub n= 7). Differences in swimming were apparent in the spatial distribution of the two social phenotypes within the

experimental arena. AB dominants exhibited strong territorial displays in which they swam freely throughout the tank, while subordinates were relegated to the bottom corner (Figure 6A, right panel). Unlike AB pairs, we found differences in swim frequency and patterns between dominant and subordinate TLN fish to be less pronounced regardless of the stability of social structure. Statistical analysis of distance traveled for all TLN fish showed a significant difference in the average number of distance traveled between dominant and subordinate fish, but no difference between group-housed vs. subordinate fish (Figure 6B1. Kruskal-Wallis test One-way ANOVA, Dunn's Multiple Comparison Test, $P < 0.0001$, [difference in rank sum: group-housed vs. Dom = -25.33, group-housed vs. Sub = -10.67, Dom vs Sub = 14.67]; group-housed $n = 12$, Dom $n = 7$, Sub $n = 7$). However, among the TLN pairs that displayed social reversals, we did not find significant differences in the average number of distance traveled (Figure 6 B2. Kruskal-Wallis test One-way ANOVA, Dunn's Multiple Comparison Test, $P < 0.0001$, [difference in rank sum: group-housed vs. Dom = -21.51, group-housed vs. Sub = -13.27, Dom vs. Sub = 8.267]; group-housed $n = 12$, Dom $n = 7$, Sub $n = 7$). Moreover, in both socially stable and unstable TLN pairs, dominants and subordinates did not display territorial behavior as was observed in AB pairs (Figure 6 B1, B2 right panels). These results suggest that disruption of proper visual social signals affects the dynamics of social interactions with negative consequences on swimming and territorial behavior.

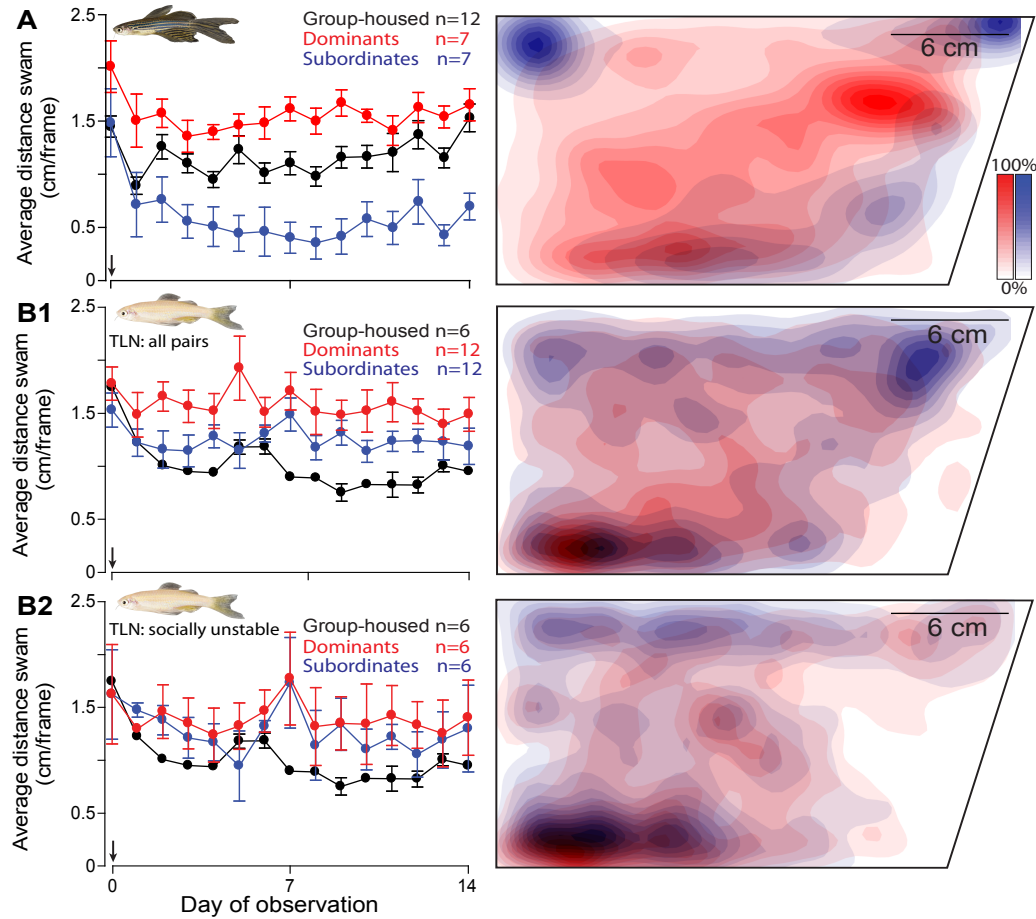


Figure 6. Visual social cues affect swimming and territorial displays. Left panels: Average distance traveled over a 1-minute period of filming of group-housed, dominant, and subordinate for (A) AB, (B1) all TLN, (B2) socially unstable TLN pairs. Right panels: Kernel heat-map estimation plots of swimming activity over a 1-minute period of filming for dominants (red) and subordinates (blue) on day 14 post pairing for each experimental condition. (A) AB condition (n= 12 group-housed, n= 7 dominants, and n= 7 subordinates). (B1) All TLNs animals (n= 10 group-housed, n= 12 dominants, n= 12 subordinates). (B2) Only TLN pairs that experienced dominance reversals (n= 12 group-housed, n= 6 dominants, n= 6 subordinates). Day 0 marks observations of animals before they were paired (Before pairing: arrow).

Visual signals affect the sensitivity of the C-start escape response

To determine whether differences in visual signals can affect the activation of the C-start escape response in TLN fish, we tested the animals' sensitivity to auditory pulses. To prevent

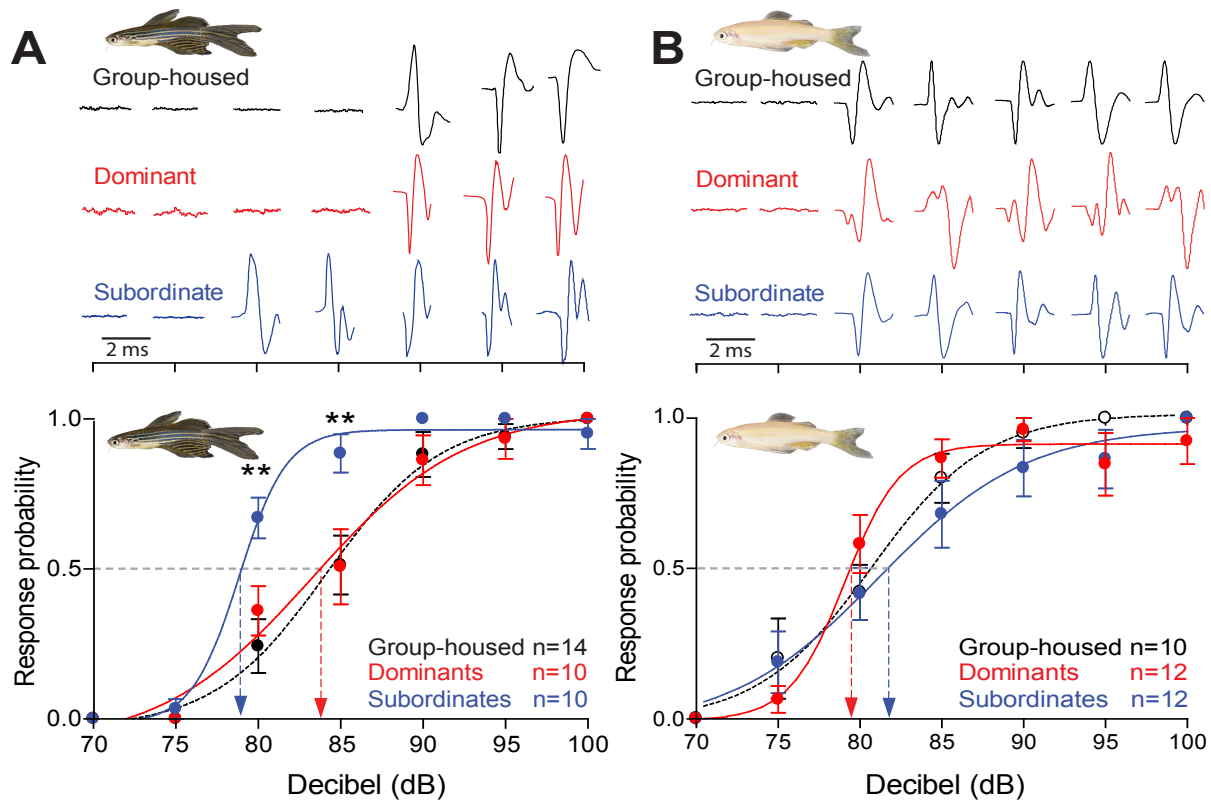


Figure 7. Improper visual social cues eliminate social differences in probability of C-start escape response. A) Individual examples of C-start field potentials from group-housed, dominant, and subordinate AB animals at increasing decibel intensities (top) and probability of C-start escape response (bottom). Probability of initiating an escape response is significantly higher in subordinates (n=10) compared to dominants (n=10) and group-housed animals (n=14) at the 85-90 dB levels. A response probability of 0.5 is indicated with the dashed arrow line. B) Individual examples of C-start field potentials from group-housed, dominant, and subordinate animals at increasing decibel intensities (top) in TLN fish. Probability of C-start escape response in TLN fish (bottom).

habituation of the escape response, we randomized the intensity of the auditory pulses while maintaining a minimum of 2-minute intervals between stimuli (see methods for details). In AB pairs, subordinate fish displayed heightened sensitivity to auditory stimulation illustrated in more frequent responses to lower auditory pulses compared to dominants (Figure 7A). Subordinate fish reached the 50% response threshold at 78.84 dB, dominants at 83.28 dB and group-housed

animals at 84.19 dB. Data was curve fitted with a non-linear regression, Boltzmann Sigmoidal curve fit; Goodness of fit R^2 for group-housed = [0.7765], dominants = [0.7675], subordinates = [0.9096]; group-housed n= 14; dominant n= 10, subordinate n= 10. This result is consistent with our previous report demonstrating status-dependent differences in the sensitivity of the escape response (Miller et al., 2017). Unlike AB pairs, the probability of the C-start escape response was similar in dominant and subordinate TLN animals (Figure 7B). Subordinate fish reached the 50% response threshold at 81.18 dB, dominants at 79.1 dB, and group-housed animals at 80.7 dB. Data was curve fitted with a non-linear regression, Boltzmann Sigmoidal curve fit; Goodness of fit R^2 for group-housed = [0.8091], dominants = [0.8434], subordinates = [0.6927]; group-housed n= 10; dominant n= 12, subordinate n= 12. Comparison of the sensitivity curves of all three social groups shows that paired-wise interactions had no effect on the response sensitivity (Figure 7B). The lack of differences in the sensitivity of the escape response in TLN fish demonstrates the importance of proper visual social cues in regulating adaptive motor behavior.

Social status regulates intensity of skin pigment

Our results show that visual cues play an important role in regulating social interactions, and disruption of proper visual information weakens social stability and leads to changes in behavior patterns and escape behavior. This result suggests that perhaps in AB fish body coloration is socially regulated and potentially serves as a mechanism to communicate social status. To test this hypothesis, we measured changes in color intensity of AB animals prior to social interactions (Day 0) and two weeks later (Day 14) (Figure 8). At first examination, we found that social interactions did not lead to a change in color intensity in dominants but a

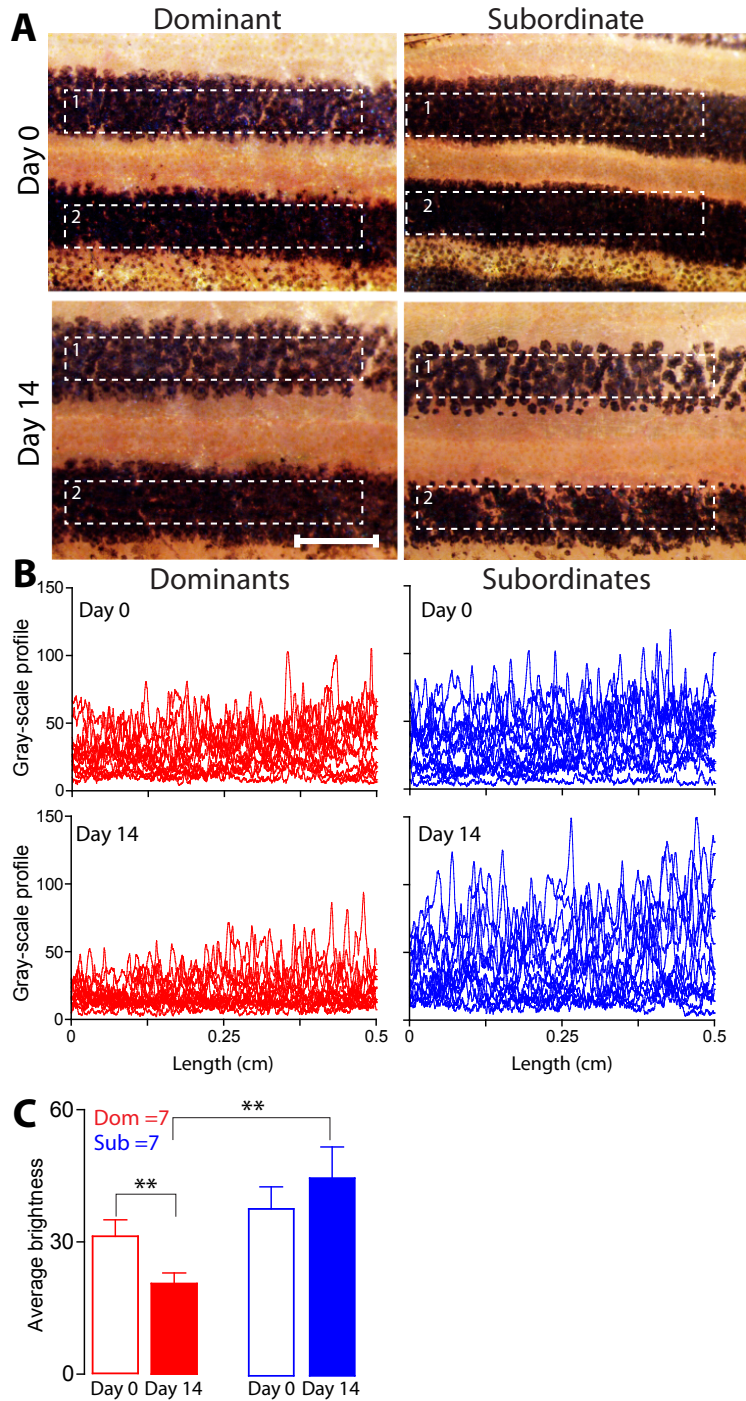


Figure 8. Social status-dependent regulation of body color intensity in zebrafish. A) Representative examples illustrating changes in intensity of body coloration. Illustrations are from of AB dominant and its subordinate counterpart on day 0 and day 14 of pairing. Dashed boxes highlight analyzed ROIs for color stripes 1 and 2 (scale bar = 125 mm). B) Plot profiles of the gray-scale intensity of all stripes and pairs tested. C) Average change in color intensity between dominant and subordinate fish on days 0 and 14 of pairing (n= 7 dominants, n= 7 subordinates).

decrease in intensity in subordinates (Figure 8A). Subordinates became lighter in coloration. This was noticeable when comparing the distribution of melanin within the melanophores between day 0 and day 14 (Figure 8A, subordinate right panel). On day 0, melanin was widely distributed within the cells preventing the ability to differentiate individual melanophores. However, by day

14 individual melanophores became more distinct, a sign of aggregation of melanin centrally within the cells. This is typically indicative of a decrease in color intensity. To verify this result we calculated the gray-scale intensity profile for each ROI (Figure 8A, dashed boxes). Intensity plot profiles showed significant differences in intensity patterns between dominants and subordinates after two weeks of interactions (Figure (8B, C). Dominants became increasingly darker with a tighter intensity profile distribution, while subordinates became lighter with a widely varied intensity profiles (Figure 8B). At day 0 color intensities did not differ between the two social groups (Figure 8C, Dom= 7, Sub = 7; Wilcoxon signed rank test, two-tailed, $P=0.4263$). However, the change in color intensities after two weeks became more pronounced and statistically significant (Figure 8C, Avg. gray intensity day 0: Dom= 31.12 ± 5.2 ; Sub= 37.9 ± 6.1 . Avg. gray intensity day 14: Dom= 20.6 ± 3.3 ; Sub= 44.8 ± 8.7 . Wilcoxon signed rank test, two-tailed, $P=0.0023$). A closer examination revealed that 6/7 dominants increased their color intensity (Wilcoxon signed rank test, two-tailed, $P=0.0166$); while 4/7 subordinates showed a decrease in intensity (Wilcoxon signed rank test, two-tailed, $P=0.1726$). Moreover, pairwise comparison of the average change in intensity indicated that dominants were darker in intensity than their subordinate counterparts (5/7 pairs). These differences were not intrinsic because intensities were similar prior to pairing, and differences only emerged because of social interaction to denote social status.

To validate this result, we paired AB with TLN fish. We predicted that if the lack of body coloration serves as a cue of social subordination, then AB fish would be more likely than TLN to be dominants. Indeed, we found that AB and TLN fish form stable social relationships in which on average AB fish are more likely to dominate their TLN counterparts (9/12 pairs AB were dominant; 3/12 TLN were dominant). These dominance relationships tended to be strong

and quick to form evident by the fact that dominance was formed by day 1 (data not shown), and nearly half of the TLN animals (4/9, 44%) were killed by their AB counterparts during the first week of pairing. This result was concordant with strong territorial displays by AB fish that limited the swimming behavior of TLN fish to only one corner of the arena. Taken together, our results suggest that pigment coloration in zebrafish is plastic, socially regulated, and it serves as a behavioral mechanism to convey information of one's social rank.

Discussion

Our results show that proper visual signals play an important role in social regulation in zebrafish. We demonstrated that the lack of proper visual displays not only leads to frequent social reversals but also affects the activation pattern of motor behavior. TLN zebrafish that lack striking skin coloration form unstable social relationships and display different escape and swim behavioral patterns compared to AB fish. Although the aggression level and pattern of social interactions was like that of AB, social relationships reversed at least once 37.5% of TLN pairs (Figure 5). As animals engage in agonistic interactions, they are relying on many different types of social information to assess their opponents' strengths. Absence of key visual signals prevents the fish from fully accepting their opponents' dominance. Our results support this notion because improper visual cues in TLN fish led to continued competition for dominance, whereas AB fish quickly established stable relationships. Although a significant number of TLN pairs failed to form stable relationships, the remaining 62.5% did, which suggests that other pertinent factors such as body size, pheromones and inherent motivational differences may compensate for the loss in proper visual cues.

In many social animals, proper assessment of the strength and aggressive motivation of an opponent determines the outcome of agonistic interactions (Huber et al., 1997; Issa et al.,

2012). Moreover, with frequent encounters “winner” and “loser” effects develop and shape the outcome of future interactions (Oliveira et al., 2011). If lighter body coloration serves as a signal of social subordination as our data suggests, then it is of no surprise that TLN pairs showed frequent social instability due to the incongruent social messages’ opponents were experiencing, pale coloration as a signal of subordination is inconsistent with displays of aggressive behavior. Conversely, when AB and TLN fish were paired, the exaggerated signal of social subordination (paleness) of the TLN fish was likely to have influenced the aggressive state of their AB counterparts and increased their motivation to fight and win subsequent encounters. The induced “winner” effect in AB fish explains the results showing quick dominance formation, higher probability of AB fish to dominate and kill their TLN counterparts, a phenomenon that is rarely observed among AB fish.

The frequent social instability in TLN pairs was evident in the lack of differences in swimming activity and territorial behavior typical of AB zebrafish pairs (Figure 6). Within few days of pairing, AB subordinates displayed a strong preference for the bottom corner of the housing tank as a form of shelter and/or avoidance strategy of their dominant counterparts (Figure 6A). However, subordinate TLN fish did not display place preference, and their swim pattern overlapped extensively with that of dominants (Figure 6B). In addition, while AB subordinates decreased their swimming activity compared to dominants and group-housed fish, TLN subordinates swam as frequently as dominants; this includes TLN subordinates of pairs that formed stable social relationships. This suggests that the individual behavioral components that constitute submissiveness can be independently regulated. In the present case, although TLN subordinates reduced their aggressive behavior, they did not reduce their swimming as was observed in AB subordinates. This suggests that aggression by dominants is necessary to

establish a social order and reduce subordinates' aggressive behavior. However, aggression alone is insufficient in changing the swimming behavior pattern, which may require additional visual cues to engage specific decision-making circuits responsible for suppressing the swimming circuit.

TLN zebrafish showed no status-dependent difference in the sensitivity of escape response to auditory stimuli (Figure 7). This was in stark contrast to ABs in which the sensitivity of the escape response of subordinates was significantly enhanced compared to dominants, and in contrast to previously reported observations showing status-dependent differences in M-cell sensitivity in cichlids (Neumeister et al., 2010; Miller et al., 2017). One possible mechanism that may explain this difference between AB and TLN animals is that integrated visual signals from the optic tectum differ in the neural message relayed to decision-making circuits including the hypothalamic dopaminergic neurons known to modulate the excitability of the M-cell (Mu et al., 2012). How the optic tectum processes, integrates and relays information relevant to body coloration is not known. It is likely that changes in body coloration may alter neuromodulatory release and excitability of downstream motor circuits such as the M-cell escape and swim circuits. It is noteworthy that the sensitivity of the escape response of all TLN fish, regardless of social status was moderately elevated and comparable to that of AB subordinate fish. Although we have no direct evidence, the instability of social relationship between TLN fish may lead to an increase in stress levels due to the mismatch in social signals. If so, then it is expected that stress hormones can modulate the activation threshold and excitability of spinal motor circuits in a social status-dependent manner. Indeed, in goldfish cortisol application increases M-cell excitability by increasing M-cell input resistance and the magnitude of M-cell postsynaptic potentials by reducing the strength of local feed-forward inhibition inputs (Bronson and Preuss,

2017). Moreover, exposing zebrafish to unpredictable chronic stress increases their cortisol levels and alters their swimming activity (Manuel et al., 2014). Taken together, this evidence lends credence to our results that proper visual displays are important in social regulation and activation of spinal motor circuits, and disruption of visual information can have profound impact on the dynamics of social interactions and nervous system function.

Status-dependent differences in the intensity of body coloration in AB animals suggest that color plasticity is an adaptive strategy and is evidence that social displays can act as a modulator for behavioral motor output. Without the ability to visually display one's social rank, TLN zebrafish are unable to successfully prioritize adaptive behaviors. This could be either due to a lack of stability of dominance relationships, or a disruption of the neuromodulatory signaling pathways that rely on the processing of visual information between members of a social group. The interplay between visual information and neuromodulation of motor circuits is not well understood. However, there is strong evidence showing that visual information activates dopaminergic brain nuclei known to modulate spinal motor circuits such as the escape and swim circuits (Mu et al., 2012). It is unlikely that only one neuromodulator predominates any one neural circuit. Rather, it is probable that excitatory and inhibitory inputs converge and prime the activation of motor circuits in a socially adaptive manner (Petrov et al., 1991; McLean & Fetcho, 2004; Moly & Hatta, 2011; Bacque-Cazenave et al., 2013, Pantoja et al., 2016). Investigating the social status-dependent changes in these signaling pathways would be a step forward for an improved understanding of the effects of social status on nervous system function.

It has not escaped our attention that our results potentially have broader implications on many zebrafish studies in which genetically engineered lines are used. In this context, utilization of different phenotypic morphs to investigate brain function, particularly studies that examine

motivated behavior, should be put in context of the animals' early development and social environment to avoid confounding results. While investigating shoaling behavior of AB and Nacre unpigmented zebrafish, Engeszer demonstrated the importance of early life experience on the preference of shoaling behavior at adulthood and determined that shoaling with similar color morph fish is not innate, rather it is learned (Engeszer et al., 2004). Our results extend on their analysis and show the implications of visual displays in regulating motor behavior patterns. However, how disruption of proper visual social cues affects the activation patterns of other brain circuits involved in motivated behavior remains unknown. Therefore, we caution that the life social history should be central in the design and interpretation of results when morphologically engineered zebrafish are used to study the neural bases of behavior.

CHAPTER 3: THE ROLE OF *slc6a3* AND *drd1b* IN REGULATING SOCIAL DOMINANCE AND MOTOR BEHAVIORS

Introduction

Social dominance relationships are ubiquitous across various species to facilitate evolutionary success (Dreher & Tremblay, 2017). Many of the underlying neural circuits responsible for the establishment of social dominance, such as the ventral tegmental area (Groppe et al., 2013), the nucleus accumbens (Shan et al., 2022), and the amygdala (Fried et al., 2001), consist of dopaminergic (DA) signaling. Our lab is motivated to understand whether there are status-dependent changes in proteins within the DA signaling cascade and if these changes are a consequence of social dominance or instead influence social rank during pairing. Using zebrafish, we find that two proteins, Dat and Drd1b show status-dependent expression. We hypothesized that changes in protein expression of these two proteins are a result of social dominance rather than a contributing factor to who becomes subordinate. We find that neither decreased expression of Dat nor Drd1b influences establishment of stable social dominance. Further we show that that these two mutations independently alter motor behavior to resemble a subordinate phenotype.

As a social species, zebrafish engage in agonistic interactions to establish a stable dominance relationship (Miller et al., 2017, Clements et al., 2018). These interactions include attacks, chases, and retreats between two paired conspecifics (Balph, 1979; Francis, 1984). Dopamine is a major neuromodulator in regions associated with social dominance relationships, where multiple components of DA signaling have been implicated in the modulation of sociability of social aggression. These include presynaptic Dat (Rodríguez et al., 2004, Adriani et al., 2010; Enter et al., 2012) and postsynaptic Drd1 (Couppis et al., 2008; Plaven-Sigray et al., 2014; Yamaguchi et al., 2017; Homberg et al., 2016) and Drd2 (Nikulina & Kapralova, 1992;

Morrison et al., 2015; Mahadevia et al., 2021). Thus, we were interested in probing how social dominance relationships alter proteins in the DA signaling cascade in zebrafish and further test whether these changes are a consequence of social dominance or if they influence which animal becomes a dominant for subordinate.

In this study, we performed qPCR and Western Blot to assess status-dependent changes in protein expression in the DA signaling cascade. Based off these results we tested whether fish with a mutation in either *slc6a3* or *drd1b* formed stable dominance relationships to discern whether these two genes are important in the establishment of social dominance. If so, we anticipate seeing an unstable or lack of social dominance in fish with these mutations. Otherwise, they will form stable dominance relationships comparable to their wild type (WT) siblings and will suggest that observed expression changes are due to stable dominance.

In addition to the establishment of stable dominance relationships, there is evidence supporting that social dominance in teleost fish lead to altered escape and swimming behavior (Neumeister et al., 2010). We will examine whether stable dominance relationships with these two mutant lines lead to status-dependent escape and swimming behavior differences. Our results highlight the importance of looking at the potential reciprocal relationship of mutations in these two genes regarding (1) the establishment of stable social dominance and (2) the effects of status-dependent locomotor behavior.

Methods

Animal Maintenance

Adult zebrafish (*Danio rerio*) AB strain of mixed sex were group-housed in 10-liter tanks (~20 animals) at the core animal facility and kept at a constant temperature at 28°C under a 14 h/10 h light/dark cycle. Fish were fed twice daily with high protein commercial food (Otohime

B2, Reed Mariculture, CA, USA), and with newly hatched artemia (Brine Shrimp Direct, UT, USA). All experiments were performed in accordance with the Institutional Animal Care and Use Committee at East Carolina University.

RNA extraction

RNA extractions were performed using a modified phenol-chloroform protocol using Trizol reagent. Brains were dissected and placed in a 1.5 mL microcentrifuge tube. Samples were homogenized by gentle sonication on ice, 400 μ L additional Trizol was added, and then samples were incubated at room temperature for 5 min. 160 μ L chloroform was added, and the samples were mixed. Samples were incubated at 4 °C for 20 minutes with intermittent mixing, followed by centrifugation. The aqueous phase was transferred to a new, sterile tube, and 500 μ L ice-cold ethanol was added. The sample was loaded onto an RNeasy spin column (Qiagen) and spun. Samples were washed with buffer RW1, followed by two washes of Buffer RPE. RNA was eluted with two, 20 μ L volumes of RNase-free water into an RNase-free microfuge tube. RNA extracts were quantified by Nanodrop 2000 (Thermo), and then stored at -80°C until ready to use.

RT synthesis of cDNA stocks: Qiagen's QuantiText Reverse Transcription Kit was used for cDNA synthesis of RNA extracts. RNA was brought to a volume of 12 μ L with RNase-free water, and to eliminate any DNA contamination, 2 μ L Qiagen gDNA wipeout buffer was added and samples incubated at 42 °C for two minutes. Samples were incubated at 42°C for 30 mins for cDNA synthesis, then three minutes at 95°C for enzyme deactivation. Samples were brought up to a final RNA concentration of 10 ng/ μ L with RNase-free water and stored at -20°C until ready for qPCR.

qPCR primer design and validation: Primers were designed using NCBI Primer-BLAST software. All primers were designed to span an exon-exon junction, minimize secondary products, produce an amplicon of 75-150 bp, and have an annealing temperature of 60-67 °C. Primers were validated to confirm amplicon size, and to determine ideal primer concentration for qPCR reactions. 20 µL PCR reactions were run using GoTaq Flexi DNA Polymerase Reagents from Promega. Primer pairs were assessed at concentrations of 500, 750, and 900 nM final concentrations for amplicon amounts, and the presence of primer-dimer secondary product. Samples were run on 2 % agarose gels containing 0.5x GelRed for visualization under UV light, and pictures were taken with Alpha Innotech's FluorChem 8900 gel imaging system. Working primer concentrations were determined to be: 500 nM for all primer pairs.

qPCR primer efficiency determination: Standard curves for primer efficiencies were prepared using an initial cDNA amount of 30 ng total cDNA, then 1/10 dilutions were prepared. 20 µL reactions of each cDNA amount and each primer pair were run in triplicate on a 96-well PCR plate from Applied Biosystems. Primer pairs were used in predetermined optimal concentrations, and 10 µL 2x Applied Biosystems Fast SYBR green reaction mix was added for a total volume of 20 µL. Real-time PCR reactions were run on an Applied Biosystems Quantistudio 12k Flex Real-Time PCR System for Ct determination. Primer efficiency standard curves were prepared using the Quantistudio 12k Flex Real-Time PCR System software and are reported in Table 2.

qPCR reaction protocol: qPCR reactions were run using Applied Biosystems Fast SYBR Green Master mix, using Applied Biosystems Quantistudio 12k Flex Real-Time PCR System. 96 well plates were used with duplicate technical replicates, and 20 µL reactions were prepared. SYBR green detection occurred during each cycle's amplification stage. Passive reference dye

ROX was used for volume standardization. All samples were compared to internal housekeeping gene *actb2* for normalization, and isolate fish served as a biological control. Three biological replicates of each social group (Dominant, Subordinate, and Isolate) were analyzed on each 96-well plate. Multiple 96-well plates were run so that each gene had n=10.

qPCR data analysis: qPCR data was analyzed using the comparative $\Delta\Delta C_t$ method. C_t values were determined for each gene of each biological replicate, and experimental genes were compared to internal housekeeping gene *actb2* for determination of ΔC_t values. $\Delta\Delta C_t$ values were determined by normalization of dominant and subordinate social groups to social isolate controls, and fold-change was determined from $\Delta\Delta C_t$ values using the appropriate primer efficiencies. Fold-change values were transformed to a log base 2 scale for graphing and statistical analysis. Individual gene's fold changes were analyzed for significance by two-sided one sample t-test from a theoretical mean of 0, while fold changes between social groups (dominant and subordinate) were analyzed using a two-sided Wilcoxon signed rank test, $\alpha = 0.05$.

Western Blot

For western blot analysis, 4 separate western blot trials were conducted with 10 brains per trial for each social phenotype except for *slc6a3* in which an additional trial was conducted (n=5). Zebrafish were anesthetized with 0.02% MS-222 (1 min) then placed in iced water (10min). Brains were dissected out, placed in a 1.5 mL microcentrifuge tube, and stored at -20°C until use. Then they were prepared using the total membrane isolation protocol. Brains were homogenized in 1 ml of resuspension buffer: 2.5 ml 2 M Sucrose, 2 ml 10x 10 mM Tris-HCl, 400 μL 0.25 M EDTA, 40 μL 20x protease cocktail inhibitor. The homogenized brains were

centrifuged (2,000 rpm, 4°C, 10 min) followed by ultra-centrifugation (37,000 rpm, 4°C, 60 min). Protein sample concentrations were determined with a Lowery protein assay.

For western blots, 10 µg of each protein sample was denatured using 4X buffer containing 10X reducing reagent at 70°C for 10 min and loaded onto a Mini-PROTEAN® TGX™ Precast Protein Gels (Bio-Rad, Hercules, CA) and run ~120min at 60V. The proteins were transferred to a 0.2 µm nitrocellulose membrane using Trans-Blot® Turbo™ RTA Mni Transfer Kit (Bio-Rad, Hercules, CA). The membrane was blocked with PBS containing 5% (w/v) nonfat dry milk and 0.1% (v/v) Tween 20 for 1 h at room temperature and then incubated with primary antibodies overnight at 4°C. After three washes with PBS containing 0.1% (v/v) Tween 20, the membrane was incubated with HRP-conjugated goat anti-mouse or goat anti-rabbit IgG (Cell Signaling) secondary antibody for 1 hr. After washing, protein detection was performed using Horseradish peroxidase (HRP) Detection kit (SuperSignal™ West Pico, Thermo Fisher Scientific) and visualized using the ChemiDoc Imaging System (Bio-Rad). Bands were quantified with ImageLab software and normalized to β-actin (Bio-Rad, Hercules, CA). Primary antibodies used were as follows: mouse anti-DRD1 (1:500 Novus Biological), Rabbit anti-DAT (1:500 ProteinTech Group Inc.), β-actin (1:1000 Cell Signaling).

Design of *slc6a3* gRNA for CRISPR/Cas9

We identified 5'GG(N₁₅)-NGG3' target sequence in exon 3 of *slc6a3* (GGATCCACTGCAACAACACCTGG) (Figure 1). The target oligonucleotide was designed with the intention of disrupting the BamHI restriction site (5'GGATCC'3) for easy identification of mutation by restriction digest. Synthesis of single guide RNAs (sgRNAs) were based on a protocol described previously (Jao et al., 2013). Cas9 protein was ordered from ThermoFisher (Cat#A36496).

Use of *drd1b* mutant line

Sanger-generated *drd1b*^{sa16476} that results in a G>A point mutation at Chr 9: 54418464 and a premature stop codon (Busch-Nentwich et al., 2013) were generously gifted from Dr. Teresa Nicolson's lab. For this dissertation, homozygous mutants, *drd1b*^{sa16476}, will be notated as *drd1b*^(-/-), heterozygous siblings, *drd1b*^{sa16476}/*drd1b*⁺, will be notated as *drd1b*^(+/-), and WT siblings, *drd1b*⁺, will be notated as *drd1b*^(+/+). Protein nomenclature follows the ZFIN nomenclature guidelines: Drd1b. Without proper confirmation of protein expression changes, all results are discussed in genomic context rather than protein expression. Further experiments need to be done to confirm changes in protein expression in *drd1b*^(-/-) compared to *drd1b*^(+/+) controls.

slc6a3 mutant line construct

To generate a founder line (F₀), embryos were collected within 10 minutes of natural spawning of in-crossed WT fish. Eggs were microinjected at one or two-cell stages. Approximately 100 ng/ul of sgRNA and 1 ng/ul of Cas9 protein were co-injected into the eggs using a capillary micropipette. To measure efficiency and lethality, a subset of sibling embryos that were not microinjected were used as controls. At 2-5 days post fertilization (dpf), 30 embryos of either injected or control fish were placed in 30 ul of 50 mM NaOH and were dissolved by placing samples into a thermocycler (Mastercycler Nexus Gradient) at 99°C for 15-minutes. Genomic DNA was amplified using Polymerase Chain Reaction (PCR). A mutant-specific primer was designed according to the mutated gene of interest (*slc6a3*-Forward-5'-TTTATGAGGCGTGGGCTTCA-3'; *slc6a3*-Reverse-5'-GGGCAAATGTCCCCACAAGT-3'; *drd1b*-Forward-5'-CCTGTGGCCATTATGCTGGT-3'; *drd1b*-Reverse-5'-AGCAAGATGGAGAATGC-3'). The PCR program was optimized for efficient identification of the mutation of interest using MyTaq Red 2x Mix (BioLine Cat #BIO-21108). Initial

denaturation of 94°C for 1 minute, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for *drd1b* or 60°C for *slc6a3* for 15 seconds, extension at 72°C for 10 seconds followed by an additional extension at 72°C for 5 minutes. Mutation rates were estimated based on comparing undigested PCR product to digested PCR product using a BamHI restriction endonuclease (ThermoFisher Cat#FD0054). The remainder of the clutches were raised to adulthood.

The remainder of the F₀ generation of injected fish were outcrossed to WT fish. To identify specific induced mutations, fins of 2-month-old fish (WT and microinjected) were clipped and placed in 50 ul of 50 mM NaOH. Genomic DNA was extracted from these samples by placing them in a thermocycler at 99°C for 15 minutes. Dissolved samples were run through the PCR protocols described above, followed by restriction digest using BamHI. PCR samples were purified and ligated into a TOPO-TA cloning vector and transformed. Cells were plated on bacterial agar plates containing ampicillin and colonies were picked, grown overnight, purified, and sent for sequencing. Mutated and WT sequences (Figure 1) were compared, and a 95 bp overall change was identified. This mutant line is being registered with ZFIN as a CRISPR-*slc6a3* with the university identifier of *ecu20*. For this dissertation homozygous mutants, *slc6a3^{ecu20/ecu20}*, will be notated as *slc6a3^(-/-)* and WT siblings, *slc6a3⁺*, will be notated as *slc6a3^(+/+)*. Protein nomenclature for *slc6a3* follows the ZFIN nomenclature guidelines: Dat. Without proper confirmation of protein expression changes, all results are discussed in genomic context rather than protein expression. Further experiments need to be done to confirm changes in protein expression in *slc6a3^(-/-)* compared to *slc6a3^(+/+)* controls.

Social Dominance Formation and Quantification

Sexually mature adult males (4-12 months of age) for each genotype (*slc6a3*^(-/-) and *slc6a3*^(+/+); *drd1b*^(-/-) and *drd1b*^(+/+)) were isolated for one week to establish a baseline among all individuals. After isolation, males were age- and size-matched for a two-week pairing period. during which pairs were filmed daily for 5 minutes using a Canon Camcorder (Digital video ZR500) to monitor changes in tank territorial preference and to quantify number of attacks from each fish during social dominance formation. Dominance rank (social dominant or subordinate) was assigned based on the total number of attacks and retreats quantified for each fish over the two-week pairing period. Fish with more frequent and consistent attacks were determined as dominants, while fish with more retreats were assigned subordinate status. If there were switches or inconsistencies between which fish attacked or retreated on any given day throughout the pairing period, fish that attacked more on the final day were assigned dominant and it was marked that the pair was not stable.

Heatmap Generation

To determine place preference in the pairing tank, the daily 5-minute videos were analyzed. Interactions during the video period were removed using Adobe Photoshop, and the video was rendered into an image sequence using 4500 frames at a 4 frame-per-second (fps). Movement of each fish was analyzed using the ImageJ tracking software RRID:SCR_003070 Manual Tracking plugin as described previously (Miller et al., 2017). Using motion tracking coordinates, *stat_density_2D* plots were generated using the *ggplot2* package in RStudio.

Measuring Escape Behavior

After the pairing, fish were temporarily separated, and behavioral testing was performed sequentially to measure the effect of social status on short-latency escape response. Each fish was placed in a testing chamber (dimensions: 11 x 4 x 3cm) filled with double distilled water

having a resistance of $\sim 15 \text{ M}\Omega \bullet \text{cm}$. High resistance water increases signal to noise ratio and was shown to have no adverse effects on the health of the fish⁴⁶. A pair of conductive electrodes placed on either side of the chamber recorded the electric field potentials. Bare electrodes were 1 mm in thickness with 3-5 mm metal exposure. Electrodes were connected to an AC differential amplifier (AM-Systems model 1700, Carlsborg, WA USA), allowing the amplification of signals 1000-fold. Electrical signals were low-pass filtered at 300 Hz and high-pass filtered at 1 KHz. Signals were digitized using a Digidata-1322A digitizer and stored using Axoscope software (Molecular Devices, Inc., Sunnyvale, CA, USA). The animals were acclimatized for 30 minutes before behavioral testing was initiated.

Measuring Swimming Behavior

Following the 30-minute acclimation period in the setup described above, the animal's spontaneous swimming behavior was recorded for 1 minute. Swimming activity was measured by counting swim bursts with Clampfit software. The "Threshold" function was used for this purpose. A swim potential was marked as a swim burst if it was at least 8 mV in total amplitude and 30-200 ms in duration (Miller et al., 2017). The swim burst onset was tabulated into a Microsoft Excel spreadsheet and results were plotted using Prism (GraphPad) software.

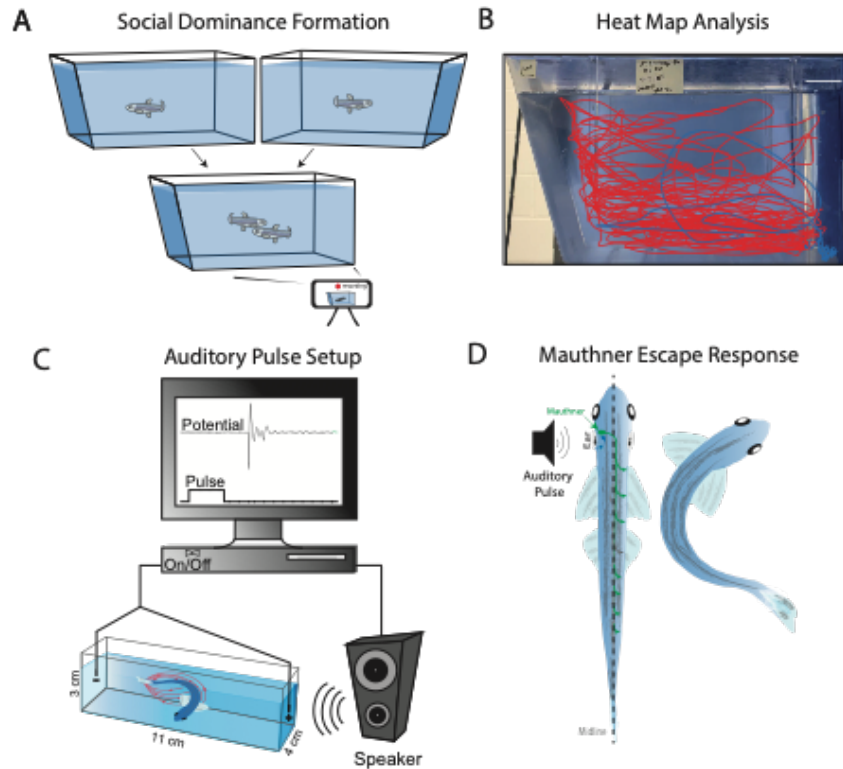


Figure 9. Experimental setup for social dominance and escape testing. (A) Social dominance formation and video collection. (B) Analysis of dominant and subordinate place preference in the pairing tank via motion tracking. (C) Escape probability setup. (D) Representation of the auditory-stimulated M-cell escape response.

Data Analysis and Statistics

Statistical analysis for attacks and escape behavior were done in RStudio using the following packages: gt, tidyverse, gridExtra, betareg, and rstatix (RStudio Team, 2011). Attack plots were made in RStudio using the ggplot2 package. Swimming data was analyzed using GraphPad software (GraphPad Inc., San Diego, USA) and IBM-SPSS (RRID:SCR_002865).

Results

slc6a3 and *drd1b* expression are downregulated in subordinates

To determine the effects of social status on DA signaling and spinal motor activity, we measured changes in expression patterns of genes involved in DA signaling. We found that

expression of *drd1b* was significantly reduced in subordinates relative to dominants and control isolates (Figure 10A). Conversely, levels of dopamine reuptake transporter (*slc6a3*) were significantly elevated in both dominants and subordinates compared to control isolates and the increase in *slc6a3* expression was significantly different between dominants and subordinates.

Dat and Drd1b protein levels are decreased in subordinates compared to dominants

Western blot analysis of protein expression mirrored our qPCR results, where Drd1b protein expression was decreased in subordinates compared to dominants, while differences in Dat protein expression between dominants and subordinates were less obvious (Figure 10B).

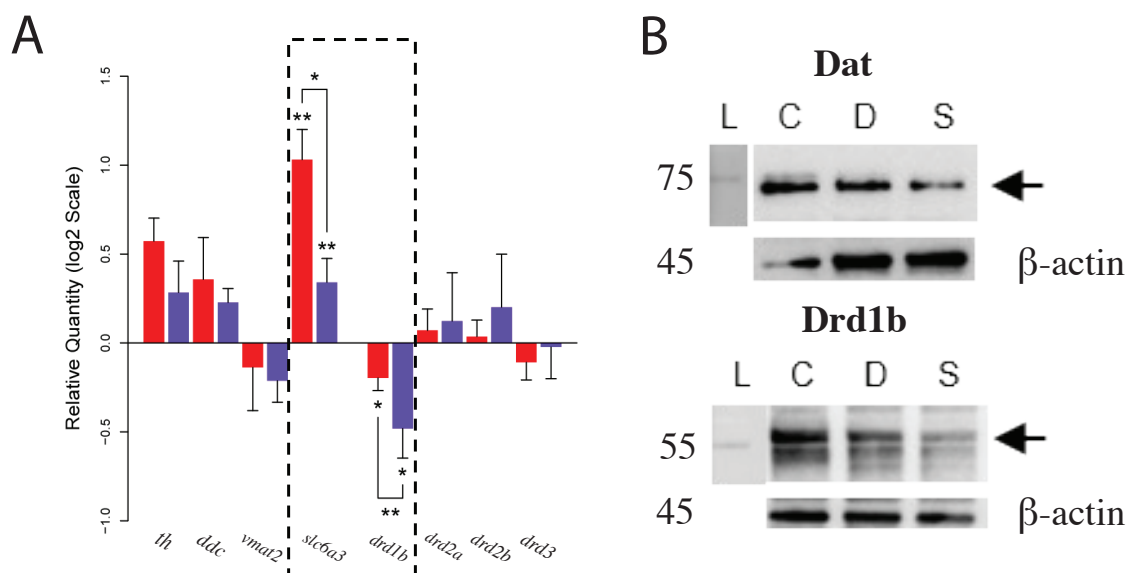


Figure 10. *slc6a3* and *drd1b* expression is decreased in subordinates compared to dominants. (A) Expression of DA pathway genes in whole brains (n=10 pairs) by qPCR using the $\Delta\Delta C_t$ method. *actb2* is used as an internal reference gene, and expression from social isolates (n=10) used for standardization. Bars represent mean and SEM. Fold changes transformed to a log base 2 scale for statistical analysis and more accurate representation of expression change data. Zero indicates no change in gene expression relative to isolate control. (B) Western blot analysis of Drd1b receptor and Dat with β -actin serving as a control. Protein expression of dominants, subordinates were normalized to WT group-housed controls as a ratio (illustrated below each band). Bar graphs represent average percent change in protein concentration of four replicates of samples (Drd1b) and five replicates of samples (Dat). Each replicate consisted of 10 brains normalized to WT group-housed fish.

Establishment of *slc6a3* mutation in Zebrafish

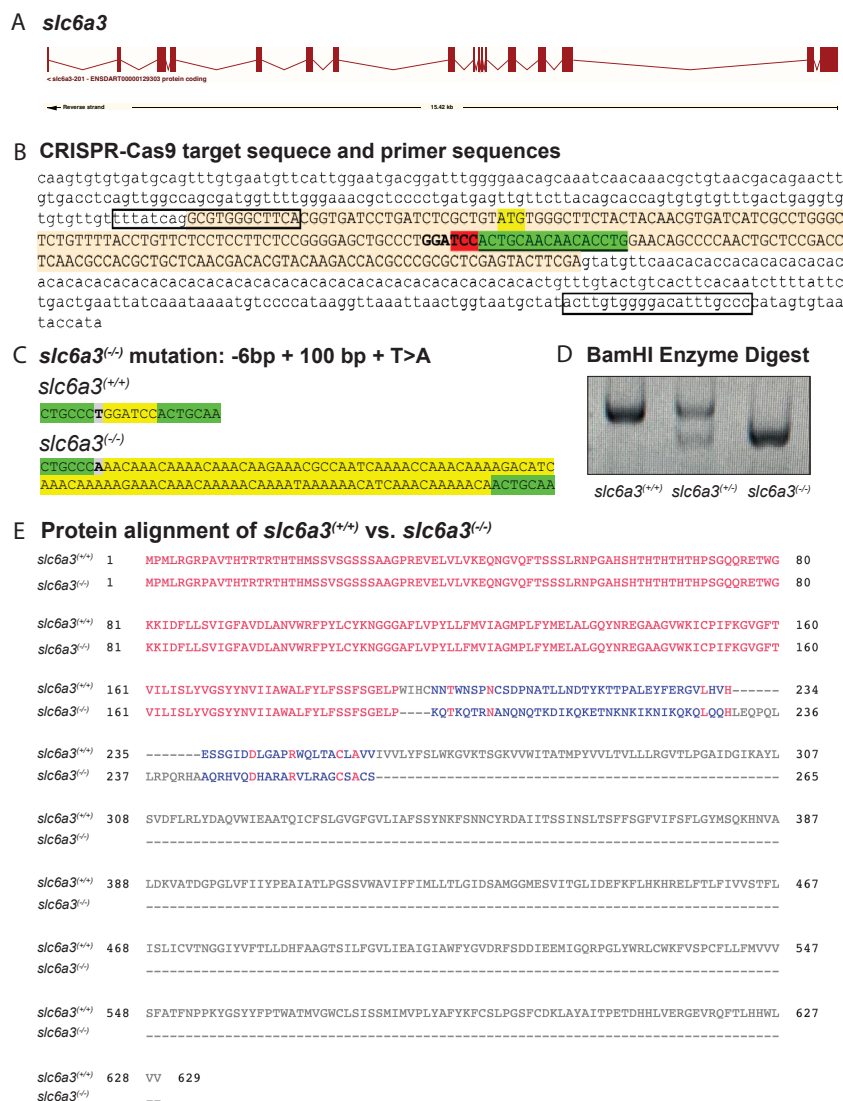


Figure 11. Establishment of *slc6a3* mutant. (A) protein coding sequence of *slc6a3* (B) Target region for CRISPR on exon three. Green indicates guide sequence. Red indicates Cas9 site. Boxed sections represent fwd and rv primers. (C) Comparison between WT and *slc6a3* sequence at mutation site. There was a deletion of 6 bp of the BamHI site, insertion of 100 bp, and a point mutation T>A. (D) Enzyme digest for identifying WT, heterozygous, and homozygous mutants. (E) Protein alignment between WT and *slc6a3*^(-/-) that results in early stop codon at AA265.

To test whether a mutation in *slc6a3* can alter the establishment and maintenance of social dominance relationships, we used CRISPR/Cas9 to generate a *slc6a3* mutant line by targeting the third exon of *slc6a3* (Figure 11A, B). This induced an overall 95 bp insertion (101 bp insertion with the 6 bp BamHI restriction site deleted and a T>A point mutation at the beginning of the gRNA target sequence) (Figure 12C). Fish were identified using an enzyme digest for BamHI (Figure 11D). Overall, this mutation leads to an early stop codon at AA265 (Figure 11E). However, confirmation at the protein level is still needed.

***slc6a3*^(-/-) leads to a stable social dominance relationship**

To test whether presumed decreased Dat via *slc6a3*^(-/-) leads to an unstable social dominance relationship we measured the number of agonistic interactions and swimming patterns between paired male mutants (Figure 12A, B). As with *slc6a3*^(+/+) controls, *slc6a3*^(-/-) showed a stable social dominance relationship that was formed on day of pairing and lasted throughout the observation period, (Figure 12A, B). Further, *slc6a3*^(-/-) showed no significant differences in aggression level as determined by the number of attacks over the 14-day pairing period compared to *slc6a3*^(+/+) controls according to Welch's t-test, $t(162.83) = -1.88$, $p = 0.06$ (Figure 12C). Although not statistically significant, a higher sample size could yield significant differences in aggressive behavior between *slc6a3*^(-/-) and *slc6a3*^(+/+). However, these results suggest that *slc6a3*^(-/-) does not alter the establishment and stability of social dominance.

***drd1b*^(-/-) leads to a stable dominance relationship**

To test whether *drd1b*^(-/-) influences the establishment and maintenance of social dominance relationships, we tracked the agonistic interactions between paired *drd1b*^(-/-), as well as *drd1b*^(+/+) (Figure 12D, E) and *drd1b*^(+/-) siblings (data not shown). As with *drd1b*^(+/+) siblings, *drd1b*^(-/-) formed social dominance relationships on day one and remained stable over the 14-day

pairing period (Figure 12D, E). When compared to *drd1b*^(+/+) sibling controls, *drd1b*^(-/-) showed no difference in the number of attacks over the pairing period according to Welch's t-test, t

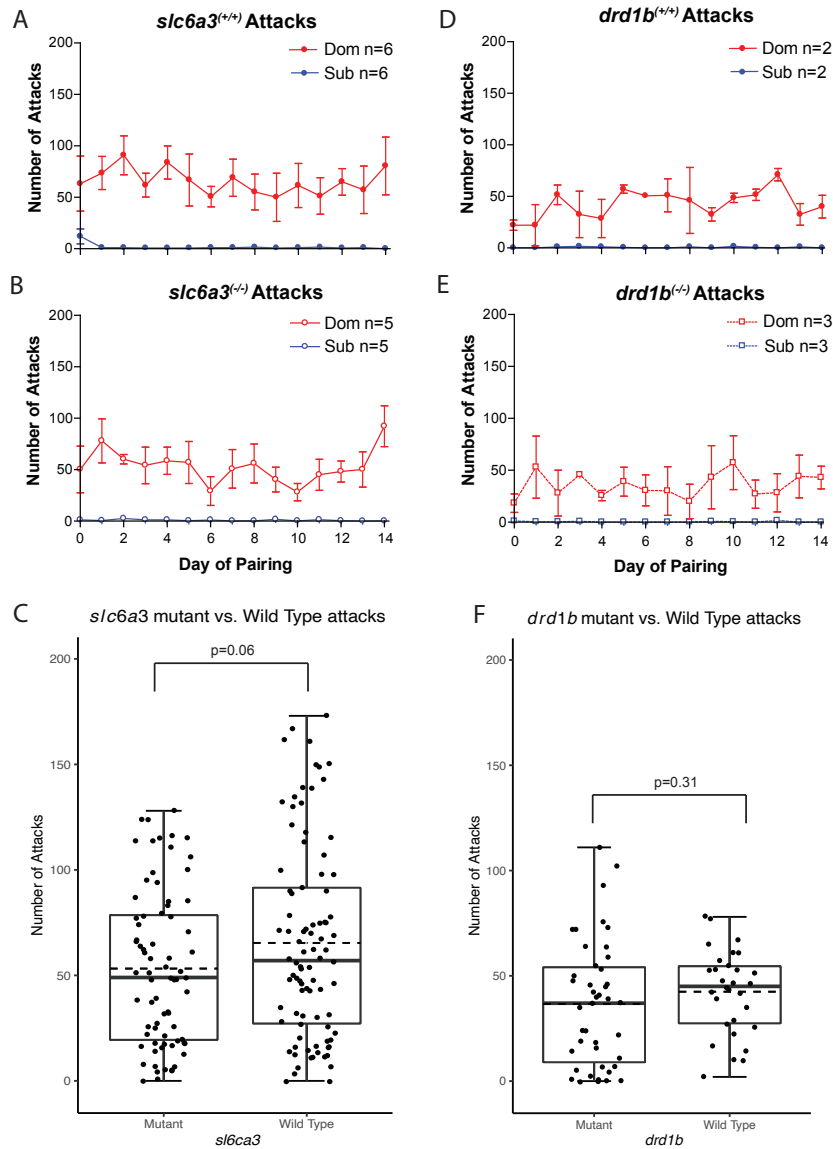


Figure 12. Social dominance formation comparison between *slc6a3*^(-/-), *drd1b*^(-/-) and WT siblings. (A-B) Total number of attacks by dominants and subordinates from both *slc6a3*^(+/+) and *slc6a3*^(-/-), over the 14-day pairing period. Both *slc6a3*^(+/+) and *slc6a3*^(-/-), presented stable dominance with no switching of dominance ranks during pairing (C-D) Total number of attacks by dominants and subordinates from *drd1b*^(+/+) and *drd1b*^(-/-) over the 14-day pairing period. Both *drd1b*^(-/-) and *drd1b*^(+/+) siblings exhibited stable dominance.

(70.89) = -1.00, $p = 0.32$ (Figure 12F). These results suggest that a mutation in *drd1b* does not influence the formation of social dominance relationships.

***slc6a3*^(-/-) fish show altered place preference in pairing tanks**

During initial observations of group-housed *slc6a3*^(-/-) we noticed an obvious swimming phenotype, in which shoaling fish exhibited bottom-dwelling behavior consistent with previous findings (Kacprzak et al., 2017). Given that WT zebrafish display a clear status-dependent place preference (Miller et al., 2017), we were interested in determining whether a mutation in *slc6a3* would affect place preference. Thus, we conducted motion tracking analysis and measured the XY coordinates of both group-housed and paired fish in their pairing tank on Day 0 and Day 14 post-pairing (Figure 9B). We observed that place preference differed between *slc6a3*^(-/-) and *slc6a3*^(+/+) controls on Day 0 (Figure 13A, B), where *slc6a3*^(-/-) fish presented extreme bottom-dwelling behavior. Further, we see that stable dominance on Day 14 does not alter this bottom-dwelling behavior (13C, D). These results suggest that a mutation in *slc6a3* alters the exploration of the tank, which is not altered by stable dominance.

***drd1b*^(-/-) show no difference in place preference compared to *drd1b*^(+/+)**

To determine whether *drd1b* expression regulates place preference behavior in adult zebrafish, we compared place preference between *drd1b*^(-/-) and *drd1b*^(+/+) at Day 0 and Day 14 of pairing by measuring the animals' XY coordinates on Day 0 and 14 post pairing. On both Day 0 and Day 14, there was no difference in preference between *drd1b*^(-/-) and *drd1b*^(+/+) (Figure 13E-G). *drd1b*^(-/-) showed healthy exploration of the tank before social dominance, and then shifted preference once dominance was established. These results suggest that a mutation in *drd1b* does not alter exploration and place preference in the tank compared to *drd1b*^(+/+).

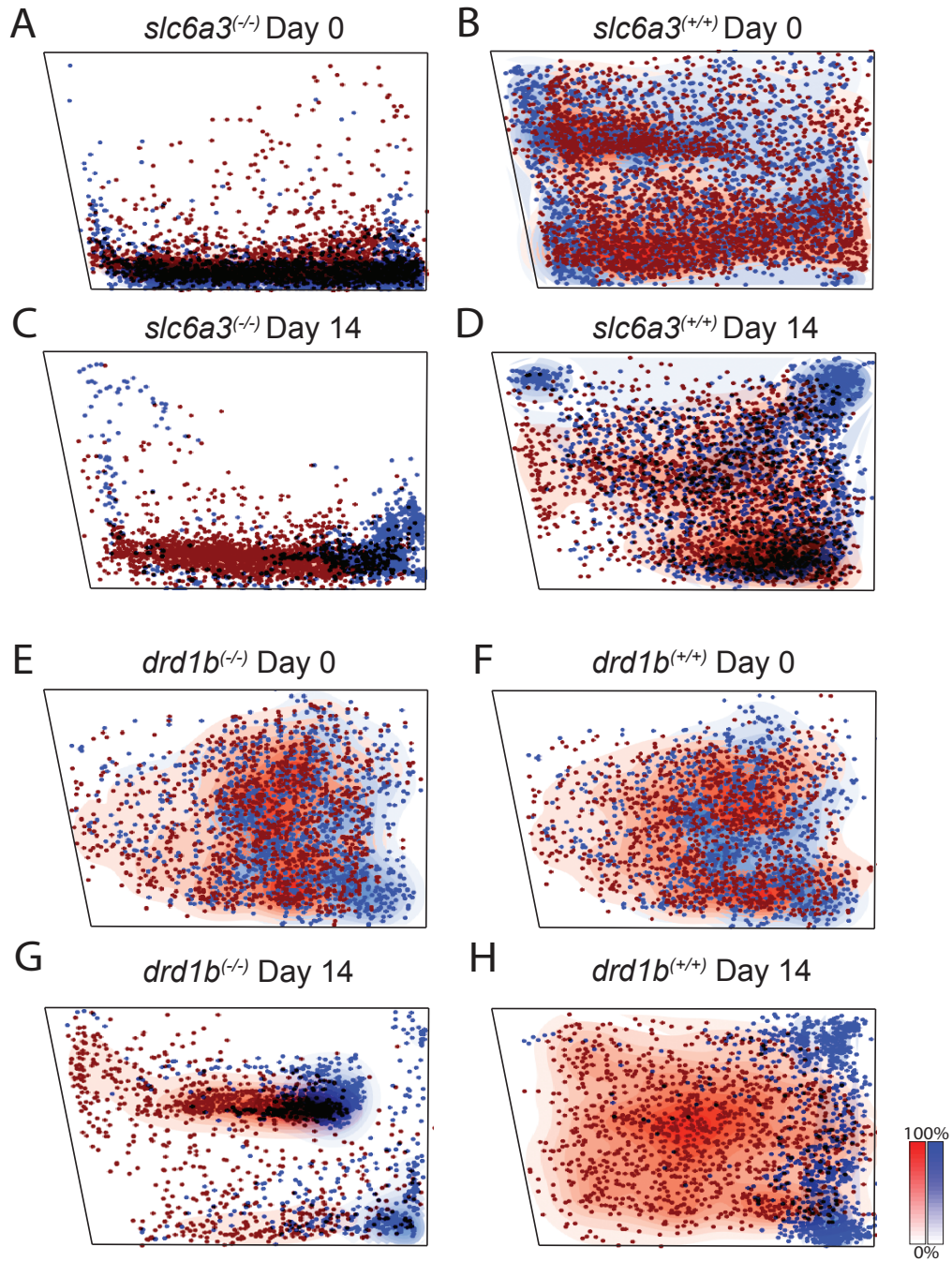


Figure 13. Comparison of place preference in pairing tanks between *slc6a3*^{-/-}, *drd1b*^{-/-} and WT siblings. (A-D) Representation of swimming activity between dominants and subordinates on Day 0 and Day 14 of pairing between *slc6a3*^{-/-} and *slc6a3*^{+/+} siblings. (E-H) Comparison between *drd1b*^{-/-} and *drd1b*^{+/+} place preference on Day 0 and Day 14.

slc6a3^{-/-} show no differences in escape activity compared to *slc6a3*^{+/+} siblings

Previously, we showed that the startle escape response is socially regulated (Miller et al., 2017). The sensitivity of the M-cell escape response was enhanced in subordinates compared to dominants. Since *slc6a3*^(-/-) showed stable social dominance relationships, but altered place preference in the pairing tanks, we wanted to determine whether this mutation influenced escape response probability and how that might change because of stable dominance. To test this, we placed individual fish of either group-housed, dominant, or subordinate social status into a small testing chamber with two recording electrodes that allowed the detection of far-field muscular potentials during escape and swimming activities (Figure 9C, D). We saw that group-housed *slc6a3*^(-/-) showed no difference in escape probability compared to *slc6a3*^(+/+) controls (Figure 14A). We ran a generalized linear model regressing response probability against decibel level between *slc6a3*^(-/-) and *slc6a3*^(+/+) groups where there was no significant difference in escape probability between group housed groups based on genotype ($p = 0.52$). Furthermore, we observed the same status-dependent shift in escape probability in *slc6a3*^(-/-) pairs as was observed in *slc6a3*^(+/+) control pairs, whereby subordinates show an increased escape probability and dominants showed no difference in escape compared to group-housed controls (Figure 14B). We ran a generalized linear mixed model regressing response probability of both genotype and social status. There was no significant difference between genotype ($p = 0.83$), but there was significance between social status ($p = 0.01$). These results support that a mutation in *slc6a3* does not alter excitability of escape behavior.

***slc6a3*^(-/-) show decreased swimming activity compared to *slc6a3*^(+/+)**

In WT zebrafish, swimming activity is socially regulated whereby dominants increase their swimming frequency while subordinates reduce their swimming activity (Miller et al., 2017). Here, we tested whether a mutation in *slc6a3* affects swimming frequency. Given that

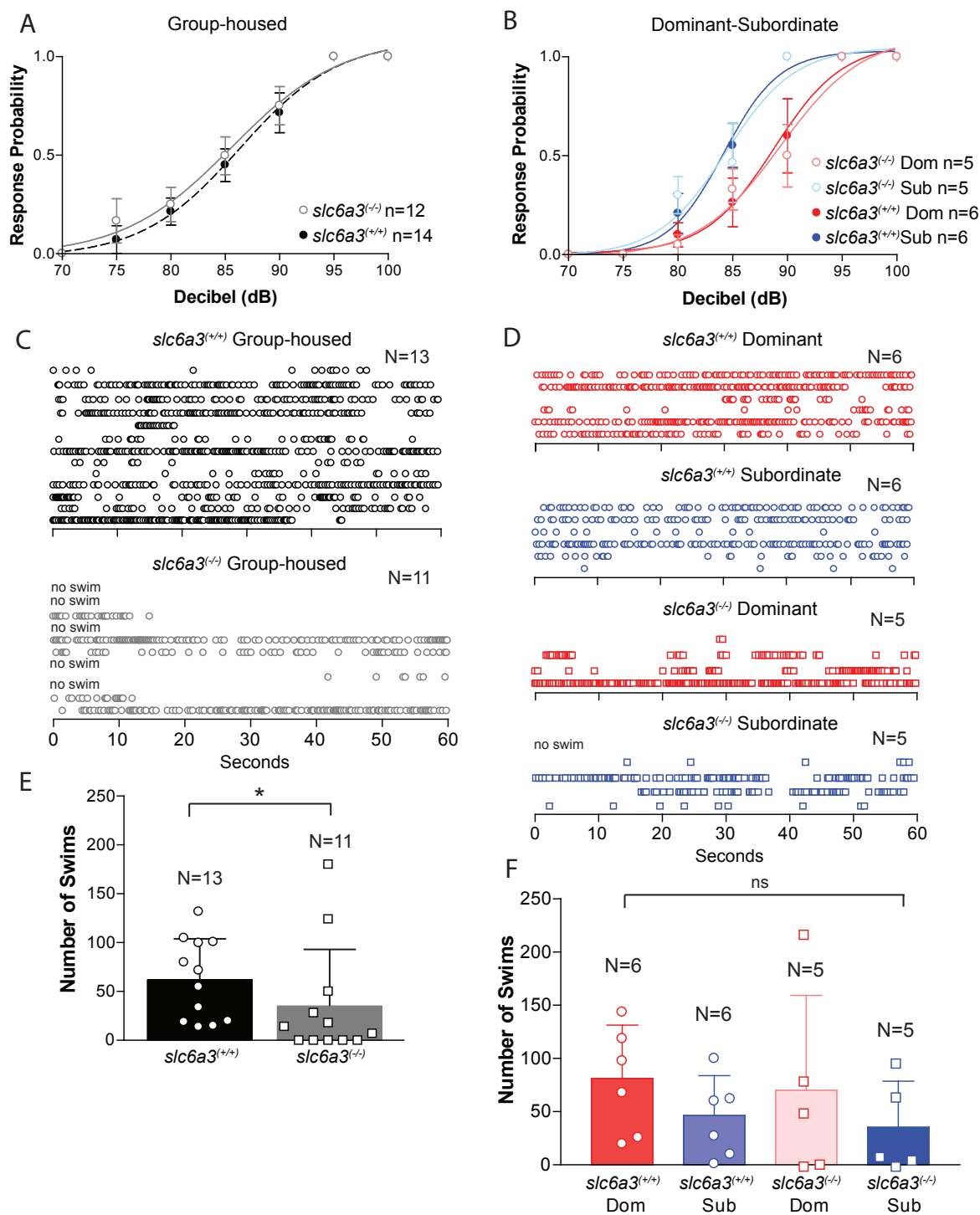


Figure 14. Escape and Swimming behavior for *slc6a3*^(-/-) and *slc6a3*^(+/+) siblings. Escape probability between group-housed *slc6a3*^(-/-) and *slc6a3*^(+/+) (A) and dominants/subordinate *slc6a3*^(-/-) and *slc6a3*^(+/+) (B). (C-D). Raster plots illustrating individual swim bursts (dots) per fish (row). (E-F) Binned swimming for group-housed *slc6a3*^(-/-) and *slc6a3*^(+/+) and binned swimming for dominants and subordinates for *slc6a3*^(-/-) and *slc6a3*^(+/+).

group-housed and paired *slc6a3*^(-/-) fish displayed anxiety-like behavior illustrated in bottom-

dwelling reminiscent of submissive behavior in WT animals, we postulated that *slc6a3*^(-/-) fish would also show reduced swimming activity as observed in WT submissive fish. Indeed, we found that in group-housed conditions, *slc6a3*^(-/-) fish significantly differed from their *slc6a3*^(+/+) counterparts (Figure 14C, E), where *slc6a3*^(-/-) showed a significant decrease in swimming frequency (Mann-Whitney Two-tailed test P=0.0327) and that *slc6a3*^(-/-) had zero swimming activity in 5/11 animals compared to 0/12 in *slc6a3*^(+/+). However, it appears that social dominance rescues the swimming frequency of *slc6a3*^(-/-) dominants. Results from a factorial ANOVA show that there is no significance in swimming between genotype, social status, or the interactions between genotype*social (p = 0.17, p = 0.67, p = 0.99, respectively). These results may be skewed by the single instance of a *slc6a3*^(-/-) dominant that presented over 200 swim bursts and was not a statistical outlier via IQR assessment. Taken together, results suggest that a mutation in *slc6a3* regulates swimming locomotor behavior and that it may be altered by social status.

***drd1b*^(-/-) show increased escape sensitivity compared to *drd1b*^(+/+) and *drd1b*^(+/-)**

Cachope and Pereda previously showed that dopaminergic modulation of M-cell excitability is mediated through the activation of Drd1 (Cachope and Pereda, 2015). To test whether a mutation in *drd1b* affects escape probability, we tested *drd1b*^(-/-), *drd1b*^(+/+) siblings, and *drd1b*^(+/-) siblings. We saw that in a group-housed setting that *drd1b*^(-/-) showed increased escape sensitivity compared to *drd1b*^(+/+) controls, whereas *drd1b*^(+/-) siblings showed an intermediate escape sensitivity (Figure 15A). We ran a generalized linear mixed model regressing response probability based on genotype. There was no significant difference between *drd1b*^(-/-) and *drd1b*^(+/+) (p = 0.08), but this non-significant result is due to a small sample size (*drd1b*^(-/-) n=3 and *drd1b*^(+/+) n=2). This trend suggests that significance may be reached with an

increased sample size. In addition there was no significance between *drd1b*^(+/-) and *drd1b*^(+/+) ($p = 0.33$), but again this non-significant result may be due to the small sample size in the *drd1b*^(+/+) siblings. After social dominance formation, we saw that social dominants in the *drd1b*^(-/-) fish maintained an elevated escape response probability (Figure 15B). We ran a generalized linear mixed model regressing the response probability based on genotype and social status and saw a significant difference in escape response based on genotype ($p = 0.0001$) and no significance based on social factor ($p = 0.91$). This is due to the increased escape response probability of dominant *drd1b*^(-/-) comparable to social subordinates. These results show that although *drd1b*^(-/-) does not influence the establishment of social dominance, a mutation in *drd1b* shifts escape probability to reflect a subordinate phenotype regardless of social rank.

***drd1b*^(-/-) showed decreased swimming behavior compared to *drd1b*^(+/-) but not *drd1b*^(+/+)**

To test whether a mutation in *drd1b* alters swimming frequency we quantified swimming frequency of group-housed *drd1b*^(-/-), *drd1b*^(+/-) and *drd1b*^(+/+) siblings (Figure 15C). We ran a one-way ANOVA for group-housed *drd1b*^(+/+), *drd1b*^(+/-), and *drd1b*^(-/-) and saw no significance ($p = 0.06$), however, this is likely due to the low sample size. The trend suggests that a higher sample size would lead to a significant decrease in *drd1b*^(-/-) swimming. The single instance of zero swimming from one *drd1b*^(+/+) fish significantly shifted swimming analysis for this group. After pairing, we observed that stable dominance rescued swimming behavior in both *drd1b*^(+/-) and *drd1b*^(-/-) dominants (Figure 15F). A one-way ANOVA resulted in no statistical significance between social groups and genotypes ($p = 0.35$). These results support that although *Drd1b*

appears to be involved in swimming frequency, that alternate mechanisms may assist in

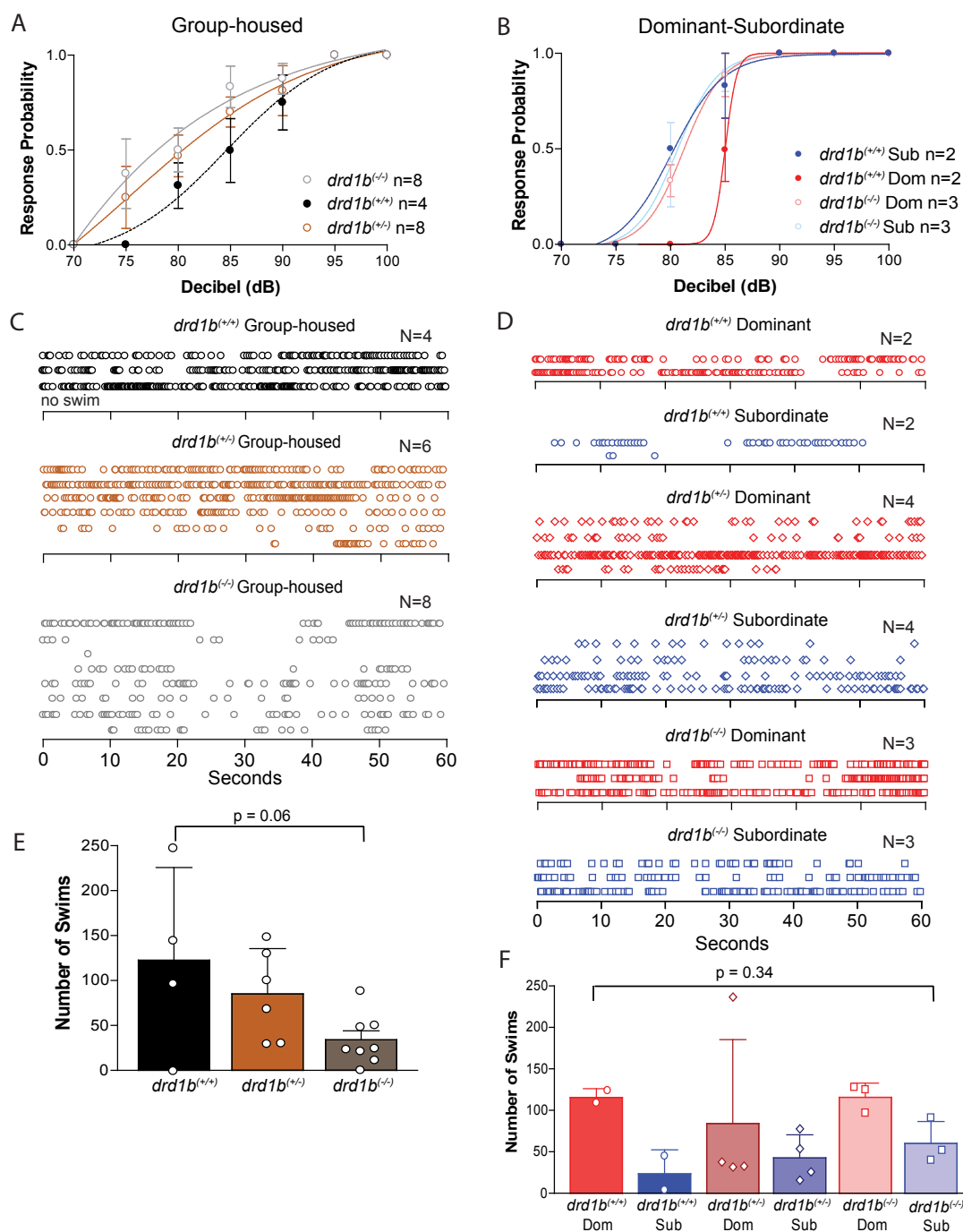


Figure 15. Escape and Swimming behavior for $drd1b^{(-/-)}$, $drd1b^{(+/+)}$, and $drd1b^{(+/-)}$ siblings. Escape probability between group-housed $drd1b^{(-/-)}$, $drd1b^{(+/+)}$, and $drd1b^{(+/-)}$ siblings (A) and dominants/subordinates (B). (C-D). Raster plots illustrating individual swim bursts (dots) per fish (row). (E-F) Binned swimming for group-housed $drd1b^{(-/-)}$, $drd1b^{(+/+)}$, and $drd1b^{(+/-)}$ and binned swimming for dominants and subordinates.

overriding decreased swimming once stable dominance has formed.

Discussion

Results from these experiments support the hypothesis that decreased *slc6a3* and *drd1b* expression may occur due to social dominance rather than influencing social dominance. Using a genetic approach, we observed that mutations in either *slc6a3* or *drd1b* do not regulate social aggression, dominance formation, and instead, regulate motor activity (escape and swim behavior) in zebrafish. Mutations in *slc6a3* and *drd1b* led to a subordinate-like motor phenotype, resulting in decreased swimming frequency and increased escape sensitivity, respectively.

Both *slc6a3*^(-/-) agonistic behavior and escape sensitivity is comparable to *slc6a3*^(+/+) controls (Figures 12, 14). Interestingly, we observed differences between *slc6a3*^(-/-) and *slc6a3*^(+/+) in swimming preference in the pairing tank (Figure 13). While *slc6a3*^(+/+) fish showed high levels of tank exploration before dominance formation, *slc6a3*^(-/-) exhibited bottom-dwelling behavior. This remains even after the establishment of a dominance relationship (Figure 12C); where both dominant and subordinate *slc6a3*^(-/-) maintain bottom-dwelling behavior. This behavior was first described in 2017, where *slc6a3*^(-/-) were reported to exhibit bottom-dwelling and thigmotaxis, reflecting an anxiety-like state (Kacprzak et al., 2017), which was replicated in an additional group of generated *slc6a3*^(-/-) (Wang et al., 2019). Further, *slc6a3*^(-/-) led to decreased tyrosine hydroxylase (TH) expression in regions known for their role in fear response (Cho & Fudge, 2010) and control of voluntary movement (Hegarty et al., 2013). Yet, our *slc6a3*^(-/-) line showed a stable dominance relationship with aggressive displays comparable to *slc6a3*^(+/+) siblings. These results support that when paired, alternate neural networks, such as the ventral tegmental area (Groppe et al., 2013; Solie et al., 2022) and nucleus accumbens (Ikemoto et al.,

1999; Kohls et al., 2013; Dölen & Malenka, 2014), that are involved in motivated social behavior may play a larger role in the motivation for forming stable dominance relationships.

We also observed a significant decrease in *slc6a3*^(-/-) swimming frequency compared to *slc6a3*^(+/+) siblings (Figure 14), suggesting that dopamine reuptake influences spinal circuits involved in rhythmic locomotor patterns. Interestingly, 45% of *slc6a3*^(-/-) control fish showed no swimming, compared to 0% in *slc6a3*^(+/+) siblings (Figure 14C), supporting previous studies that claim Dat mutants exhibit an anxiety-like phenotype (Kacprzak et al., 2017). In previous studies, Dat expression has been implicated in many cognitive processes (Rodríguez et al., 2004; Erixon-Lindroth et al., 2005; Brehmer et al., 2009), but there is some conflict between the role of Dat on locomotion. Studies in primates (Yue et al., 2012) and rats (Nikolaus et al., 2014) show that decreased DAT leads to decreased motor behavior, whereas studies in mice provide evidence that decreased DAT leads to hyperactivity (Jones et al., 1998; Spielwoy et al., 2009). Our results provide evidence that a mutation in *slc6a3* leads to decreased locomotor circuit activity in zebrafish.

In addition to Dat, our study has provided evidence that a mutation in *drd1b* does not influence social dominance formation or place preference. Group-housed and paired *drd1b*^(-/-) showed a WT phenotype in both aggression levels and place preference (Figure 12, 13). Interestingly, *drd1b*^(-/-) showed an increased escape probability regardless of social phenotype (Figure 14). This escape probability is comparable to *drd1b*^(+/+) subordinates, where the underlying motor circuit responsible for the escape response is activated more readily. These results support that Drd1b expression changes as a function of social subordination.

Although not statistically significant, swimming frequency was also altered in *drd1b*^(-/-), where mutants exhibited decreased swimming frequency compared to *drd1b*^(+/-) and *drd1b*^(+/+).

siblings. We suspect that this lack of significance is due to the small sample size in the *drd1b*^(+/+) group (n=4), but the results show a clear trend in the reduced swimming frequency between genotypes. Our results support the notion that decreased *Drd1b* leads to lowered locomotion in other species, ranging from the honeybee (Mustard et al., 2010), rodents (Bardgett & Henry, 1999), and primates (Beninger et al., 1991).

In conclusion, although the dopamine pathways have been implicated in social dominance formation (Qu et al., 2017; Shan et al., 2022), we show that mutations in both *slc6a3* and *drd1b* do not influence aggression or dominance formation in zebrafish. However, this study highlights key differences between *slc6a3*^(-/-) and *drd1b*^(-/-) regarding motor behavior. *slc6a3*^(-/-) impacts swimming behavior: both swimming frequency and place preference in their housing tank, while *drd1b*^(-/-) affects both M-cell escape circuit excitability and swimming frequency. Taken together, our study has provided evidence that *slc6a3* and *drd1b* expression is regulated in a status-dependent manner, and that these changes control locomotor changes observed in subordinate animals.

Although these results are exciting, it is necessary to confirm altered protein expression in the *slc6a3* and *drd1b* mutants. I would advise that these mutant lines be confirmed at the protein level through western blot analysis or mass spectrometry to make claims regarding how differences in protein expression of these mutant lines influence the observed behavioral phenotypes.

An additional caveat of this study is measuring auditory-evoked escape response probability for these DA-specific mutations. DA signaling plays a known role in modulating sensitivity of the auditory pathway (d'Aldin et al., 1995; Darrow et al., 2006). Initial investigations in the guinea pig provided evidence that both DRD1 and DRD2 receptors led to an

inhibition of the afferent dendrites that synapse onto hair cells (Oestreicher et al., 1997). Further investigation supports the inhibitory neuromodulatory effects of DA via DRD1 and DRD2 on auditory nerve activity (Ruel et al., 2001; Sun & Salvi, 2001; Perelmutter et al., 2019). An additional study, however, suggests that DRD1 enhances the probability of depolarization of these cells in response to auditory stimulus in guinea pigs (Niu & Canlon, 2006). This study used the DRD1 antagonist, SKF-83566, which has been shown to also inhibit DAT and impact the levels of DA in the synaptic cleft (Stouffer et al., 2011). Interestingly, in studies performed in mice and larval zebrafish, provide evidence that decreased DRD1/Drd1b activity led to increased excitability of the auditory system (Maison et al., 2012; Toro et al., 2015). Our *drd1b*^(-/-) fish show enhanced auditory-evoked escape probability, which supports what is known about the effect of DRD1 on auditory nerve fibers in guinea pigs, but not in larval zebrafish or mice studies. To tease apart how the auditory pathway is altered in our *drd1b*^(-/-) fish, I would suggest to record from the hair cells in this mutant line in larval and, if possible, adult fish. This would provide us with information on how this chronic loss of *drd1b* influences this sensory pathway in addition to acute changes in Drd1b activity as seen in pharmacological studies.

In addition to post-synaptic receptors, pre-synaptic DAT activity is reported as essential for the maintenance of spontaneous activity of auditory nerves as well as in their response to sound stimulation (Pogarell et al., 2004; Ruel et al., 2006). Our *slc6a3*^(-/-) fish showed no change in auditory evoked escape response compared to *slc6a3*^(+/+) siblings. These differences may be due to chronic loss of Dat in the *slc6a3*^(-/-) fish, and thus, looking at other mechanisms for DA clearance in the *slc6a3*^(-/-) fish would allow for a better understanding as to why our fish displayed no change in response probability because of auditory stimulation.

CHAPTER 4: SOCIALLY MEDIATED SHIFT IN NEURAL CIRCUIT ACTIVATION REGULATED BY SYNERGISTIC MODULATION

Introduction

Social status-dependent modulation of neural circuits has been investigated extensively in vertebrate and invertebrate systems (Edwards & Kravitz, 1999; Whitaker et al., 2011). Social factors can shift the balance between behaviorally relevant outputs by modulating the excitability of individual underlying neural networks. However, the effects of social status on the interplay between multiple neuromodulatory networks responsible for balancing the excitation of behaviorally relevant output remains uncharacterized. Using zebrafish, we investigated how social dominance influences a change in excitability in the Mauthner-mediated escape circuit, focusing on the dynamic interplay between multiple neuromodulatory networks. We found that social experience regulates the synergistic interaction of dopaminergic, glycinergic and GABAergic inputs to control spinal locomotor circuit activity, manifesting in status-dependent altered locomotor behavior.

Zebrafish are social animals and agonistic interactions among conspecifics often culminate with the formation of stable dominance relationships. These social relationships are based on aggression, with dominant animals subjugating low ranked subordinates, consequently altering escape and swimming behavior (Miller et al., 2017). In teleost fish, the short-latency startle escape response is mediated by the Mauthner cells (M-cells), a pair of neurons located in the hindbrain (Figure 3A, B). Each M-cell receives unilateral auditory input from the VIIIth nerve and innervates contralateral fast motor neurons in the spinal cord (Eaton et al., 2001; Korn & Faber, 2005). Activation of the M-cell leads to the simultaneous activation of spinal fast motor neurons that control the startle reflex and inactivation of slow motor neurons that control

swimming. The net effect is an uninterrupted startle escape response away from the perceived threat (Eaton et al., 2001). Swimming is coordinated by a distributed network of neurons in the brainstem and spinal cord. It is initiated in the mesencephalic locomotor region, which innervates reticulospinal neurons in the hindbrain to activate central pattern generators in the spinal cord, leading to a rhythmic oscillatory behavior (Roberts et al., 2008).

Both locomotor circuits receive local and far-projecting excitatory and inhibitory modulatory inputs that control their excitability and activity phase transitions (Mu et al., 2012; Medan & Preuss, 2014; McPherson et al., 2016) (Figure 3B). One such input, dopamine, is a key neuromodulator implicated in aggression, motivation, and regulating excitability of locomotor circuits (Cachope et al., 2007; Song et al., 2015). Dopamine neurons from the hypothalamus, A11 region, project to the hindbrain and spinal cord of teleosts and provide sole dopaminergic neuromodulation of M-cell excitability and central pattern generators involved in swimming regulation (Wenzel & Cheer, 2018). Thus, dopamine is a key target for investigating the underlying mechanism of how social status alters network excitability of locomotor circuits.

In addition to dopamine, inhibitory GABAergic and glycinergic inputs provide tonic feedforward and feedback regulation of the M-cell for added control of locomotor activity (Oda et al., 1995; Hatta et al., 2001; Tabor et al., 2018). While the independent involvement of this neuromodulator (DA), and these neurotransmitters (GABA and glycine) in regulating motor circuits has been investigated, the mechanism of how their synergistic modulation is affected by social factors to optimize motor output remains unknown.

Here we tested the hypothesis that status-dependent changes in locomotor behavior are a result of modified dopamine signaling onto local motor circuits. Further, dopamine signaling indirectly modulates the M-cell escape response through activation of an inhibitory interneural

network. This work culminates in describing a mechanism by which social status induces network flexibility among neuromodulators to cooperatively regulate network excitability of the escape and swim circuits. Together, our empirical and neurocomputational results provide a novel account of how social experience induces functional plasticity on the cooperative action of a multitude of neuromodulators to control locomotor behavioral outputs.

Methods

Animal maintenance

Adult zebrafish (*Danio rerio*) AB strain of mixed sex were group-housed in 10 liters (~20 animals) at core animal facility and kept at a constant temperature at 28°C under a 14 h/10 h light/dark cycle. Fish fed twice daily with high protein commercial food (Otohime B2, Reed Mariculture, CA, USA), and once daily with newly hatched artemia (Brine Shrimp Direct, UT, USA). All experiments were performed in accordance with the Institutional Animal Care and Use Committee at East Carolina University.

Social isolation and pairing

To minimize residual effects of prior social experience, male zebrafish (6-12 months old) were isolated physically and visually in individual tanks for 1 week. Following isolation, fish of equal size and age were paired in new tanks over a 2-week period to establish a social dominance relationship. Aggressive interactions were observed daily to determine and follow stability of social dominance as was previously established (Miller et al., 2017).

Experimental setup for locomotor behavior

After the pairing phase was complete, fish were temporarily separated, and behavioral testing was performed sequentially. Each fish was placed in a testing chamber (dimensions: 11 x 4 x 3cm) filled with double distilled water having a resistance of ~15 M Ω • cm. High resistance

water increases signal to noise ratio and was shown to have no adverse effects on the health of the fish⁴⁶. A pair of conductive electrodes placed on either side of the chamber recorded the electric field potentials. Bare electrodes were 1 mm in thickness with 3-5 mm metal exposure. Electrodes were connected to an AC differential amplifier (AM-Systems model 1700, Carlsborg, WA USA), allowing the amplification of signals 1000-fold. Electrical signals were low-pass filtered at 300 Hz and high-pass filtered at 1 KHz. Signals were digitized using a Digidata-1322A digitizer and stored using Axoscope software (Molecular Devices, Inc., Sunnyvale, CA, USA). The animals were acclimatized for 30 minutes before behavioral testing was initiated. Swimming behavior was recorded following acclimation. Immediately after, startle responses were taken and recorded.

Determination of startle sensitivity

Auditory pulses consisting of phasic 1 ms sine waves were generated using Audacity open-source audio editor and recorder software (audacityteam.org). Sound intensity was calibrated using a decibel meter (Sinometer, MS6700). Activation of the Mauthner-mediated escape is an all or nothing response with a short latency from stimulus (5-15 ms), and startles were only included if they fell within this range. Non-Mauthner mediated responses > 15 ms were excluded (Eaton et al. 2001). Pulse intensity ranged from 70-100 dB with 5 dB increments and delivery was randomized with a minimum of 2-minute intervals between stimuli. Each pulse intensity was delivered 3-5 times, and response probability for each intensity was tabulated then averaged across animals.

Measurement of swimming activity

Following the 30-minute acclimation period, the animal's swimming behavior was recorded for 1 minute. The same methods of data acquisition, amplification, digitization, and

storage were used as previously stated. Swimming activity was measured by counting swim bursts with Clampfit software. The “Threshold” function was used for this purpose. A potential was marked as a swim burst if it was at least 8 mV in total amplitude and 30-200 ms in duration. The timing of each swim burst was saved into a Microsoft Excel spreadsheet in reference to the recording start time.

Pharmacology

Control experiments were performed on day 14 post pairing during which both startle and swimming behaviors were tested and measured. The next day (day 15) fish were injected intraperitoneally with either the appropriate drug a sham of saline and their startle and swimming behaviors measured again. Sham injected controls showed no change in behavior (data not shown). Immediately after injections the pairs were returned to their pairing tanks but separated with a divider for a period of 1.5 hours post-injection of recovery. After the recovery period, the animals were transferred to the behavioral testing chamber and given 30 minutes of acclimation period.

For injection, capillary tubing was used, having the dimensions 1.0 mm OD x 0.5 mm ID x 100 mm in length. These were pulled using Flaming/Brown Micropipette Puller (Model P-87, Sutter Instrument Co). The tip of the micropipette was broken off with a razor blade, before loading with the drug solution. Loaded micropipettes were placed in Pneumatic PicoPump PV 820 for drug administration. A 0.02 % tricaine solution was used to anaesthetize the animal prior to injection. A stock solution of 20 mM of each drug was prepared and loaded into the capillary injection needle. A total volume of 2 μ L injected solution based on average animal body weight for 14 μ g to total weight based on drug molecular weight. L-DOPA; SCH 23390 (DRD1 antagonist), Dihydroxidine (DRD1 agonist); Bicuculline Methobromide (GABA_A antagonist);

and Strychnine (Glycine antagonist). All drugs were purchased from Tocris Bioscience except Strychnine, which was purchased from VWR.

Transgenic Lines

The *TgPAC(slc6a3:EGFP)* line was generously gifted to us by Dr. Teresa Nicolson. Correct EGFP labeling of each ROI was confirmed with Anti-TH staining (PA1-4679) (data not shown). The *Tg(GlyT2a:GAL4,UAS:GFP)* line was generated in our lab from an in-cross of separate lines. *Tg(GlyT2a:GAL4)* was generously gifted by Dr. Kurt Marsden and the *Tg(UAS:GFP)* in the background *kca33Tg/+(AB)* was ordered from ZIRC (Cat ID: ZL624).

Data Analysis and Statistics

Startle and swimming data were analyzed using Prism software (GraphPad Inc., San Diego, USA) and IBM-SPSS (RRID:SCR_002865). Effects of treatments were first subjected to two-way analysis of variance testing (ANOVA) factors as treatment and decibel followed by least significant difference (LSD) post hoc test for all multiple comparisons. Group comparisons were first subjected to one-way ANOVA or mixed design (a mixture of one between-group and repeated measures variables) ANOVA followed by Tukey's HSD post hoc test for all multiple comparisons or paired t-test. For startle data, nonlinear regressions were performed and fitted using the Boltzmann sigmoidal equation: $Y = Bottom + (Top - Bottom)/(1 + \exp((V50 - X)/Slope))$. Antibody expression and cell count data were analyzed in Microsoft Excel. Group comparisons were first subjected to one-way ANOVA followed by a t-Test: Two-Sample Assuming Unequal Variances.

Results

Dopaminergic modulation of locomotor circuits is socially regulated

We found that blockage of the Drd1 receptor activity using the selective antagonist, SCH 23390, moderately increased startle sensitivity in group-housed and significantly increased startle in dominants with no observable change in subordinates (Figure 16A-C). Conversely, SCH 23390 significantly reduced swimming in group-housed and dominants while subordinates

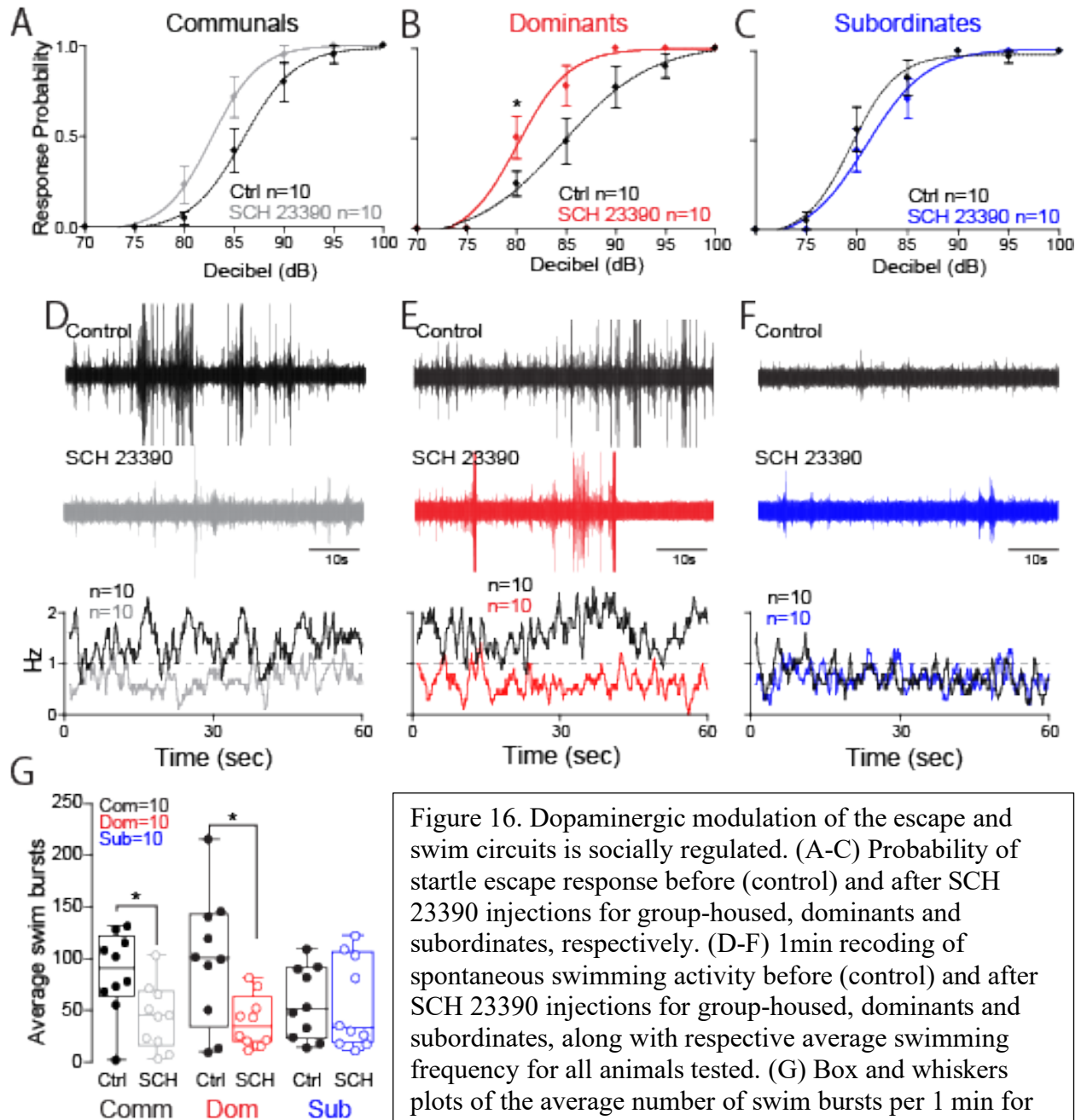


Figure 16. Dopaminergic modulation of the escape and swim circuits is socially regulated. (A-C) Probability of startle escape response before (control) and after SCH 23390 injections for group-housed, dominants and subordinates, respectively. (D-F) 1min recoding of spontaneous swimming activity before (control) and after SCH 23390 injections for group-housed, dominants and subordinates, along with respective average swimming frequency for all animals tested. (G) Box and whiskers plots of the average number of swim bursts per 1 min for each social phenotype (Com n=10; n=10 pairs).

showed no change (Figure 16D-G and Appendix D). Differences in Dat expression levels (Figure 10A, B) suggested that DA availability might work in concert with these proteins in regulating locomotor activity. We addressed this possibility by augmenting DA levels via injection of L-DOPA. We found that L-DOPA led to opposing effects on startle sensitivity in the two social phenotypes (Appendix C). In dominants, L-DOPA increased startle sensitivity, while in subordinates startle sensitivity was decreased. Collectively, these results indicate that pre- and postsynaptic regulation in DA signaling pathway regulate M-cell excitability. Interestingly, in seeing that blocking Drd1b activation led to increased, rather than decreased, M-cell sensitivity, we hypothesized that the status-dependent DA regulation of M-cell excitability occurs indirectly through an inhibitory interneuron. To identify what inhibitory input DA regulates we pharmacologically blocked either GABA_A or glycine receptors.

GABAergic regulation of M-cell excitability is socially regulated

To examine the contribution of GABAergic input in regulating the startle response, we blocked GABA_A receptors using bicuculline. We found that bicuculline did not affect startle sensitivity in both group-housed and dominant animals while it significantly decreased startle response in subordinates (Figure 17A-C). In group-housed (n=8), there was a significant main effect of decibel [$F(6, 42)=1.01e+2$, $p<1.0e-16$], but no effect of treatment [$F(1, 7)=2.80e-1$, $p>0.05$] and no effect of treatment*decibel interaction [$F(6, 42)=7.31e-1$, $p>0.05$]. In dominants (n=9), there was a significant main effect of decibel [$F(6, 48)=1.27e+2$, $p<1.0e-16$], but no effect of treatment [$F(1, 8)=1.44$, $p>0.05$] and no effect of treatment*decibel interaction [$F(6, 48)=1.58$, $p>0.05$]. In subordinates (n=9), there were significant main effects of treatment [$F(1, 8)=1.18e+1$, $p=8.92e-3$], decibel [$F(6, 48)=7.53e+1$, $p<1.0e-16$] and treatment*decibel interaction [$F(6, 48)=3.41$, $p=6.92e-3$]. Bicuculline significantly decreased the startle response

for subordinate animals. In particular, post-hoc test showed that there was a significant difference at 85 dB [$t(8)=4.60$, $p=1.75e-3$]. Asterisks denote statistical difference between control and experimental condition at the specified dB level: * $p<0.05$, ** $p<0.005$; Paired sample t-test.

Although startle response was not affected in group-housed and dominants, swimming frequency declined significantly in dominants with no change in group-housed and subordinates

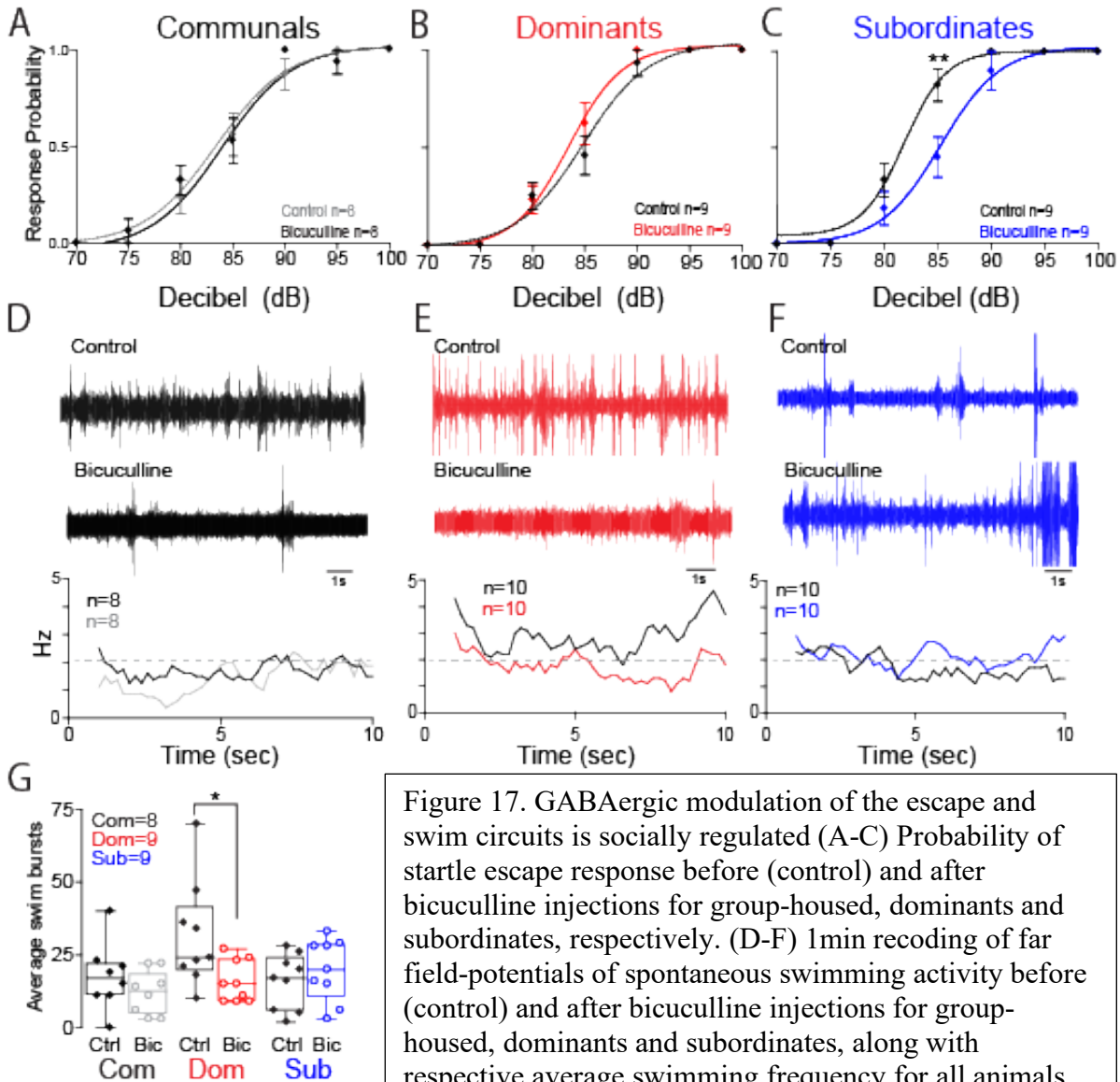


Figure 17. GABAergic modulation of the escape and swim circuits is socially regulated (A-C) Probability of startle escape response before (control) and after bicuculline injections for group-housed, dominants and subordinates, respectively. (D-F) 1min recoding of far field-potentials of spontaneous swimming activity before (control) and after bicuculline injections for group-housed, dominants and subordinates, along with respective average swimming frequency for all animals tested. (G) Box and whiskers plots of the average number of swim bursts per 1 min for each social phenotype.

(Figure 17D-G and Appendix D) In group-housed ($n=8$), there was no effect of bicuculline [two-sided Wilcoxon signed rank test, $p>0.05$]. In dominants ($n=9$) there was an effect of bicuculline [two-sided Wilcoxon signed rank test, $p=3.13e-2$]. In subordinates ($n=9$) there was no effect of bicuculline [two-sided Wilcoxon signed rank test, $p>0.05$]. The results suggested that GABAergic regulation of the startle response is socially mediated, and the decrease of startle in subordinates points to an unidentified pathway that suppresses M-cell excitability when GABAergic input is blocked. The other likely candidate that provides inhibition to M-cells is glycine (Roberts et al., 2008).

Glycinergic regulation of M-cell excitability is socially regulated

We blocked glycinergic input using strychnine and found that strychnine significantly increased startle sensitivity in dominants and marginally increased startle response in group-housed but not in subordinates (Figure 18A-C). In group-housed ($n=10$), there was a marginal main effects of treatment [$F(1, 9)=3.71$, $p=8.62e-2$], but there were significant main effect of decibel [$F(6, 54)=1.53e+2$, $p<1.0e-16$] and significant effect of treatment*decibel interaction [$F(6, 54)=3.71$, $p=3.67e-3$]. Strychnine marginally increased startle response for group-housed animals. In particular, post hoc test showed that there was a significant difference of the startle responses at 85 dB [$t(9)=2.51$, $p=3.32e-2$]. In dominants ($n=12$), there were significant main effect of treatment [$F(1, 11)=1.42e+1$, $p=2.30e-3$], decibel [$F(6, 66)=7.72e+1$, $p<1.0e-16$], and treatment*decibel interaction [$F(6, 66)=4.04$, $p=1.66e-3$]. Strychnine significantly increased the startle response for dominant animals. In particular, post hoc test showed that there was a marginal difference of the startle responses at 75 dB [$t(11)=1.82$, $p=9.60e-2$], but there were significant differences at 80 dB [$t(11)=2.65$, $p=2.24e-2$] and 85 dB [$t(11)=2.95$, $p=1.33e-2$]. In subordinates ($n=12$), there was a significant main effect of decibel [$F(6, 66)=5.07e+1$, $p<1.0e-$

16], but no effect of treatment [$F(1, 11)=1.60$, $p>0.05$] and no effect of treatment*decibel interaction [$F(6, 66)=1.28$, $p>0.05$].

While the probability of the startle response was increased in group-housed and dominants in the presence of strychnine, swimming frequency was significantly reduced in both group-housed and dominants with little change in subordinates (Figure 18D-G and Appendix D).

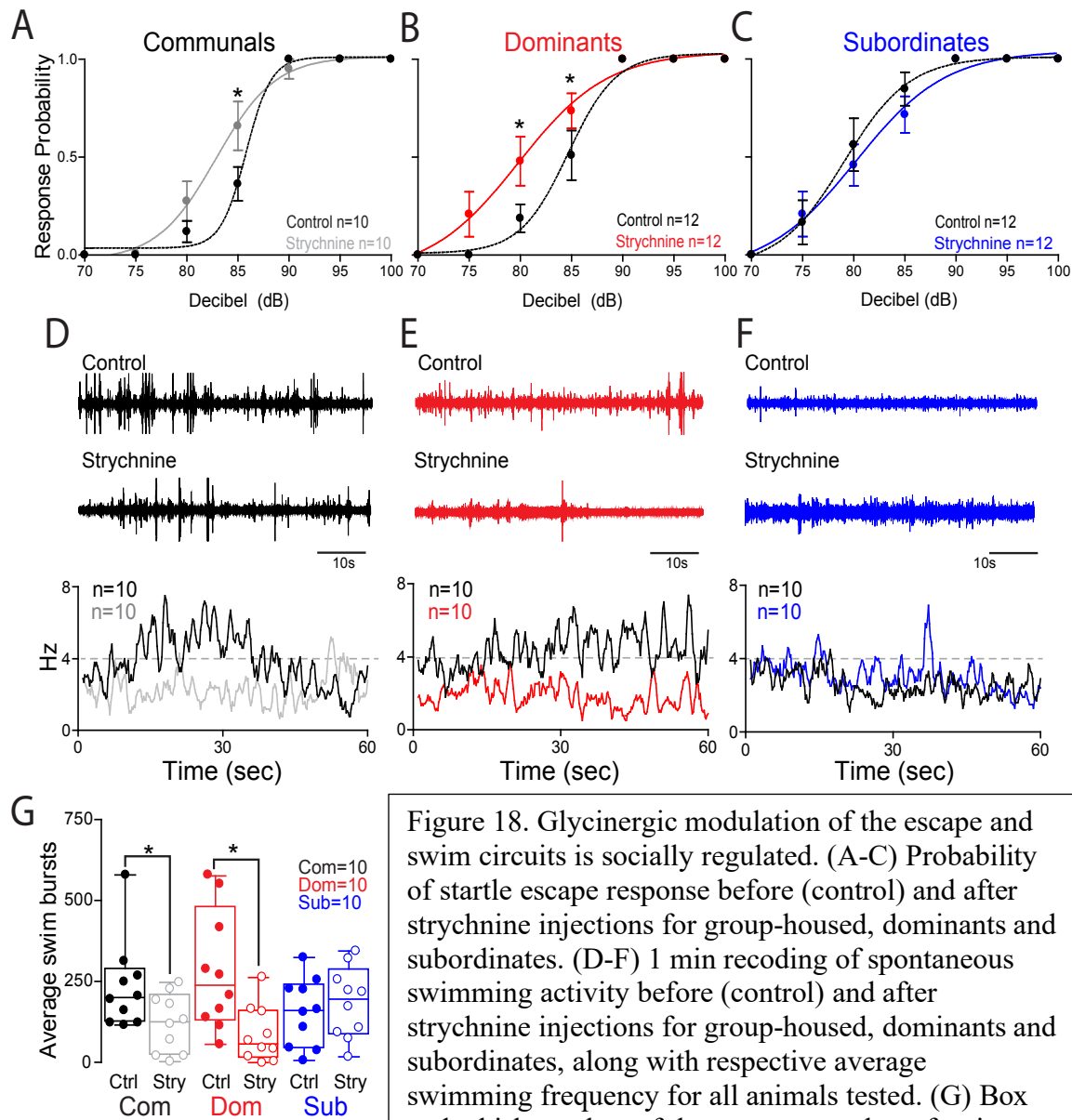


Figure 18. Glycinergic modulation of the escape and swim circuits is socially regulated. (A-C) Probability of startle escape response before (control) and after strychnine injections for group-housed, dominants and subordinates. (D-F) 1 min recoding of spontaneous swimming activity before (control) and after strychnine injections for group-housed, dominants and subordinates, along with respective average swimming frequency for all animals tested. (G) Box and whiskers plots of the average number of swim bursts per 1 min for each social phenotype.

In group-housed and dominants (n=10), there were significant effects of strychnine [two-sided Wilcoxon signed rank test, $p=4.88e-2$ for group-housed; $p=1.37e-2$ for dominants]. In subordinates (n=10), there was no effect of strychnine [two-sided Wilcoxon signed rank test, $p>0.05$].

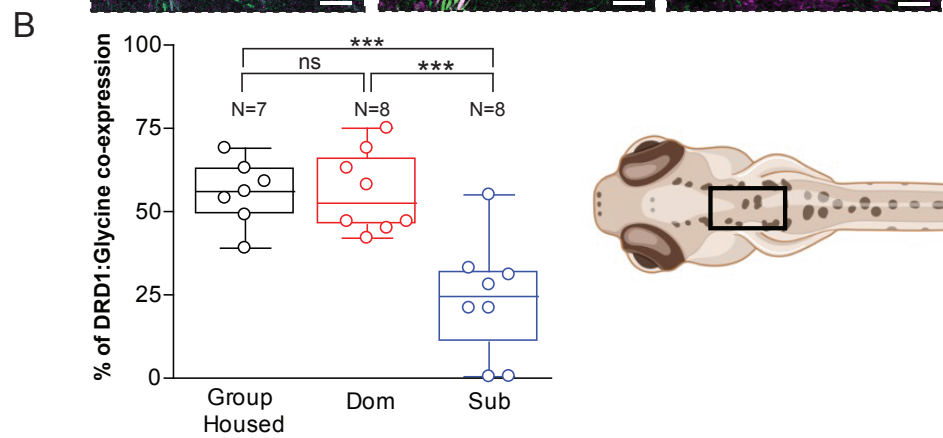
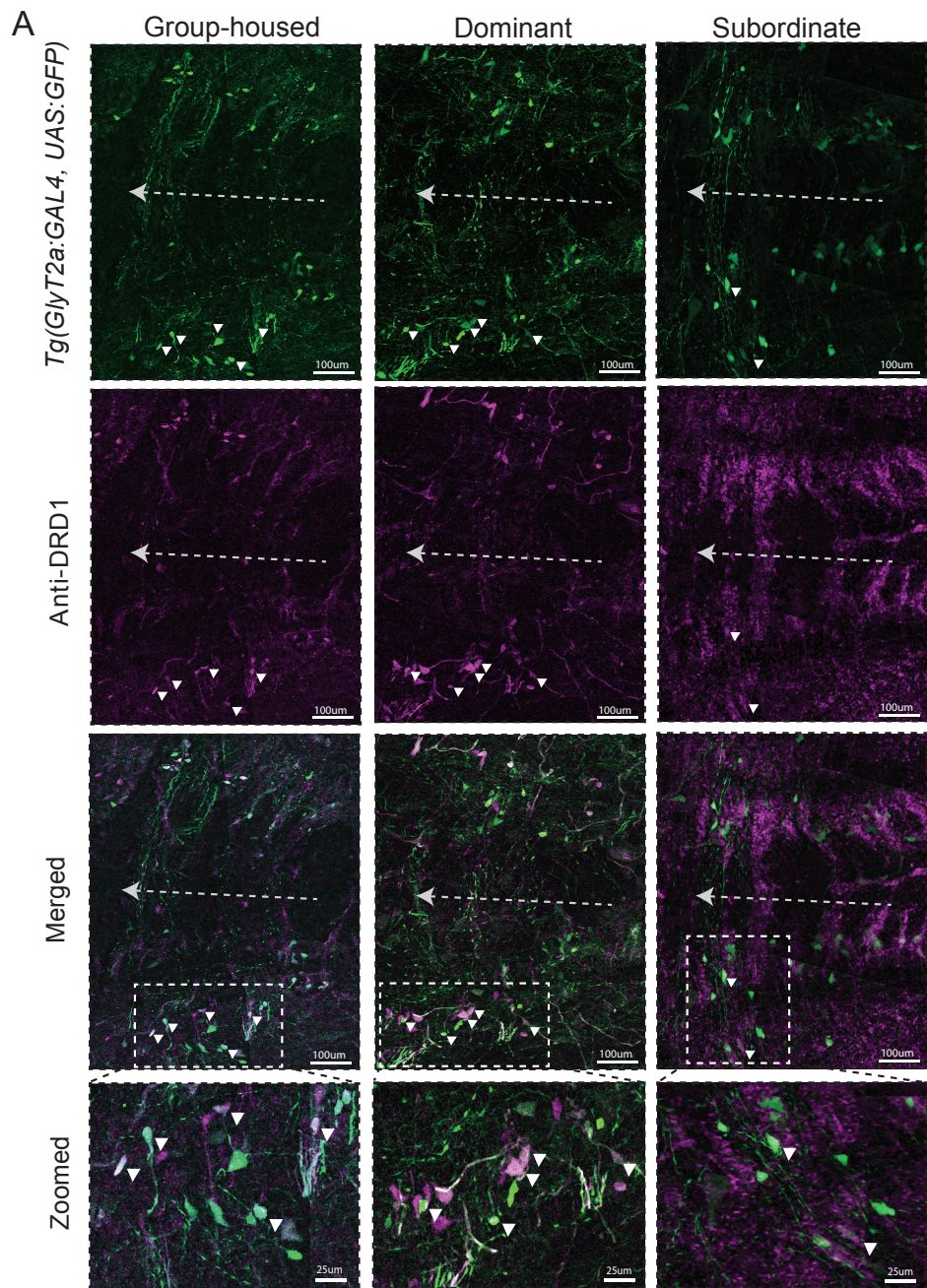
These results suggest that glycinergic modulation of M-cell excitability is weaker in subordinates compared to dominants and that this inhibitory population is the conduit for status-dependent DA regulation of M-cell excitability.

Status-dependent *Drd1* expression on hindbrain glycine neurons

To discern whether DA neuromodulation of the M-cells occurs through glycinergic interneurons, we stained for DRD1 on transgenic GFP- labeled hindbrain glycine neurons, *Tg(GlyT2a:GAL4,UAS:GFP)*. We were unable to visualize the M-cells in this transgenic line and determined from previous studies a landmark of where the M-cells are located based on commissural axonal projections of glycine neurons at the level of the M-cells (Moly & Hatta, 2011). Nevertheless, we counted the number of glycine neurons that have axons crossing the midline as well as the number of *Drd1*-expressing glycine cells in this same region to quantify whether there were differences co-expression (Figure 19A). We found that co-expression was significantly different among the groups, where subordinates showed significant decrease in co-expression compared to dominants (One-way ANOVA, $p = 0.0002$; Tukey's Multiple Comparison Test, Group-housed vs Sub $p<0.001$; Dom vs Sub $p < 0.001$; Group-housed $n = 7$, Dominant $n = 8$, Subordinate $n = 8$) (Figure 19B). In addition, there was no significant differences in total number of *Tg(GlyT2a:GAL4,UAS:GFP)* neurons between groups as determined by a one-way ANOVA ($F(2,20) = 1.997$, $p=0.16$ (data not shown). Although there is a significant difference between dominants and subordinates, without the M-cells as a landmark,

we can only speculate that these neurons are important for feed-forward inhibition. Regardless, if this is the nucleus of interest, these results provide evidence that Drd1 expression on glycinergic neurons that provide inhibition to the M-cells is status-dependent.

Figure 19 (Below). Hindbrain glycinergic neurons show status dependent Drd1 co-expression. (A) Representative confocal optical sections (60 μm thickness) of group-housed, dominant, and subordinates. The top row of images shows glycine neurons in GFP, the second row shows Drd1 staining, the third row shows glycine and Drd1 merged, and the bottom row shows zoomed-in images of the region-of-interest. Co-expressing neurons will appear white in color (arrowheads). (B) Graph comparing the percentage of co-expression between group-housed, dominant, and subordinate animals. Subordinates show a significant decrease in co-expression. One-way ANOVA, $p = 0.0002$; Tukey's Multiple Comparison Test, Group-housed vs Sub $p < 0.001$; Dom vs Sub $p < 0.0001$; Group-housed $n = 7$, Dominant $n = 8$, Subordinate $n = 8$. Scale bar = 100 μm ; zoomed images scale bars = 25 μm



Social regulation of hypothalamic dopaminergic posterior tuberculum

The capacity of social experience to regulate neuromodulatory inputs to control locomotor circuits prompted us to examine whether social status can also induce morphological plasticity. The dopaminergic system is of particular interest because some of the hypothalamic dopaminergic nuclei, specifically the posterior tuberculum (PT), are known to receive visual cues, and convey that information by modulating the escape and swim circuits (Figure 20A) (Mu et al., 2014). The PT is divided into four sub-nuclei based on morphological characteristics, projection patterns and function: Anterior PT rostral (PTar), Anterior PT caudal (PTac), Posterior PT posterior (PTp), Posterior tuberal (PTN) (Haehnel-Taguchi et al., 2018). While the PTp and PTN consist of small cells with no spinal cord projections; the PTar and PTac are homologous to the A11 of mammalian systems, with large soma size and are known to be the only dopaminergic nuclei that project caudally into the spinal cord. Given the morphological and functional similarities of the PTar and PTac and their proximity, we made no distinction in our analysis in differentiating them (Parker et al., 2013). We posited that the PT is not merely a passive conveyor of social information; rather, social cues can also alter the PT morphologically.

To determine differences in DA neuronal number, we used the *TgPAC(slca6a3:EGFP)* transgenic zebrafish line that expresses EGFP specifically in Dat positive neurons (Xi et al., 2011) (Figure 20A). Digital analysis of confocal images showed that the number of Dat positive neurons in the PT was significantly higher in group housed compared to subordinates and dominants compared to subordinates, but there was no significance in the number of Dat positive neurons between dominants and group-housed (Figure 20B, C; one-way ANOVA $p = 0.001$, post-hoc Welch's two sample t-test: Group Housed vs Sub $p=0.003$, Dom vs Sub $p=0.001$,

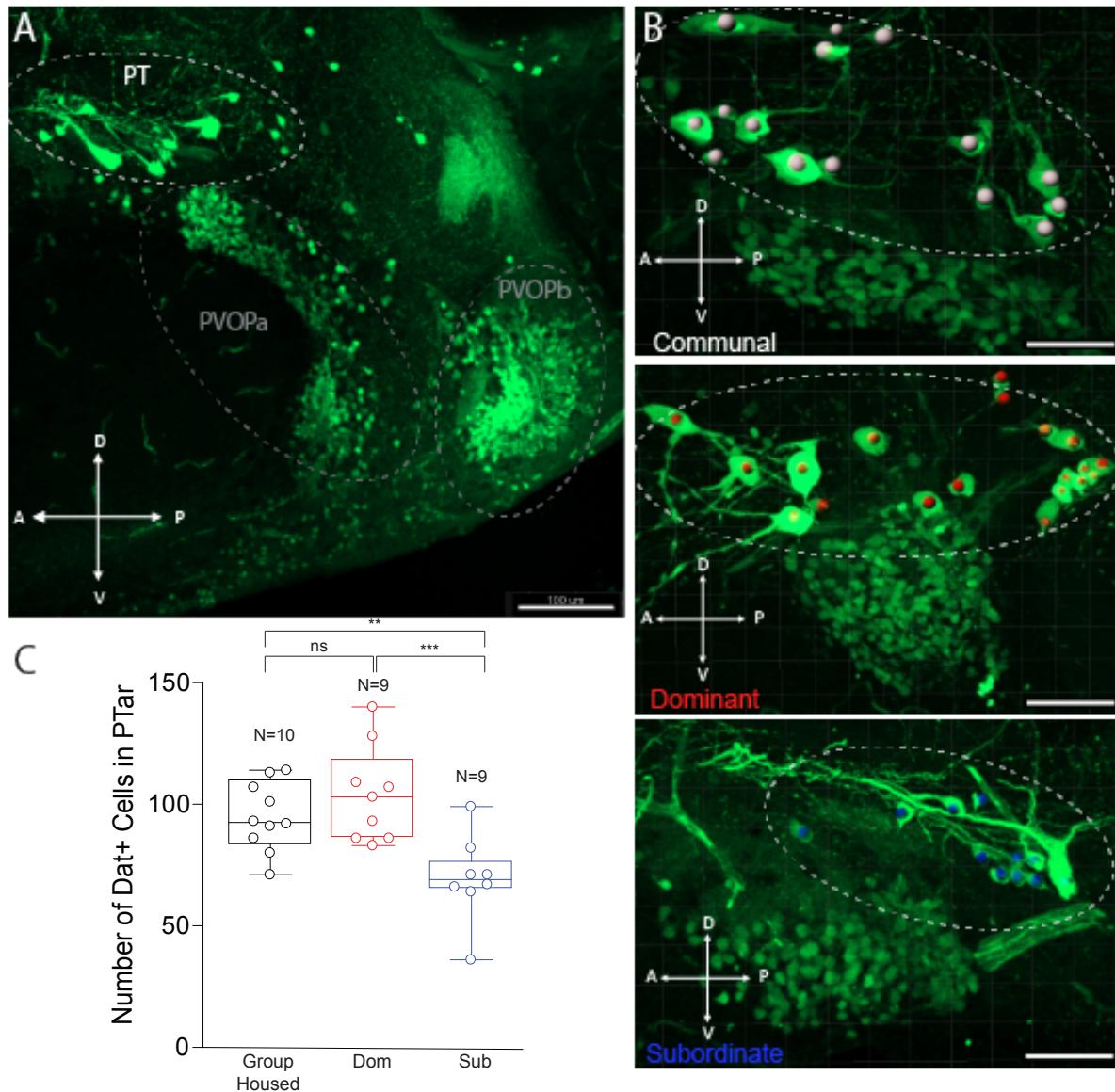


Figure 20. Socially induced morphological plasticity of hypothalamic DA neurons. (A) Overall view confocal image z-projection (30µm depth) of hypothalamic DAT-EGFP expressing neurons highlighting (ovals) the PTac, PVOPa, PVOPB sub-regions. (B) Representative confocal image projections of the PTac and PTar sub-regions from a group-housed, dominant and subordinate animal highlighting (ovals) the DAT-EGFP expressing cells. Superimposed atop of the confocal projections are the digitally identified somas (circles). (C) Summary graph of the number of DA neurons identified in the PTac and PTar regions. One-way ANOVA $p=0.001$; with post-hoc Welch's two sample t-test: Group Housed vs Sub $p=0.003$, Dom vs Sub $p=0.001$, Group Housed vs Dom $p=0.280$.

Group Housed vs Sub $p=0.280$; Group-housed $n=10$, Dom $n=9$, Sub $n=9$). The results suggest that social defeat decreases the number of Dat positive neurons in hypothalamic dopaminergic

PT nucleus.

Discussion

We used an integrative approach to examine how social factors shape the synergistic interactions of multiple neuromodulators (DA, GABA, glycine) to regulate the activity of two mutually exclusive locomotor programs: escape and swim circuits. Three principle conclusions can be drawn from this study. First, the balance in activation of the escape and swim circuits is socially regulated by shifting synaptic strength of excitatory and inhibitory inputs. Second, this shift in circuit activation is synergistic. Synaptic connections are adaptively strengthened and weakened accordingly to promote the proper activation of a specific motor program. Third, the rebalance in circuit excitability is underlined by social status-dependent biochemical expression in DA signaling pathways.

Our results suggest the presence of synaptic connections between the dopaminergic and glycinergic neurons (Yao et al., 2016) and the possibility of connections between GABAergic and glycinergic neurons. In dominant animals, the synaptic pathway of the dopaminergic→glycinergic→M-cell is strong, while the GABAergic→glycinergic pathway is weak. The synergistic strengthening DA system reinforces the excitatory pathway onto the glycinergic neurons. Collectively, this cooperative action of the strong DA input and weakening of the GABAergic input culminate with a net excitation of glycinergic neurons, thus, decreasing M-cell excitability. This conclusion is supported by the evidence that blockage of either the DRD1 or the glycine receptors shifts the balance of circuit activation from swimming to escape (Figure 16 and 18). Conversely, in subordinates the synaptic drive of the dopaminergic→glycinergic→M-cell is weak, allowing for a stronger inhibitory drive from the GABAergic→ glycinergic pathway. This rebalance decreases the release of glycine and

enhances M-cell excitability. This is supported by the result that the probability of escape response was reduced significantly when GABA_A receptors were blocked (Figure. 17). Concurrently, the weakening of the DA pathway due to the decrease of *Drd1* expression further weakens the glycine activation and ensures the removal of inhibitory input onto the M-cells. Although the pharmacology results support a GABA→connection, it is important to note that there is yet to be evidence for GABA receptors on glycinergic neurons that provide feedforward inhibition to the M-cells. So, to support these claims, future experiments need to be completed.

Our results suggest that the shift in network excitability is mediated, in part, by a status-dependent expression of DRD1 on the glycinergic neurons. In quantifying expression of *Drd1* on glycine neurons in group-housed, dominant, and subordinate *Tg(GlyT2a:GAL4,UAS:GFP)* fish, we saw that subordinates show a significant decrease in expression (Figure 19). This decline in *Drd1* expression in subordinates on the glycinergic neurons is likely to decrease dopaminergic→glycinergic synaptic strength and DA's ability to activate the glycinergic neurons, thus, removing the M-cell from inhibition. (Figure 20A, B). Although we see these expression changes on glycine neurons, it is important to point out that this nucleus is presumed to be interest based on previous studies done in larval fish (Moly & Hatta, 2011), and that further analysis with the M-cells labeled can help to support these findings.

Coupled with biochemical signaling plasticity, our results show that social experience can induce morphological plasticity of the hypothalamic PT nucleus. Subordinates showed a significant decrease in *Dat* positive neurons in the PT nucleus relative to dominants (Figure 20). However, the results do not provide any information of how these morphological changes translate into functional differences in PT excitability, and how DA release regulates post synaptic targets. One potential mechanism is that an increase or decrease in DA cell number

would correspond with a shift in synaptic interconnectivity of the PT network. An increase in cell number would increase synaptic connectivity, strengthening synaptic connections of the PT network and its excitability. Conversely a decline in cell number would translate into fewer connections and a weakening in PT excitability. Future work is necessary to test this model and to further examine how descending social cues from higher brain centers (e.g., the habenular nuclei) implicated in social regulation impinge on PT excitability and morphological plasticity to regulate locomotor behavior (Chou et al., 2016).

One caveat to this study is that the antibody used for DRD1 immunohistochemistry still requires expression pattern analysis in zebrafish. Investigating the immunogen that was given on ThermoFisher's website against the zebrafish genome suggested non-specific binding to *Drd7*, *Drd1*, and *Drd1b*. In lieu of successful expression pattern analysis of this antibody, I would use the provided peptide sequence for monoclonal antibodies against *Drd1b* in Toro et al., (2015), to confirm the presence of *Drd1b* on hindbrain glycine neurons.

In conclusion, our findings show that social regulation of locomotor behavior is mediated by synergistic interactions of multiple neuromodulatory signaling pathways that shift motor circuit dynamics to optimize locomotor selection according to social rank. This serves as an adaptive behavioral strategy as animals rise and fall in social dominance and is likely an evolutionarily conserved cellular mechanism employed by other social species.

CHAPTER 5: REFLECTIONS AND FUTURE EXPERIMENTS

The goal of this dissertation was to discern what might influence stable dominance relationship formation and how social status regulates neuromodulation of motor circuits using zebrafish. Results from this dissertation show (1) visual communication is critical to form stable social dominance and zebrafish stripe intensity is plastic and socially regulated, (2) *slc6a3* and *drd1b* expression is socially regulated, (3) that decreased *slc6a3* and *drd1b* expression results in altered synergistic neuromodulation underlying motor circuits, and that (4) this decreased DA signaling may be due to morphological plasticity of hypothalamic nuclei. This discussion will summarize my findings and propose a new working model for future investigation.

Zebrafish use stripe intensity to enforce stable social dominance relationships

A variety of terrestrial and aquatic species alter their size, coloration, plumage, or appendage variation to resemble either a dominant or subordinate phenotype (Krames et al., 1969; Greenberg & Crews, 1990; Chen & Fernald, 2011). Zebrafish form stable social dominance relationships, and display aggression by erecting dorsal (Saverino & Gerlai, 2008) and caudal fins (Gerlai et al., 2000), and displaying mouth opening behavior (Kaleuff et al., 2013). In addition, zebrafish display colorful stripes and can regulate pattern expression (Yamaguchi et al., 2007). In chapter two, I presented evidence confirming that stripe intensity reflects social dominance in zebrafish (Figure 9). These results complement findings in both terrestrial (Ducrest et al., 2008) and other aquatic species (Dijkstra et al., 2007; Bachmann et al., 2017) highlighting the importance of visual cues in social communication during dominance hierarchy formation.

Body stripe color of dominant fish increased in intensity, while stripe color of subordinates remained unchanged (Figure 9). These results show that zebrafish convey social information by altering body stripe intensity in a status-dependent manner. In addition, zebrafish use stripes as a

visual cue to reinforce stability of a dominance relationship. Paired TLN fish that lack body coloration and typical body stripes show a significant increase in social instability compared to AB pairs. TLN pairs switched dominance 33% of the time when paired with another TLN conspecific. While pairing of TLN fish with AB fish resulted in AB fish dominating 75% of the time (Figure 6). Disruption in the ability of zebrafish to convey proper visual information has far reaching consequences in altering the animals' motor activity. Unstable dominance in TLNs correlates with altered escape behavior compared to ABs, where status-dependent escape probability was eliminated in TLNs. Both dominant and subordinate TLNs exhibited an increased escape probability comparable to an AB subordinate phenotype (Figure 8). Interestingly, group-housed TLN fish showed an inherent enhanced escape probability, much different from AB group-housed fish, highlighting a caveat of this experimental design. Previous studies have shown distinct behavioral differences between lines of zebrafish at the larval (Lange et al., 2013; Vignet et al., 2013) and adult stage (Egan et al., 2009; Vignet et al., 2013). Studies in other species have shown decreased agonistic interactions between albino compared to pigmented animals (Slavík et al., 2016). Our study highlights the necessity of vigilance in the use of zebrafish mutants with varying skin coloration, particularly in studies that examine motivated behavior. It is possible that inherent genetic differences observed between TLN and AB zebrafish strains influenced TLN fish (1) not forming stable social dominance, and (2) enhanced escape behavior sensitivity.

To better answer whether zebrafish stripe intensity is used to reinforce stable social dominance, future research would benefit from mutant line constructs that lack the melanophores associated with the dark stripe patterns of fish in an AB background. The prediction would be that genetically decreased stripe intensity would result in an unstable dominance relationship.

Further, this unstable relationship would eliminate status-dependent differences in escape behavior. There is the possibility to subcutaneously inject dye in zebrafish (Cheung et al., 2014). However, this method does not provide the same specificity as genetic approaches. With the addition of these experiments, we can strengthen our claim that zebrafish stripe intensity is altered by stable dominance relationship and the status-dependent change in stripe intensity helps to reinforce dominance relationships.

Decreased *slc6a3* and *drd1b* expression do not decrease stable social dominance

DA plays an important role in sociability, reward and reinforcement, and movement (van Erp & Miczack, 2000; Jay TM, 2003; Wise, 2008). Results from this dissertation showed key elements in the DAergic pathway are socially regulated, whereby expression of both *slc6a3* and *drd1b* in subordinates was significantly reduced compared to dominants (Figure 10). This prompted me to ask whether the regulation of agonistic interactions and stable dominance formation was due to these observed differences in expression of *slc6a3* or *drd1b* using zebrafish lines with mutations induced in each gene.

Neither *slc6a3*^(-/-) nor *drd1b*^(-/-) fish lines altered the establishment of a stable dominance, and paired conspecifics showed comparable levels of aggression as WT siblings (Figure 10). These results are contrary to what has been established in a variety of species, where either decreased DAT or DRD1 led to increased and decreased aggression levels, respectively (Rodriguez et al., 2004, Adriani et al., 2010; Enter et al., 2012; Couppis et al., 2008; Plaven-Sigra et al., 2014; Yamaguchi et al., 2017; Homberg et al., 2016). Another point of conflict between our results and what is known about decreased DAT activity in mammals is that, although statistically insignificant, our *slc6a3*^(-/-) fish show a trend toward being less aggressive in the number of attacks during pairing (Figure 12). Since these results differ from what is known about the role of

DAT in aggression of mammals, I would suggest investigating how this mutation in *slc6a3* of zebrafish alters different brain regions associated with aggression and compare these changes to those known in mammals. Although there was no significant difference in aggression levels between *drd1b*^(-/-) and *drd1b*^(+/+) siblings, a higher sample size would give us a better idea if this holds true or is a product of a low sample size.

Further, social hierarchies in either mammalian DAT knockout (Rodríguez et al., 2004) or mutants (DiCarlo et al., 2020) become unstable within the first few days of pairing; yet our *slc6a3*^(-/-) fish displayed stable dominance (Figure 12). These results suggest the possibility for alternate signaling pathways involved in the establishment and maintenance of social dominance in zebrafish. It would be interesting to do RNA-sequencing to see what alternate genetic changes may be occurring because of losing an important part of DA signaling, *slc6a3* or *drd1b*. From there, we could determine if other signaling systems are compensating for this disrupted DA signaling that may allow for a stable dominance to form in these mutated fish.

Although it appears that these two proteins do not alter aggression by total number of attacks, it would be prudent to examine different aspects of agonistic behavior, such as quantifying the intensity of each aggressive encounter during video tracking or administering the mirror assay. With these tests, we can highlight possible subtle differences in both intensity and type of aggression (e.g., overt aggression; attack attempts, or restrained aggression, such as erection of fins). However, since mutations in both *slc6a3* and *drd1b* do not appear to regulate the stability of social dominance in zebrafish, this led me to question whether stable dominance in both mutant lines led to altered motor behavior, as observed previously in WT.

Status-dependent neuromodulation of spinal motor circuits

Stable dominance relationships correlate with increased escape probability and decreased swimming in subordinate zebrafish (Miller et al., 2017), and both DRD1 and DAT have been implicated in regulating locomotion in mice (Jaber et al., 1997; Crawford et al., 1997). My goal was to discern how a mutation in the zebrafish genes, *slc6a3* and *drd1b*, alter escape and swimming behavior and to determine how the relationship between DA neuromodulation results in status-dependent motor circuit excitability. This dissertation provides evidence that *slc6a3*^(-/-) fish display bottom-dwelling behavior and decreased swimming frequency and that *drd1b*^(-/-) fish show a significant increase in escape circuit excitability and decreased swimming frequency. Further *Drd1b* regulates M-cell activation through an inhibitory interneuron, and subordinates decrease DA signaling onto spinal motor circuits through decreased DA signaling from the hypothalamic PTar region (Figure 20).

The effects of mutated slc6a3 on place preference and swimming behavior

In agreement with other established *Dat* knockout lines (Kacprzak et al., 2017; Wang et al., 2019) my CRISPR-*slc6a3* mutant line displayed bottom-dwelling swimming in the pairing tank, which persisted regardless of social dominance (Figure 13). Further, *slc6a3*^(-/-) fish exhibited significantly less swimming compared to *slc6a3*^(+/+) siblings during the 1-minute acquisition before escape testing (Figure 14). These results are contrary to mice (Jaber et al., 1997), *Drosophila* (van er Voet et al., 2005), and humans (Gill et al., 1997), where either a DAT knockout or polymorphism is associated with hyperactivity. Although inconsistent with other species, my results support previous findings that decreased *Dat* in larval and adult group-housed zebrafish is associated with decreased swimming (Kacprzak et al., 2017; Wang et al., 2020).

Unlike swimming, *slc6a3*^(-/-) fish showed no variation from *slc6a3*^(+/+) siblings in escape response probability regardless of social phenotype (Figure 14). Possible interpretation suggests

this mutation does not alter escape behavior. However, it is important to disentangle the nuanced differences in assays to test M-cell excitability. In our assay, the two-minute inter-stimulus interval (ISI) may have been sufficient for DA to be cleared from the synapse instead of reuptake into the presynaptic terminal before the next stimulus, resulting in no change in M-cell excitability between *slc6a3*^(-/-) and *slc6a3*^(+/+) siblings. To address whether there is altered DA clearance in *slc6a3*^(-/-) fish, habituation experiments will be informative; whereby presenting suprathreshold auditory pulses at a higher frequency to decrease the time that the recycling machinery has between pulses to remove DA from the synaptic cleft. This will result in more DA binding to post-synaptic receptors and a faster rate of habituation compared to WT controls. Additionally, we could perform prepulse inhibition (PPI) experiments to determine whether the mutation in *slc6a3* alters sensorimotor gating and the ability of *slc6a3*^(-/-) to filter out non-salient stimuli (Curzon et al., 2009). Dopamine has been shown to be involved in PPI of the acoustic startle response (Mansbach et al., 1988; Zhang et al., 2000), so performing these tests may provide more detailed explanations regarding the rate of saturation of DA in *slc6a3*^(-/-) and how that might influence activation of the M-cells.

Importantly, a mutation in *slc6a3* may result in a lack of protein throughout development, which could induce homeostatic compensatory mechanisms that override DA saturation because of Dat absence into adulthood. One possible scenario is regulation of receptor expression on postsynaptic membranes. DA receptors are readily trafficked to and from the membrane to reflect the supply of a neurotransmitter in the synaptic cleft (Wolf et al., 2003) and decreased transmembrane DRD1 receptor expression has been seen in DAT knockout mice (Dumartin et al., 2000). An increase in synaptic DA due to chronically decreased Dat may result in Drd1b receptor trafficking away from the membrane in zebrafish, resulting in a WT escape phenotype.

A previous study has shown that acute ablation of DA neurons via microinjection of 6-hydroxydopamine (6-OHDA), resulted in locomotor deficiencies (Vijayanathan et al., 2017). Additionally, acute pharmacological blockage of Dat activation leads to further depression of the escape response in group-housed WT fish (Appendix E). Taken together, we can hypothesize that a chronic decrease in *slc6a3* leads to compensatory mechanisms regarding the startle escape circuit. To test this hypothesis, we could perform *in-situ hybridization (ISH)*, antibody staining, or generate transgenic lines probing for increased astrocytes, *vmat2*, degradative enzymes, or DA-receptor expression changes. Additional complementary experiments include qPCR, or Western Blot. Together, these experiments would provide insights to better interpret why *slc6a3*^{-/-} fish display escape circuit activation comparable to WT siblings.

Decreased drd1b and altered swimming and escape behavior

The role of DRD1b in locomotor behavior has been well established in mammals (Crawford et al., 1997; Muller et al., 2010; Rial et al., 2020; Nakamura et al., 2021). In this study, *drd1b*^{-/-} fish decreased swimming frequency and an increased sensitivity in escape regardless of social phenotype (Figure 15). These results suggest that the Drd1b plays a more prominent modulatory role in status-dependent motor circuit excitability. Interestingly, decreasing DRD1b activity correlates with increased startle behavior. Using pharmacological and electrophysiological methods, DA has been ascribed an excitatory role for the M-cells (Pereda et al., 1992, Mu et al., 2012), but histological analysis using a Tol-56 reporter line in adult zebrafish showed that the Drd1 is not expressed on M-cells (Appendix F). These results suggest that status-dependent changes in DA work indirectly through an inhibitory interneuron to regulate M-cell excitability. This has been previously reported, where blocking Drd1 activation led to enhanced visually evoked M-cell startle behavior (Yao et al., 2016), and that inhibitory interneurons are involved

during visually evoked escape. My goal was to expand on these results to discern which inhibitory input is the conduit for DA modulation of the M-cell circuit in a status-dependent manner. There are two established inhibitory inputs onto the M-cells: GABA and glycine (Diamond & Roper, 1973; Lee & Eaton, 1993; see. Korn & Faber, 2005). I initially hypothesized that, based solely on location of an abundance of receptors near the VIIIth nerve on the lateral dendrite (Figure 3B), that GABA would be the inhibitory interneuron modulated by DA. Alternatively, there is a dense population of glycine receptors near the axon hillock, which could instead mean that glycine neurons may be the inhibitory interneurons subject to DA modulation.

Exploring possible inhibitory interneurons mediated by DA signaling

To determine whether DA mediates M-cell excitability through GABAergic inhibition, I pharmacologically blocked activation of GABAergic type A receptors (GABA_A) with bicuculline. If GABA was activated by Drd1, then blockage of GABA_A would produce a similar change in excitability as *drd1b*^(-/-). Yet, blocking GABA_A led to no noticeable change in dominant escape excitability, but decreased escape probability in subordinates. These results are perplexing because there is known inhibition from GABA_A on M-cell lateral dendrites (Diamond et al., 1973; Lee & Eaton, 1993), where bicuculline decreases inhibitory post synaptic potentials (IPSPs). Since our results are contradictory to the established literature on GABAergic inhibition of the M-cells, I would begin by repeating our antagonist experiment to ensure reproducibility. In addition to the pharmacological approach taken in this study, we can test a genetic knockout of GABA_A receptor. However, if these results persist, this may suggest an unknown modulatory role of GABA receptors on either other inhibitory inputs such as regulating glycine inhibition of the M-cells, or regulation of higher brain circuits responsible for M-cell excitability. Although an exciting avenue to explore, our results suggest that GABA is not the conduit through which DA

regulates M-cell excitability. This result prompted me to adjust my focus to glycinergic inhibition.

Blocking glycine receptors (GlyRs), using strychnine, resulted in no change in escape sensitivity in subordinates, but a significant increase in escape probability of dominants (Figure 18). These results support that glycine is the conduit for auditory evoked DA modulation of M-cell excitability and provide further support for conclusions in larval zebrafish where the M-cells receive glycinergic input from hindbrain glycine neurons (Moly & Hatta, 2011), and that these neurons are activated by Drd1 (Yao et al., 2016).

To visualize whether there are status-dependent changes in Drd1 expression on hindbrain glycine neurons, I stained for Drd1 in *Tg(GlyT2a:GAL4,UAS:GFP)* fish. We were unable to localize which specific nuclei to target based on M-cell location. However, other labs have shown feedforward inhibition of the M-cells occurs from glycinergic neurons surrounding the M-cells and project a bundle of axons contralaterally across the midline (Koyama et al., 2011; Moly & Hatta, 2011). Using this landmark to narrow down our focus, we quantified the number of co-expressing *Tg(GlyT2a:GAL4,UAS:GFP)* and Drd1-stained neurons in this region and found a significant decrease in co-expression in subordinates (Figure 19). Although exciting, the conclusions we draw from these results should be conservative due to a few caveats. The first being that we have no visual evidence of the location of the M-cells in relation to the glycinergic neurons. The second being that expression patterns of this antibody have not been verified in zebrafish, and thus, could non-specifically label other targets. I have attempted to verify this antibody in 5 dpf larval fish but was unsuccessful. In lieu of staining, I searched the listed immunogen from the ThermoFisher website on UniProt (GFWPFGSFC) and blasted the protein sequence against *danio rerio*. There was a 100% match with three DA receptors: Drd1b, Drd1,

and Drd7. This investigation supports the need for expression pattern comparison with this antibody. In acknowledgment of these caveats, our preliminary results suggest that social dominance alters Drd1 expression on hindbrain glycine neurons that may provide feedforward inhibition to regulate M-cell excitability.

Decreased number of Dat⁺ neurons in the subordinate hypothalamic PTar region

Having seen both status-dependent DA modulation of the escape circuit and altered Drd1 expression on hindbrain glycine neurons that are likely to provide feedforward inhibition to the M-cells, I was curious whether the hypothalamic region responsible for providing descending DA input onto this circuit (PTar) was altered by social dominance. Using the *TgPAC(slc6a3:EGFP)* line, we found that Dat⁺ cells in PTar were significantly decreased in subordinate fish (Figure 20). One caveat to this method is that these cells were not labeled with the nuclear marker, DAPI, so it is unclear whether these cells are undergoing apoptosis or if they're simply no longer expressing the EGFP transgene. Moreover, the PTar region has been shown to co-express glutamate in rats (Kawano et al., 2006), mice (Fougère et al., 2021) and in zebrafish at the larval and juvenile stage (Filippi et al., 2014). To investigate the possibility that the PTar region turns off DA signaling in favor of glutamate signaling, I generated a double transgenic line *TgPAC(slc6a3:EGFP),Tg(VGlut2:RFP)* to probe regions in the hypothalamus for co-expression. The PTar neurons in adult zebrafish lack co-expression of glutamate and dopamine (Appendix G). Therefore, it is unlikely that these cells will switch their transmitter identity and suggests that the number of neurons within the PTar nucleus is indeed prone to social regulation.

Conclusions and future directions

Results from experiments in this dissertation have culminated into a new working model that forms a basis for future hypotheses. This conclusion will discuss this new working model and provide a series of proposed experiments to examine more deeply the connection between social dominance and altered neuromodulatory mechanisms onto motor circuits.

Proposed Model

In the normal, control condition, zebrafish receive DAergic input from the hypothalamic PTar region to hindbrain glycinergic nuclei. This signal activates Drd1b receptors on these hindbrain glycinergic neurons, increasing inhibitory feedforward signal from glycine to the M-cells and leading to an overall decreased sensitivity of the M-cells to an incoming auditory stimulus (Figure 21A). However, by becoming a social subordinate, DA signaling from the PTar nucleus is reduced due to fewer DAT⁺ cells (Figure 20). This decreases the amount of DA available in the DA→glycine synaptic cleft, resulting in less membrane bound Drd1b on glycine neurons due to potential receptor trafficking away from the membrane via GRK binding or PKA phosphorylation. Consequently, this reduces the excitability of the inhibitory glycine neurons, and results in less feedforward inhibition of the M-cells and ultimately increased sensitivity of the M-cells to incoming excitation from the VIIIth nerve (Figure 21B).

Startle escape probability of *drd1b*^(-/-) fish was significantly increased regardless of social phenotype. Further, initial analysis suggests that hindbrain glycinergic neurons that may be important for feedforward inhibition of the M-cells show decreased Drd1 expression in subordinates (Figure 19). In addition, we have evidence that there is decreased DA input from the hypothalamic PTar nucleus in subordinates (Figure 20). These results tie into the observed decreased Dat in subordinates, whereby losing the number of presynaptic terminals present

would consequently lead to reduced Dat expression. To test the effects of potential decreased Dat on motor behavior I generated a *slc6a3*^(-/-) line.

Results from *slc6a3*^(-/-) were a bit less straightforward than the *drd1b*^(-/-). I observed that a mutation in *slc6a3* does not affect startle escape in mutant group-housed fish. Few possibilities may account for this result. The first is that both group-housed WT and group-housed *slc6a3*^(-/-)

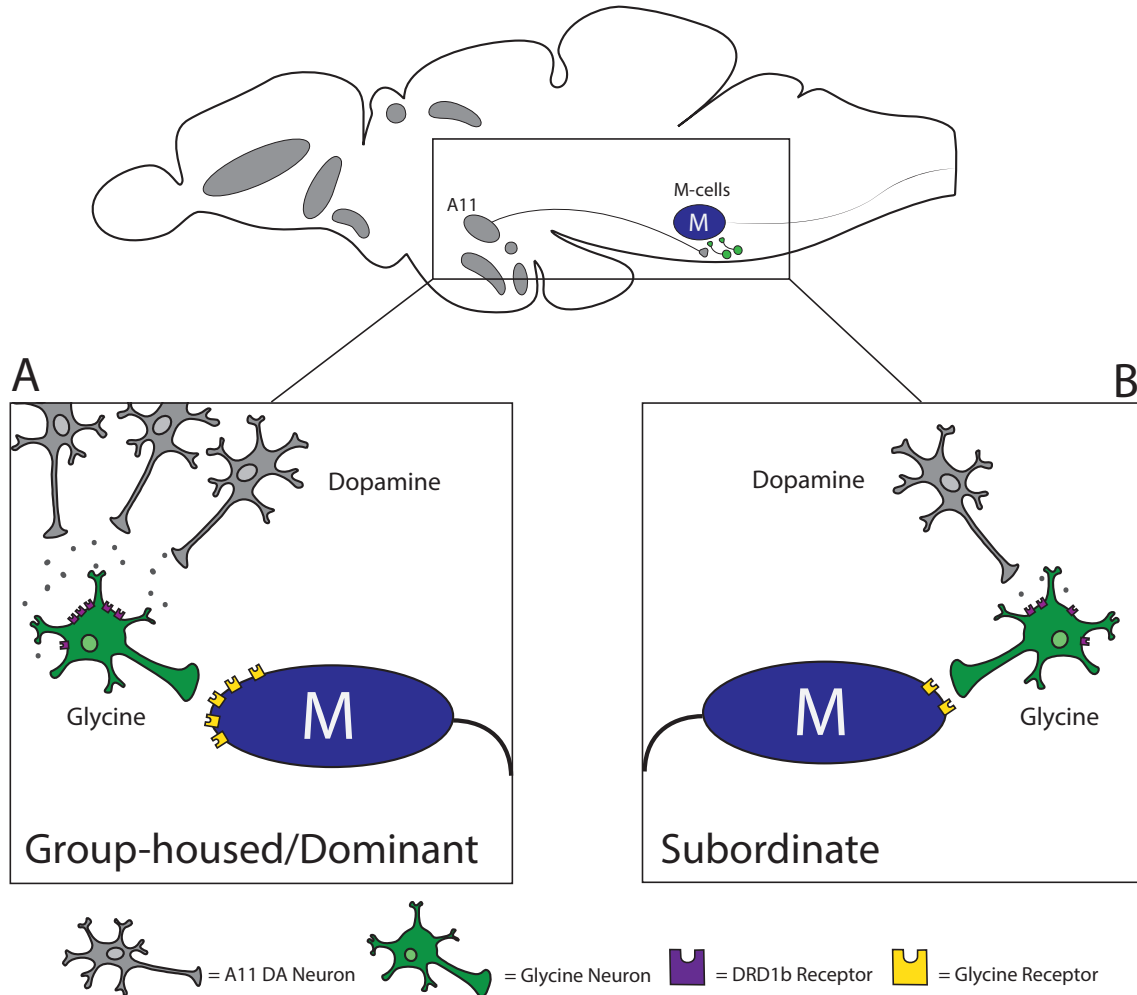


Figure 21. Working Model Illustrating status-dependent signaling onto the M-cell circuit. (A) In group housed or dominants fish, hypothalamic DA neurons (PTar) activate hindbrain glycine neurons the *Drd1b*. This increased inhibitory drive from glycine onto the M-cells and results in decreased M-cell excitability. (B) By becoming a social subordinate, the DA input from the PTar is decreased. This causes a decrease in membrane bound *Drd1b* on hindbrain glycine neurons, and results in decreased inhibition from glycine to the M-cells. These changes result in more easily excitable M-cells and an increased escape probability in subordinates.

fish presumably have the same amount of DA PTar input. The only presynaptic difference is an excess of DA without proper clearance in the *slc6a3*^(-/-), rather than decreased DA input from PTar. This idea is further supported in that when *slc6a3*^(-/-) fish form dominance relationships, there is an increased escape probability in *slc6a3*^(-/-) subordinates. As a subordinate, these *slc6a3*^(-/-) have decreased DA input onto the glycinergic cells and show the same shift in excitability as WT subordinates. From these results and the proposed model, there is plenty that can be done to probe the possibility of the model versus alternative mechanisms.

Future Directions

A caveat to our current examination of Drd1 staining on hindbrain glycinergic neurons is that we do not have the M-cells as a landmark. Visualizing the M-cells in adults using either immunohistochemistry (IHC) or a transgene has proven difficult. To strengthen our argument that feedforward hindbrain glycine neurons exhibit status-dependent Drd1 expression is to replicate this experiment where the M-cells are labeled. One such way is to perform multiplexed *in-situ* hybridization, as described in Choi et al., 2018, to probe for the M-cells, glycine neurons, and Drd1 expression. One concern with adults is finding the proper slice thickness to find a balance between thickness that will ensure penetration by the probes, can successfully be imaged, and that can incorporate all targets. I would suggest trying different sectioning planes in addition to find the best visualization. This experiment would provide either support for or counterevidence to our claim that there is decreased Drd1 expression on glycine cells involved in feedforward inhibition of M-cell excitability.

In addition, there are alternative ways to test the expression of Drd1 on glycinergic neurons. One mechanism known to cause internalization of GPCRs are G protein-coupled receptor kinases (GRKs) (Gurevich & Gurevich, 2019). GRK4 α has been shown to selectively bind to

Drd1 independently of activation of these receptors and induce receptor internalization (Rankin et al., 2006). Thus, staining for GRK could indicate whether there is increased Drd1b internalization in subordinates.

An additional interesting question is whether there are changes in glycine receptor availability on the M-cells in subordinates. If subordinates have decreased glycine activation due to decreased DA signaling, this may induce GlyR receptor internalization on the M-cells. It is known that GlyRs on the M-cells can exhibit recycling in and out of the membrane (Ogino et al., 2019), and so staining for GlyR in an M-cell transgenic line could provide evidence that glycine signal onto the M-cells is altered in subordinates.

Finally, the PTar region exhibits the ability for neurogenesis into adulthood (Vijayanathan et al., 2017), and so there is a possibility for these neurons to undergo cycles of apoptosis and neurogenesis based on social experience. To determine if social subordination leads to cell death future studies would benefit from utilizing apoptotic markers, such as Caspase-3 or TUNEL, in dominants and subordinates. One caveat is that cell death would most likely occur throughout the 14-day pairing period rather than on a single day. This makes sampling at different times points imperative for being able to tease apart when cell death is occurring. This would give us a broader look at if these cells are undergoing apoptosis and at what time point during social interactions apoptosis occurs. Taken together, results from these proposed experiments will give us more detailed information about the nature of the status-dependent plasticity of the PTar nucleus.

In conclusion, the work from this dissertation provides evidence that (1) social dominance alters stripe coloration, (2) that *slc6a3* and *drd1b* expression is regulated in a status-dependent manner, (3) that decreased expression of *slc6a3* and *drd1b* results in altered

synergistic neuromodulation underlying motor circuits, and that (4) these changes may extend from decreased DA signaling from the hypothalamic PTar nucleus. These results, along with proposed future experiments, provide a detailed look into the status-dependent connection between multiple brain regions, and further, the mechanistic changes that result in altered synergistic neuromodulation of motor circuits.

REFERENCES

- Abdallah, K., Monconduit, L., Artola, A., Luccarini, P., Dallel, R. (2015). GABAergic inhibition or dopamine denervation of the A11 hypothalamic nucleus induces trigeminal analgesia. *Pain*, 156(4), 644–655. <https://doi.org/10.1097/j.pain.0000000000000091>.
- Abril-de-Abreu, R., Cruz, A. S., Oliveira, R. F. (2015). Social dominance modulates eavesdropping in zebrafish. *Royal Society open science*, 2(8), 150220.
- Adriani, W., Boyer, F., Leo, D., Canese, R., Podo, F., Perrone-Capano, C., ... & Laviola, G. (2010). Social withdrawal and gambling-like profile after lentiviral manipulation of DAT expression in the rat accumbens. *International Journal of Neuropsychopharmacology*, 13(10), 1329–1342.
- AlSubaie, R., Wee, R. W., Ritoux, A., Mishchanchuk, K., Passlack, J., Regester, D., MacAskill, A. F. (2021). Control of parallel hippocampal output pathways by amygdalar long-range inhibition. *eLife*, 10, e74758. <https://doi.org/10.7554/eLife.74758>.
- Andres, K.H., von Düring, M., Veh, R.W. (1999). Subnuclear organization of the rat habenular complexes. *The Journal of Comparative Neurology*, 407 (1): 130–50. doi:10.1002/(SICI)1096-9861(19990428)407:1<130::AID-CNE10>3.0.CO;2-8. PMID 10213193.
- Anfora G, Rigosi E, Frasnelli E, Ruga V, Trona F, Vallortigara G. (2011). Lateralization in the Invertebrate Brain: Left-Right Asymmetry of Olfaction in Bumble Bee, *Bombus terrestris*. *PloS one*, 6(4): e18903. <https://doi.org/10.1371/journal.pone.0018903>.
- Araki, M., Hasegawa, T., Komatsuda, S., Nagayama, T. (2013). Social status-dependent modulation of LG-flip habituation in the crayfish. *The Journal of experimental biology*, 216(Pt 4), 681–686. <https://doi.org/10.1242/jeb.075689>.
- Bacque-Cazenave, J., Issa, F.A., Edwards, D.H., Cattaert, D. (2013). Spatial segregation of excitatory and inhibitory effects produced by 5-HT cell on depressor motoneurons of the crayfish walking leg postural circuit. *J. Neurophysiol.*, 109, 2793-2802.
- Baez, R. & Schultz, W. (2013). The role of the striatum in social behavior. *Frontiers in Neuroscience*, <https://doi.org/10.3389/fnins.2013.00233>.
- Balph, M.H. (1979). Flock stability in relation to social dominance and agonistic behavior in wintering dark-eyed juncos. *The Auk*, 96(4):714-722. Doi: <https://doi.org/10.1093/auk/96.4.714>.
- Banner, A., & Shamay-Tsoory, S. (2018). Effects of androstadienone on dominance perception in males with low and high social anxiety. *Psychoneuroendocrinology*, 95, 138–144. <https://doi.org/10.1016/j.psyneuen.2018.05.032>.
- Barrot, M., Wallace, D.L., Bolanos, C.A...Nestler, E.J. (2005). Regulation of anxiety and initiation of sexual behavior by CREB in the nucleus accumbans. *PNAS*, 102(23):8357-8362.
- Bátora, D., Zsigmond, Á., Lőrincz, I. Z., Szegvári, G., Varga, M., & Málnási-Csizmadia, A. (2021). Subcellular Dissection of a Simple Neural Circuit: Functional Domains of the Mauthner-Cell During Habituation. *Frontiers in neural circuits*, 15, 648487. <https://doi.org/10.3389/fncir.2021.648487>.

- Bayram, H. L., Franco, C., Brownridge, P., Claydon, A. J., Koch, N., Hurst, J. L., Beynon, R. J., & Stockley, P. (2020). Social status and ejaculate composition in the house mouse. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 375(1813), 20200083. <https://doi.org/10.1098/rstb.2020.0083>.
- Beauchamp, G.K. (1974). The perineal scent gland and social dominance in the male guinea pig. *Physiology & Behavior*, 13(5):669-673.
- Beltrao, P., Marques, C.I., Cardoso, C.G., Gomes, A.C.R. (2021). Plumage colour saturation predict long-term, cross-seasonal social dominance in a mutually ornamented bird. *Animal Behaviour*, 182:239-250.
- Benton, D., Dalrymple-Alford, J.C., Brain, P.F. (1980). Comparisons of measures of dominance in the laboratory mouse. *Anim. Behav.*, 28:1274-1279.
- Bernau, N. A., Puzdrowski, R. L., Leonard, R. B. (1991). Identification of the midbrain locomotor region and its relation to descending locomotor pathways in the Atlantic stingray, *Dasyatis sabina*. *Brain Res.*, 557, 83–94. doi:10.1016/0006-8993(91)90119-G.
- Bernstein, I.S. (1976). Dominance, aggression, and reproduction in primate societies. *Journal of Theoretical Biology*, 60(2):459-472.
- Bicks, L.K., Koike, H., Akbarian, S., Morishita, H. (2015). Prefrontal cortex and social cognition in mouse and man. *Frontiers in Psychology*, <https://doi.org/10.3389/fpsyg.2015.01805>.
- Bielsky, I. F., Hu, S. B., Ren, X., Terwilliger, E. F., & Young, L. J. (2005). The V1a vasopressin receptor is necessary and sufficient for normal social recognition: a gene replacement study. *Neuron*, 47(4), 503–513. <https://doi.org/10.1016/j.neuron.2005.06.031>.
- Blanchard, D. C., Sakai, R. R., McEwen, B., Weiss, S. M., & Blanchard, R. J. (1993). Subordination stress: behavioral, brain, and neuroendocrine correlates. *Behavioural brain research*, 58(1-2):113–121 [https://doi.org/10.1016/0166-4328\(93\)90096-9](https://doi.org/10.1016/0166-4328(93)90096-9).
- Blanchard, R., Hori K., Tom, P., Blanchard, D.C. (1988). Social dominance and individual aggressiveness. *Aggressive Behavior*, 14(3):195-203.
- Bodian, D. (1937). The structure of the vertebrate synapse. A study of the axon endings on Mauthner's cell and neighboring centers in the goldfish. *Journal of Compar. Neuro.*, 68(1):117-159.
- Brehmer, Y., Westerberg, H., Bellander, M., Fürth, D., Karlsson, S., & Bäckman, L. (2009). Working memory plasticity modulated by dopamine transporter genotype. *Neuroscience letters*, 467(2), 117-120.

- Bronson, D. R., & Preuss, T. (2017). Cellular Mechanisms of Cortisol-Induced Changes in Mauthner-Cell Excitability in the Startle Circuit of Goldfish. *Frontiers in neural circuits*, 11, 68. <https://doi.org/10.3389/fncir.2017.00068>.
- Bunnell, B. N. (1966). Amygdaloid lesions and social dominance in the hooded rat. *Psychonomic Science*, 6(3), 93-94.
- Busch-Nentwich, E., Kettleborough, R., Dooley, C. M., Scahill, C., Sealy, I., White, R., ... & Stemple, D. L. (2013). Sanger institute zebrafish mutation project mutant data submission. *ZFIN direct data submission*.
- Buske, C., & Gerlai, R. (2011). Shoaling develops with age in Zebrafish (*Danio rerio*). *Progress in neuro-psychopharmacology & biological psychiatry*, 35(6), 1409–1415. <https://doi.org/10.1016/j.pnpbp.2010.09.003>.
- Cabelguen, J. M., Bourcier-Lucas, C., Dubuc, R. (2003). Bimodal locomotion elicited by electrical stimulation of the midbrain in the salamander *Notophthalmus viridescens*. *J. Neurosci.*, 23, 2434–2439.
- Canfield, J. G., & Rose, G. J. (1996). Hierarchical sensory guidance of mauthner-mediated escape responses in goldfish (*Carassius auratus*) and cichlids (*Haplochromis burtoni*). *Brain, behavior and evolution*, 48(3), 137–156. <https://doi.org/10.1159/000113193>.
- Carlsson, A. (1993). Thirty years of dopamine research. *Advances in neurology*, 60, 1–10.
- Carlsson, A., Lindqvist, M., & Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature*, 180(4596), 1200. <https://doi.org/10.1038/1801200a0>.
- Casey, E., Avale, M. E., Kravitz, A., & Rubinstein, M. (2022). Partial Ablation of Postsynaptic Dopamine D2 Receptors in the Central Nucleus of the Amygdala Increases Risk Avoidance in Exploratory Tasks. *eNeuro*, <https://doi.org/10.1523/ENEURO.0528-21.2022>.
- Chen, C-C. & Fernald, R.D. (2011). Visual information alone changes behavior and physiology during social interactions in a cichlid fish (*Astatotilapia burtoni*). *PloS one*. 6(5): e20313.
- Cheung, E., Chatterjee, D., & Gerlai, R. (2014). Subcutaneous dye injection for marking and identification of individual adult zebrafish (*Danio rerio*) in behavioral studies. *Behavior research methods*, 46(3), 619–624. <https://doi.org/10.3758/s13428-013-0399-x>
- Choi, H., Schwarzkopf, M., Fornace, M. E., Acharya, A., Artavanis, G., Stegmaier, J., Cunha, A., & Pierce, N. A. (2018). Third-generation *in situ* hybridization chain reaction: multiplexed, quantitative, sensitive, versatile, robust. *Development (Cambridge, England)*, 145(12), dev165753. <https://doi.org/10.1242/dev.165753>.
- Chou, M. Y., Amo, R., Kinoshita, M., Cherng, B. W., Shimazaki, H., Agetsuma, M., Shiraki, T., Aoki, T., Takahoko, M., Yamazaki, M., Higashijima, S., & Okamoto, H. (2016). Social conflict resolution regulated by two dorsal habenular subregions in zebrafish. *Science (New York, N.Y.)*, 352(6281), 87–90. <https://doi.org/10.1126/science.aac9508>.
- Cinque, S., Zoratto, F., Poleggi, A., Leo, D., Cerniglia, L., Cimino, S., Tambelli, R., Alleva, E., Gainetdinov, R. R., Laviola, G., & Adriani, W. (2018). Behavioral Phenotyping of Dopamine Transporter Knockout Rats: Compulsive Traits, Motor Stereotypies, and Anhedonia. *Frontiers in psychiatry*, 9, 43. <https://doi.org/10.3389/fpsy.2018.00043>.

- Cioni, C., de Palma, F., & Stefanelli, A. (1989). Morphology of afferent synapses in the Mauthner cell of larval *Xenopus laevis*. *The Journal of comparative neurology*, 284(2), 205–214. <https://doi.org/10.1002/cne.902840205>.
- Civelli, O., Bunzow, J.R., Grandy, D.K. (1993). Molecular diversity of the dopamine receptors. *Ann. Rev. Pharmacol. Toxicol.*, 32:281-307.
- Corrales-Parada, C. D., Morandi-Raikova, A., Rosa-Salva, O., & Mayer, U. (2021). Neural basis of unfamiliar conspecific recognition in domestic chicks (*Gallus Gallus domesticus*). *Behavioural brain research*, 397, 112927. <https://doi.org/10.1016/j.bbr.2020.112927>.
- Couppis, M. H., Kennedy, C. H., & Stanwood, G. D. (2008). Differences in aggressive behavior and in the mesocorticolimbic DA system between A/J and BALB/cJ mice. *Synapse*, 62(10), 715–724.
- Crawford, C. A., Drago, J., Watson, J. B., & Levine, M. S. (1997). Effects of repeated amphetamine treatment on the locomotor activity of the dopamine D1A-deficient mouse. *Neuroreport*, 8(11), 2523-2527.
- Curzon P, Zhang M, Radek RJ, et al. (2009). The Behavioral Assessment of Sensorimotor Processes in the Mouse: Acoustic Startle, Sensory Gating, Locomotor Activity, Rotarod, and Beam Walking. In: Buccafusco JJ, editor. *Methods of Behavior Analysis in Neuroscience*. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; Chapter 8. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK5236/>.
- d'Aldin, C., Eybalin, M., Puel, J. L., Charachon, G., Ladrech, S., Renard, N., & Pujol, R. (1995). Synaptic connections and putative functions of the dopaminergic innervation of the guinea pig cochlea. *European archives of oto-rhino-laryngology*, 252(5), 270-274.
- Dagda, R. K., & Das Banerjee, T. (2015). Role of protein kinase A in regulating mitochondrial function and neuronal development: implications to neurodegenerative diseases. *Reviews in the neurosciences*, 26(3), 359–370. <https://doi.org/10.1515/revneuro-2014-0085>.
- Darrow, K. N., Simons, E. J., Dodds, L., & Liberman, M. C. (2006). Dopaminergic innervation of the mouse inner ear: evidence for a separate cytochemical group of cochlear efferent fibers. *Journal of Comparative Neurology*, 498(3), 403-414.
- Davis, M., & Sheard, M. H. (1974). Habituation and sensitization of the rat startle response: effects of raphe lesions. *Physiology & behavior*, 12(3), 425–431. [https://doi.org/10.1016/0031-9384\(74\)90120-6](https://doi.org/10.1016/0031-9384(74)90120-6).
- Day, J.J & Carelli, R.M. (2011). The nucleus accumbens and pavlovian reward learning. *Neuroscientist*. 13(2):148-159.
- Dhingra, N. K., Lakshmana, M. K., Meti, B. L., & Raju, T. R. (1996). Subordination induced decrease in 5-hydroxytryptamine and dopamine levels in the frontal cortex—a study using worker-parasite relationship in rats as a model. *Indian journal of physiology and pharmacology*, 40(3), 213–219.
- Diamond, J., & Huxley, A. F. (1968). The activation and distribution of GABA and L-glutamate receptors on goldfish Mauthner: an analysis of dendritic remote inhibition. *The Journal of physiology*, 194(3), 669–723. <https://doi.org/10.1113/jphysiol.1968.sp008432>.

- Diamond, J., & Roper, S. (1973). Analysis of Mauthner cell responses to iontophoretically delivered pulses of GABA, glycine and L-glutamate. *The Journal of physiology*, 232(1), 113-128.
- Diamond, J., Roper, S., & Yasargil, G. M. (1973). The membrane effects, and sensitivity to strychnine, of neural inhibition of the Mauthner cell, and its inhibition by glycine and GABA. *The Journal of physiology*, 232(1), 87-111.
- DiCarlo, G. E., Aguilar, J. I., Matthies, H. J., Harrison, F. E., Bundschuh, K. E., West, A., ... & Galli, A. (2020). Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors. *The Journal of clinical investigation*, 129(8), 3407-3419.
- DiDomenico, R., & Eaton, R. C. (1988). Seven principles for command and the neural causation of behavior. *Brain, behavior and evolution*, 31(3), 125–140. <https://doi.org/10.1159/000116580>.
- Dijkstra, P. D., Hekman, R., Schulz, R. W., & Groothuis, T. G. (2007). Social stimulation, nuptial colouration, androgens and immunocompetence in a sexual dimorphic cichlid fish. *Behavioral Ecology and Sociobiology*, 61(4), 599-609.
- Dölen, G., & Malenka, R. C. (2014). The emerging role of nucleus accumbens oxytocin in social cognition. *Biological psychiatry*, 76(5), 354-355.
- Drea, C. M., Davies, C. S., Greene, L. K., Mitchell, J., Blondel, D. V., Shearer, C. L., Feldblum, J. T., Dimac-Stohl, K. A., Smyth-Kabay, K. N., & Clutton-Brock, T. H. (2021). An intergenerational androgenic mechanism of female intrasexual competition in the cooperatively breeding meerkat. *Nature communications*, 12(1), 7332. <https://doi.org/10.1038/s41467-021-27496-x>.
- Du, H., Deng, W., Aimone, J. B., Ge, M., Parylak, S., Walch, K., Zhang, W., Cook, J., Song, H., Wang, L., Gage, F. H., & Mu, Y. (2016). Dopaminergic inputs in the dentate gyrus direct the choice of memory encoding. *Proceedings of the National Academy of Sciences of the United States of America*, 113(37), E5501–E5510. <https://doi.org/10.1073/pnas.1606951113>.
- Ducrest, A. L., Keller, L., & Roulin, A. (2008). Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends in ecology & evolution*, 23(9), 502–510. <https://doi.org/10.1016/j.tree.2008.06.001>.
- Dumartin, B., Jaber, M., Gonon, F., Caron, M. G., Giros, B., & Bloch, B. (2000). Dopamine tone regulates D1 receptor trafficking and delivery in striatal neurons in dopamine transporter-deficient mice. *Proceedings of the National Academy of Sciences*, 97(4), 1879-1884.
- Dyakonova, V. E., Schürmann, F., & Sakharov, D. A. (1999). Effects of serotonergic and opiodergic drugs on escape behaviors and social status of male crickets. *Die Naturwissenschaften*, 86(9), 435–437. <https://doi.org/10.1007/s001140050647>.
- Eaton, R.C., Bombardieri, R.A., Meyer, D.L. (1977). Mauthner-initiated startle response in teleost fish. *J. Exp. Biol.*, 66, 65-81.
- Eaton, R.C., DiDomenico, R., & Nissanov, J. (1991). Role of the Mauthner cell in sensorimotor integration by the brain stem escape network. *Brain, behavior and evolution*, 37(5), 272–285. <https://doi.org/10.1159/000114365>.

- Eaton, R.C., Lee, R.K., & Foreman, M.B. (2001). The Mauthner cell and other identified neurons of the brainstem escape network of fish. *Progress in neurobiology*, 63(4), 467–485. [https://doi.org/10.1016/s0301-0082\(00\)00047-2](https://doi.org/10.1016/s0301-0082(00)00047-2).
- Eberhart, J.A., Keverne, E.B., Meller, R.E. (1980). Social influences on plasma testosterone levels in male Talapoin monkeys. *Hormones and Behavior*, 14:247-266.
- Edwards, D.H., & Spitzer, N. (2006). 6. Social dominance and serotonin receptor genes in crayfish. *Current topics in developmental biology*, 74, 177–199. [https://doi.org/10.1016/S0070-2153\(06\)74006-6](https://doi.org/10.1016/S0070-2153(06)74006-6).
- Egan, R. J., Bergner, C. L., Hart, P. C., Cachat, J. M., Canavello, P. R., Elegante, M. F., ... & Kalueff, A. V. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural brain research*, 205(1), 38-44.
- Eichenbaum, H., Otto, T., & Cohen, N. J. (1992). The hippocampus—what does it do? *Behavioral and neural biology*, 57(1), 2-36.
- Eidelberg, E., Walden, J. G., Nguyen, L. H. (1981). Locomotor control in macaque monkeys. *Brain*, 104, 647–663. [10.1093/brain/104.4.647-a](https://doi.org/10.1093/brain/104.4.647-a).
- El Kholy, S., Wang, K., El-Seedi, H. R., & Al Naggar, Y. (2021). Dopamine Modulates Drosophila Gut Physiology, Providing New Insights for Future Gastrointestinal Pharmacotherapy. *Biology*, 10(10), 983. <https://doi.org/10.3390/biology10100983>.
- Elsworth J.D., & Roth, R.H. (1997). Dopamine synthesis, uptake, metabolism, and receptors: Relevance to gene therapy of Parkinson's Disease. *Exp. Neurology*, 144:4-9.
- Emery, N. J., Capitanio, J. P., Mason, W. A., Machado, C. J., Mendoza, S. P., and Amaral, D. G. (2001). The effects of bilateral lesions of the amygdala on dyadic social interactions in rhesus monkeys (*Macaca mulatta*). *Behav. Neurosci.*, 115, 515–544. Doi: 10.1037/0735-7044.115.3.515.
- Engeszer R.E., Ryan M.J., Parichy D.M. (2004). Learned social preference in zebrafish *Curr. Bio.*, 14, 881–884.
- Enter, D., Colzato, L. S., & Roelofs, K. (2012). Dopamine transporter polymorphisms affect social approach–avoidance tendencies. *Genes, Brain and Behavior*, 11(6), 671-676.
- Erixon-Lindroth, N., Farde, L., Wahlin, T. B. R., Sovago, J., Halldin, C., & Bäckman, L. (2005). The role of the striatal dopamine transporter in cognitive aging. *Psychiatry Research: Neuroimaging*, 138(1), 1-12.
- Faber, D. S., & Korn, H. (1987). Voltage-dependence of glycine-activated Cl⁻ channels: a potentiometer for inhibition? *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 7(3), 807–811. <https://doi.org/10.1523/JNEUROSCI.07-03-00807.1987>.
- Falkenburger, B. H., Barstow, K. L., & Mintz, I. M. (2001). Dendrodendritic inhibition through reversal of dopamine transport. *Science (New York, N.Y.)*, 293(5539), 2465–2470. <https://doi.org/10.1126/science.1060645>.
- Fanciullacci, M., Alessandri, M., & Del Rosso, A. (2000). Dopamine involvement in the migraine attack. *Functional Neurology*, 15(3), 171-181.

- Feldman, J. M., Boyd, A. E., 3rd, & Lebovitz, H. E. (1971). Structural determinants of catecholamine action on in vitro insulin release. *The Journal of pharmacology and experimental therapeutics*, 176(3), 611–621.
- Förster D., Dal Maschio M., Laurell E., Baier H. (2017). An optogenetic toolbox for unbiased discovery of functionally connected cells in neural circuits. *Nat. Commun.*, 8, 116.
- Fougère, M., van der Zouwen, C. I., Boutin, J., & Ryczko, D. (2021). Heterogeneous expression of dopaminergic markers and Vglut2 in mouse mesodiencephalic dopaminergic nuclei A8–A13. *Journal of Comparative Neurology*, 529(7), 1273–1292.
- Francis, R.C. 1984. The effects of bidirectional selection for social dominance on agonistic behavior and sex ratios in the paradise fish (*Macropodus Opercularis*). *Behaviour.*, 90(1-3):25-44.
- Freese, J. L., and Amaral, D. G. (2009). “Neuroanatomy of the primate amygdala,” in *The Human Amygdala*, eds P. J. Whalen and E. A. Phelps (New York, NY: Guilford Press), 3–42.
- Fujii R. (2000). The regulation of motile activity in fish chromatophores. *Pigment Cell Res.*, 13, 300–19.
- Fujimoto, S., Hirata, B., & Nagayama, T. (2011). Dominance hierarchy-dependent behavioural plasticity of crayfish avoidance reactions. *The Journal of experimental biology*, 214(Pt 16), 2718–2723. <https://doi.org/10.1242/jeb.057752>.
- Fuxjager M.J., Marler C.A. How and why the winner effect forms: Influences of contest environment and species differences. *Behavioral Ecology*, 21(1):37-45.
- Garcia-Rill E., Houser C. R., Skinner R. D., Smith W., Woodward D. J. (1987). Locomotion-inducing sites in the vicinity of the pedunculopontine nucleus. *Brain Res. Bull.*, 18, 731–738. [10.1016/0361-9230\(87\)90208-5](https://doi.org/10.1016/0361-9230(87)90208-5).
- Gerlai, R., Lahav, M., Guo, S., & Rosenthal, A. (2000). Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacology, biochemistry, and behavior*, 67(4), 773–782. [https://doi.org/10.1016/s0091-3057\(00\)00422-6](https://doi.org/10.1016/s0091-3057(00)00422-6).
- Geyer, M. A., & Tapson, G. S. (1988). Habituation of tactile startle is altered by drugs acting on serotonin-2 receptors. *Neuropsychopharmacology*, 1(2), 135–147. [https://doi.org/10.1016/0893-133x\(88\)90005-x](https://doi.org/10.1016/0893-133x(88)90005-x).
- Gill, M., Daly, G., Heron, S., Hawi, Z., & Fitzgerald, M. (1997). Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism. *Molecular psychiatry*, 2(4), 311–313. <https://doi.org/10.1038/sj.mp.4000290>.
- Gjedde, A., Kumakura, Y., Cumming, P., Linnet, J., & Møller, A. (2010). Inverted-U-shaped correlation between dopamine receptor availability in striatum and sensation seeking. *Proceedings of the National Academy of Sciences of the United States of America*, 107(8), 3870–3875. <https://doi.org/10.1073/pnas.0912319107>.
- Gold, M. S., Levine, J. D., & Correa, A. M. (1998). Modulation of TTX-R INa by PKC and PKA and their role in PGE2-induced sensitization of rat sensory neurons in vitro. *Journal of Neuroscience*, 18(24), 10345–10355.

- Goodson, J. L., & Kingsbury, M. A. (2013). What's in a name? Considerations of homologies and nomenclature for vertebrate social behavior networks. *Hormones and behavior*, 64(1), 103–112. <https://doi.org/10.1016/j.yhbeh.2013.05.006>.
- Goodson, J. L., Eibach, R., Sakata, J., & Adkins-Regan, E. (1998). Effect of septal lesions on male song and aggression in the colonial zebra finch (*Taeniopygia guttata* *Spizella pusilla*). *Behav. Brain Res.*, 101(1), 167–180.
- Greenberg N. & Crews D. (1990). Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinesis*. *General and Compar Endocrin.*, 77(2)246-255.
- Greenberg, D., Snyder, K. P., Filazzola, A., Mastromonaco, G. F., & Schoof, V. (2022). Hormonal correlates of male dominance rank, age, and genital colouration in vervet monkey (*Chlorocebus pygerythrus*). *General and comparative endocrinology*, 316, 113948. <https://doi.org/10.1016/j.ygcen.2021.113948>.
- Grether G.F. (2011). The neuroecology of competitor recognition. *Integr. Comp. Biol.*, 51, 807–818.
- Groppe, S. E., Gossen, A., Rademacher, L., Hahn, A., Westphal, L., Gründer, G., & Spreckelmeyer, K. N. (2013). Oxytocin influences processing of socially relevant cues in the ventral tegmental area of the human brain. *Biological psychiatry*, 74(3), 172–179.
- Grosenick, L., Clement, T. S., and Fernald, R. D. (2007). Fish can infer social rank by observation alone. *Nature*, 445, 429–432. Doi: 10.1038/nature05511.
- Gunaydin L.A., Grosenick L., Finkelstein J.C., Kauvar I.V., Fenno L.E., Adhikari A., Lammel S., Mirzabekov J.J., Airan R.D., Zalocusky K.A. Natural neural projection dynamics underlying social behavior. *Cell.*, 2014(157):1535–1551.
- Gurevich, V. V., & Gurevich, E. V. (2019). GPCR Signaling Regulation: The Role of GRKs and Arrestins. *Frontiers in pharmacology*, 10, 125. <https://doi.org/10.3389/fphar.2019.00125>.
- Haehnel-Taguchi, M., Fernandes, A. M., Böhler, M., Schmitt, I., Tittel, L., & Driever, W. (2018). Projections of the Diencephalospinal Dopaminergic System to Peripheral Sense Organs in Larval Zebrafish (*Danio rerio*). *Frontiers in neuroanatomy*, 12, 20. <https://doi.org/10.3389/fnana.2018.00020>.
- Hale, M. E., Katz, H. R., Peek, M. Y., & Fremont, R. T. (2016). Neural circuits that drive startle behavior, with a focus on the Mauthner cells and spiral fiber neurons of fishes. *Journal of neurogenetics*, 30(2), 89–100.
- Hartwig, H. G., Brisson, P., Lyncker, I., & Collin, J. P. (1980). Aminergic systems in ulmonated gastropod molluscs. III. Microspectrofluorometric characterization of the monoamines in the reproductive system. *Cell and tissue research*, 210(2), 223–234. <https://doi.org/10.1007/BF00237611>.
- Hatamoto K. and Shingyoji C. (2008). Cyclical training enhances the melanophore responses of zebrafish to background colours. *Pigment Cell Melanoma Res.*, 21, 397–406.
- Henneman E. (1957). Relation between size of neurons and their susceptibility to discharge. *Science (New York, N.Y.)*, 126(3287), 1345–1347. <https://doi.org/10.1126/science.126.3287.1345>.

- Herberholz J, Issa FA, Edwards DH (2001). Patterns of neural circuit activation and behavior during dominance hierarchy formation in freely behaving crayfish. *The Journal of Neuroscience*, 21(8):2759-2767.
- Hoglund, E., Balm, P. H., & Winberg, S. (2000). Skin darkening, a potential social signal in subordinate arctic charr (*Salvelinus alpinus*): the regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides. *Journal of Experimental Biology*, 203(11), 1711-1721.
- Höjesjö J., Axelsson M., Dahy R., Gustavsson L. and Johnsson J.I. (2015). Sight or smell - Behavioral and heart rate response in subordinate rainbow trout exposed to cues from dominant fish. *PeerJ*, 3, e1169.
- Holmes, M.M., Rosen, G.J., Jordan, C.L., de Vries, F.J., Goldman, B.D., Forger, N.G. (2007). Social control of brain morphology in a eusocial mammal. *PNAS*. 104(25): 10548-10552.
- Homberg, J. R., Olivier, J. D., VandenBroeke, M., Youn, J., Ellenbroek, A. K., Karel, P., ... & Ellenbroek, B. A. (2016). The role of the dopamine D1 receptor in social cognition: studies using a novel genetic rat model. *Disease models & mechanisms*, 9(10), 1147-1158.
- Horová, E., K. Brandlová, and M. Gloneková. (2015). The first description of dominance hierarchy in captive giraffe: not loose and egalitarian, but clear and linear. *PloS one*, 10: e0124570. <https://doi.org/10.1016/j.yhbeh.2016.05.020>.
- Huber R., Smith K., Delago A., Isaksson K., Kravitz E.A. (1997). Serotonin and aggressive motivation in crustaceans: altering the decision to retreat. *Proc. Natl. Acad. Sci. USA*, 94, 5939-42.
- Hughes R. N. (1984). Cholinergic and monoaminergic substrates of startle habituation. *Life sciences*, 34(22), 2101–2105. [https://doi.org/10.1016/0024-3205\(84\)90308-4](https://doi.org/10.1016/0024-3205(84)90308-4).
- Huntingford F.A. and Turner A.K. (1987). Chapman and Hall Animal Behaviour Series. *Chapman and Hall: London*. ISBN 0-412-28750-1. 448 pp.
- Hutter S., Hettyey A., Penn D.J., Zala S.M. (2012). Ephemeral Sexual Dichromatism in Zebrafish (*Danio rerio*). *Ethology*, 118, 1208-1218.
- Ikebuchi, M., Hasegawa, T., & Bischof, H. J. (2009). Amygdala and socio-sexual behavior in male zebra finches. *Brain, behavior and Evolution*, 74(4), 250-257.
- Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Research Reviews*, 31(1), 6-41.
- Issa F.A., Adamson D.J., Edwards D.H. (1999). Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*. *J. Exp. Biol.*, 202, 3497-506.
- Issa F.A., Drummond J., Cattaert D., and Edwards D.H. (2012). Neural circuit reconfiguration by social status. *J. Neurosci.*, 32, 5638-5645.
- Issa F.A., Edwards D.H. (2006). Ritualized submission and the reduction of aggression in an invertebrate. *Current Biology*, 16(22):2217-2221.
- Issa F.A., O'Brien G., Kettunen P., Sagasti A., Glanzman D.L., Papazian D.M. (2011). Neural circuit activity in freely behaving zebrafish (*Danio rerio*). *J. Exp. Biol.*, 214, 1028-1038.

- Jaber, M., Jones, S., Giros, B., & Caron, M. G. (1997). The dopamine transporter: a crucial component regulating dopamine transmission. *Movement disorders: official journal of the Movement Disorder Society*, 12(5), 629-633.
- Jay T. M. (2003). Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. *Progress in neurobiology*, 69(6), 375–390. [https://doi.org/10.1016/s0301-0082\(03\)00085-6](https://doi.org/10.1016/s0301-0082(03)00085-6).
- John L., Rick I.P., Vitt S., Thunken T. (2021). Body coloration as a dynamic signal during intrasexual communication in a cichlid fish. *BMC Zoology*, 6(9): <https://doi.org/10.1186/s40850-021-00075-9>.
- Johnsson, J.I. (1997). Individual Recognition Affects Aggression and Dominance Relations in Rainbow Trout (*Oncorhynchus mykiss*). *Ethology*, 104, 267-282.
- Kacprzak, V., Patel, N. A., Riley, E., Yu, L., Yeh, J. J., & Zhdanova, I. V. (2017). Dopaminergic control of anxiety in young and aged zebrafish. *Pharmacology, biochemistry, and behavior*, 157, 1–8. <https://doi.org/10.1016/j.pbb.2017.01.005>.
- Kalueff, A. V., Gebhardt, M., Stewart, A. M., Cachat, J. M., Brimmer, M., Chawla, J. S., Craddock, C., Kyzar, E. J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., Gilman, C. P. (2013). Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish*, 10(1), 70–86. <https://doi.org/10.1089/zeb.2012.0861>.
- Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in pharmacological sciences*, 35(2), 63–75. <https://doi.org/10.1016/j.tips.2013.12.002>.
- Kaspter, C., Colombo, M., Aubin-Horth, N., Taborsky, B. (2018). Brain activation patterns following a cooperation opportunity in a highly social cichlid fish. *Physiology & Behavior*, 195:37-47.
- Kawano, M., Kawasaki, A., Sakata-Haga, H., Fukui, Y., Kawano, H., Nogami, H., & Hisano, S. (2006). Particular subpopulations of midbrain and hypothalamic dopamine neurons express vesicular glutamate transporter 2 in the rat brain. *The Journal of comparative neurology*, 498(5), 581–592. <https://doi.org/10.1002/cne.21054>.
- Keeler, B. E., Baran, C. A., Brewer, K. L., & Clemens, S. (2012). Increased excitability of spinal pain reflexes and altered frequency-dependent modulation in the dopamine D3-receptor knockout mouse. *Experimental neurology*, 238(2), 273–283. <https://doi.org/10.1016/j.expneurol.2012.09.002>.
- Kikkawa J., Smith, J.N.M., Prys-Jones, R., Fisk, P., Catterall, C. (1986). Determinants of social dominance and inheritance of agonistic behavior in an island population of silvereyes, *Zosterops lateralis*. *Behav. Ecol. & Sociobio.*, 19:165-169.
- Kim, J. Y., Tillu, D. V., Quinn, T. L., Mejia, G. L., Shy, A., Asiedu, M. N., Murad, E., Schumann, A. P., Totsch, S. K., Sorge, R. E., Mantyh, P. W., Dussor, G., & Price, T. J. (2015). Spinal dopaminergic projections control the transition to pathological pain plasticity via a D1/D5-mediated mechanism. *The Journal of neuroscience : The official journal of the Society for Neuroscience*, 35(16), 6307–6317. <https://doi.org/10.1523/JNEUROSCI.3481-14.2015>.

- Klein, M.O., Battagello, D.S., Cardoso, A.R. et al. Dopamine: Functions, Signaling, and Association with Neurological Diseases. *Cell Mol Neurobiol.*, 39, 31–59 (2019). <https://doi.org/10.1007/s10571-018-0632-3>.
- Knoch, S., Whiteside, M. A., Madden, J. R., Rose, P. E., & Fawcett, T. W. (2022). Hot-headed peckers: thermographic changes during aggression among juvenile pheasants (*Phasianus colchicus*). *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 377(1845), 20200442. <https://doi.org/10.1098/rstb.2020.0442>.
- Kohashi T. and Oda Y. (2008). Initiation of Mauthner- or non-Mauthner-mediated fast escape evoked by different modes of sensory input. *J. Neurosci.*, 28, 10641-10653.
- Kohls, G., Perino, M. T., Taylor, J. M., Madva, E. N., Cayless, S. J., Troiani, V., ... & Schultz, R. T. (2013). The nucleus accumbens is involved in both the pursuit of social reward and the avoidance of social punishment. *Neuropsychologia*, 51(11), 2062-2069.
- Korn H. and Faber D.S. (2005). The Mauthner cell half a century later: a neurobiological model for decision-making? *Neuron*, 47, 13-28.
- Korzan W.J., Robison R.R., Zhao S. and Fernald R.D. (2008). Color change as a potential behavioral strategy. *Horm. Behav.*, 54, 463-470.
- Koyama, M., Kinkhabwala, A., Satou, C., Higashijima, S. I., & Fetcho, J. (2011). Mapping a sensory-motor network onto a structural and functional ground plan in the hindbrain. *Proceedings of the National Academy of Sciences*, 108(3), 1170-1175.
- Kozorovitskiy, Y., & Gould, E. (2004). Dominance hierarchy influences adult neurogenesis in the dentate gyrus. *Journal of Neuroscience*, 24(30), 6755-6759.
- Krames, L., Carr, W.J. & Bergman, B. (1969). A pheromone associated with social dominance among male rats. *Psychon Sci*, 16, 11–12 <https://doi.org/10.3758/BF03331885>.
- Krasne, F. B., Shamsian, A., & Kulkarni, R. (1997). Altered excitability of the crayfish lateral giant escape reflex during agonistic encounters. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 17(2), 709–716. <https://doi.org/10.1523/JNEUROSCI.17-02-00709.1997>.
- Kroes, R.A., Panksepp, J., Burgdorf, J., Otto, N.J., Moskal, J.R. Modeling depression: dominance-submission gene expression patterns in rat neocortex. *Neuroscience*, 137(1):37-49.
- Lacoste, A. M., Schoppik, D., Robson, D. N., Haesemeyer, M., Portugues, R., Li, J. M., Randlett, O., Wee, C. L., Engert, F., & Schier, A. F. (2015). A convergent and essential interneuron pathway for Mauthner-cell-mediated escapes. *Current biology*, 25(11), 1526–1534. <https://doi.org/10.1016/j.cub.2015.04.025>.
- Lambert A.M., Bonkowsky J.L. and Masino M.A. (2012). The Conserved Dopaminergic Diencephalospinal Tract Mediates Vertebrate Locomotor Development in Zebrafish Larvae. *J. Neurosci.*, 32, 13488-13500.
- Lange, M., Neuzeret, F., Fabreges, B., Froc, C., Bedu, S., Bally-Cuif, L., & Norton, W. H. (2013). Inter-individual and inter-strain variations in zebrafish locomotor ontogeny. *PloS one*, 8(8), e70172.

- Larson, E. T., O'Malley, D. M., & Melloni, R. H., Jr (2006). Aggression and vasotocin are associated with dominant-subordinate relationships in zebrafish. *Behavioural brain research*, 167(1), 94–102. <https://doi.org/10.1016/j.bbr.2005.08.020>.
- Lee, R. K., Finger, T. E., & Eaton, R. C. (1993). GABAergic innervation of the Mauthner cell and other reticulospinal neurons in the goldfish. *The Journal of comparative neurology*, 338(4), 601–611. <https://doi.org/10.1002/cne.903380409>.
- Leo D., Sukanov I., Zoratto F., Illiano P., Caffino L...Gainetdinov RR. (2018). Pronounced hyperactivity, cognitive dysfunctions, and BDNF dysregulation in dopamine transporter knock-out rats. *Journal of Neuroscience*, 28(8) doi: 10.1523/jneurosci.1931-17.2018.
- Li, C., Liu, S., Lu, X., & Tao, F. (2019). Role of descending dopaminergic input in pain modulation. *Current Neuropharmacology*, 17(12), 1176–1182.
- Lister J.A., Robertson C.P., Lepage T., Johnson S.L. and Raible D.W. (1999). nacre encodes a zebrafish microphthalmia-related protein that regulates neural-crest-derived pigment cell fate. *Development*, 12, 3757–3767.
- Liu, C., & Kaeser, P. S. (2019). Mechanisms and regulation of dopamine release. *Current opinion in neurobiology*, 57, 46–53. <https://doi.org/10.1016/j.conb.2019.01.001>.
- Liu, K. S., & Fetcho, J. R. (1999). Laser ablations reveal functional relationships of segmental hindbrain neurons in zebrafish. *Neuron*, 23(2), 325–335. [https://doi.org/10.1016/s0896-6273\(00\)80783-7](https://doi.org/10.1016/s0896-6273(00)80783-7).
- Logan D.W., Burn S.F. and Jackson I.J. (2006). Regulation of pigmentation in zebrafish melanophores. *Pigment Cell Res.*, 19, 206–213.
- Lord J.P., Moser R.M.,Buonocore E.M...Lucas O. (2021). Dominance hierarchies in marine invertebrates. *Biol. Bull.*, 240:2-15.
- Lorent, K., Liu, K. S., Fetcho, J. R., & Granato, M. (2001). The zebrafish space cadet gene controls axonal pathfinding of neurons that modulate fast turning movements. *Development (Cambridge, England)*, 128(11), 2131–2142. <https://doi.org/10.1242/dev.128.11.2131>.
- Lozano-Montes, L., Astori, S., Abad, S., Guillot de Suduiraut, I., Sandi, C., & Zalachoras, I. (2019). Latency to Reward Predicts Social Dominance in Rats: A Causal Role for the Dopaminergic Mesolimbic System. *Frontiers in behavioral neuroscience*, 13, 69. <https://doi.org/10.3389/fnbeh.2019.00069>.
- Madeira, N., & Oliveira, R. F. (2017). Long-term social recognition memory in zebrafish. *Zebrafish*, 14(4), 305–310.
- Maguire S.M., DeAngelis R., Dijkstra P.D., Jordan A., Hofmann H.A. (2021). Social network dynamics predict hormone levels and behavior in highly social cichlid fish. *Horm Behav.*, doi: <https://doi.org/10.1016/j.yhbeh.2021.104994>.
- Mahadevia, D., Saha, R., Manganaro, A., Chuhma, N., Ziolkowski-Blake, A., Morgan, A. A., Dumitriu, D., Rayport, S., & Ansorge, M. S. (2021). Dopamine promotes aggression in mice via ventral tegmental area to lateral septum projections. *Nature communications*, 12(1), 6796. <https://doi.org/10.1038/s41467-021-27092-z>.

- Maison, S. F., Liu, X. P., Eatock, R. A., Sibley, D. R., Grandy, D. K., & Liberman, M. C. (2012). Dopaminergic signaling in the cochlea: receptor expression patterns and deletion phenotypes. *Journal of Neuroscience*, 32(1), 344-355.
- Mansbach, R. S., Geyer, M. A., & Braff, D. L. (1988). Dopaminergic stimulation disrupts sensorimotor gating in the rat. *Psychopharmacology*, 94(4), 507-514. <https://doi.org/10.1007/BF00212846>.
- Manuel R., Gorissen M., Zethof J., Ebbesson L.O., van de Vis H., Flik G. and van den Bos R. (2014). Unpredictable chronic stress decreases inhibitory avoidance learning in Tuebingen long-fin zebrafish: stronger effects in the resting phase than in the active phase. *J. Exp. Biol.*, 217, 3919-3928.
- Marlinsky V. V., Voitenko L. P. (1991). The effect of procaine injection into the medullary reticular formation on forelimb muscle activity evoked by mesencephalic locomotor region and vestibular stimulation in the decerebrated guinea-pig. *Neuroscience*, 45, 753-759. 10.1016/0306-4522(91)90287-X.
- Marmura M.J. (2012). Dopamine antagonists in treatment of migraine. *Curr Treat Options Neurol.*, 14(1):27-35.
- Marsden, K. C., & Granato, M. (2015). In Vivo Ca(2+) Imaging Reveals that Decreased Dendritic Excitability Drives Startle Habituation. *Cell reports*, 13(9), 1733-1740. <https://doi.org/10.1016/j.celrep.2015.10.060>.
- Martinez, D., Orlowska, D., Narendran, R...Kleber, H. D. 2010. Dopamine type 2/3 receptor availability in the striatum and social status in human volunteers. *Biol. Psychiatry*, 67(3):275-278.
- Mascaro, O., and Csibra, G. (2012). Representation of stable social dominance relations by human infants. *Proc. Natl. Acad. Sci. U.S.A.*, 109, 6862-6867. doi: 10.1073/pnas.1113194109.
- Matsumoto, M., & Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature*, 447(7148), 1111-1115. <https://doi.org/10.1038/nature05860>.
- McLean D.L., Fetcho JR. (2004). Relationship of tyrosine hydroxylase and serotonin immunoreactivity to sensorimotor circuitry in larval zebrafish. *J Comp Neurol.*, 480, 57-71.
- Medan V. and Preuss T. (2014). The Mauthner-cell circuit of fish as a model system for startle plasticity. *J. Physiol. Paris*, 108, 129-140.
- Menon, R., Sub, T., Elias de Moura Olivera, V., Neumann, I.D., Bludau A. (2021). Neurobiology of the lateral septum: regulation of social behavior. *Trends in Neuroscience*, 45(1):27-40.
- Michopoulos, V., Reding, K. M., Wilson, M. E., & Toufexis, D. (2012). Social subordination impairs hypothalamic-pituitary-adrenal function in female rhesus monkeys. *Hormones and behavior*, 62(4):389-399. <https://doi.org/10.1016/j.yhbeh.2012.07.014>.
- Miller, T. H., Clements, K., Ahn, S., Park, C., Hye Ji, E., & Issa, F. A. (2017). Social Status-Dependent Shift in Neural Circuit Activation Affects Decision Making. *The Journal of neuroscience : The official journal of the Society for Neuroscience*, 37(8), 2137-2148. <https://doi.org/10.1523/JNEUROSCI.1548-16.2017>.

- Miln, C., Ward, A., & Seebacher, F. (2021). Social rank and not physiological capacity determines competitive success in zebrafish (*Danio rerio*). *Royal Society open science*, 8(4), 210146. <https://doi.org/10.1098/rsos.210146>.
- Miyai, C. A., Sanches, F. H. C., Costa, T. M., Colpo, K. D., Volpato, G. L., & Barreto, R. E. (2011). The correlation between subordinate fish eye colour and received attacks: a negative social feedback mechanism for the reduction of aggression during the formation of dominance hierarchies. *Zoology*, 114(6), 335-339.
- Moly, P. K., & Hatta, K. (2011). Early glycinergic axon contact with the Mauthner neuron during zebrafish development. *Neuroscience research*, 70(3), 251–259. <https://doi.org/10.1016/j.neures.2011.03.003>.
- Montagrin, A., Saiote, C., & Schiller, D. (2018). The social hippocampus. *Hippocampus*, 28(9), 672-679.
- Moore, A.J., Ciccone, W.J. & Breed, M.D. (1988). The influence of social experience on the behavior of male cockroaches, *Nauphoeta cinerea*. *J Insect Behav.*, 1, 157–168. <https://doi.org/10.1007/BF01052235>.
- Morrison, T. R., Ricci, L. A., & Melloni, R. H. (2015). Dopamine D2 receptors act upstream of AVP in the latero-anterior hypothalamus to modulate adolescent anabolic/androgenic steroid-induced aggression in Syrian hamsters. *Behavioral neuroscience*, 129(2), 197–204. <https://doi.org/10.1037/bne0000044>.
- Mu, Y., Li, X. Q., Zhang, B., & Du, J. L. (2012). Visual input modulates audiomotor function via hypothalamic dopaminergic neurons through a cooperative mechanism. *Neuron*, 75(4), 688–699. <https://doi.org/10.1016/j.neuron.2012.05.035>.
- Müller, M., Olivier, J., & Homberg, J. (2010). Knockout and mutant rats. In *Transgenic and Mutant Tools to Model Brain Disorders* (pp. 13-31). Humana Press.
- Muller, M.N., Wrangham, R.W. (2003). Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*). *Behav Ecol Sociobiol.*, 44:332-340.
- Musienko P. E., Zelenin P. V., Lyalka V. F., Orlovsky G. N., Deliagina T. G. (2008). Postural performance in decerebrated rabbit. *Behav. Brain Res.*, 190, 124–134. [10.1016/j.bbr.2008.02.011](https://doi.org/10.1016/j.bbr.2008.02.011).
- Nader, M. A., Nader, S. H., Czoty, P. W., Riddick, N. V., Gage, H. D., Gould, R. W., Blaylock, B. L., Kaplan, J. R., Garg, P. K., Davies, H. M., Morton, D., Garg, S., & Reboussin, B. A. (2012). Social dominance in female monkeys: dopamine receptor function and cocaine reinforcement. *Biological psychiatry*, 72(5), 414–421. <https://doi.org/10.1016/j.biopsych.2012.03.002>.
- Nakamura, Y., Longueville, S., Nishi, A., Hervé, D., Girault, J. A., & Nakamura, Y. (2021). Dopamine D1 receptor-expressing neurons activity is essential for locomotor and sensitizing effects of a single injection of cocaine. *European Journal of Neuroscience*, 54(4), 5327-5340.
- Nakayama, H., & Oda, Y. (2004). Common sensory inputs and differential excitability of segmentally homologous reticulospinal neurons in the hindbrain. *The Journal of neuroscience : The official journal of the Society for Neuroscience*, 24(13), 3199–3209. <https://doi.org/10.1523/JNEUROSCI.4419-03.2004>.

- Narvaes, R., & Martins de Almeida, R. M. (2014). Aggressive behavior and three neurotransmitters: dopamine, GABA, and serotonin—A review of the last 10 years. *Psychology & Neuroscience*, 7(4), 601–607. <http://dx.doi.org/10.3922/j.psns.2014.4.20>.
- Neckameyer, W., O'Donnell, J., Huang, Z., & Stark, W. (2001). Dopamine and sensory tissue development in *Drosophila melanogaster*. *Journal of neurobiology*, 47(4), 280–294. <https://doi.org/10.1002/neu.1035>.
- Neumeister, H., Whitaker, K. W., Hofmann, H. A., & Preuss, T. (2010). Social and ecological regulation of a decision-making circuit. *Journal of neurophysiology*, 104(6), 3180–3188. <https://doi.org/10.1152/jn.00574.2010>.
- Nieuwenhuys, A., & Oudejans, R. R. (2012). Anxiety and perceptual-motor performance: toward an integrated model of concepts, mechanisms, and processes. *Psychological research*, 76(6), 747–759. <https://doi.org/10.1007/s00426-011-0384-x>.
- Nikulina, E. M., & Kapralova, N. S. (1992). Role of dopamine receptors in the regulation of aggression in mice; relationship to genotype. *Neuroscience and behavioral physiology*, 22(5), 364–369. <https://doi.org/10.1007/BF01186627>.
- Niu, X., & Canlon, B. (2006). The signal transduction pathway for the dopamine D1 receptor in the guinea-pig cochlea. *Neuroscience*, 137(3), 981–990.
- Norbury, A., & Husain, M. (2015). Sensation-seeking: Dopaminergic modulation and risk for psychopathology. *Behavioural brain research*, 288, 79–93. <https://doi.org/10.1016/j.bbr.2015.04.015>.
- Novick, A. M., Forster, G. L., Hassell, J. E., Davies, D. R., Scholl, J. L., Renner, K. J., & Watt, M. J. (2015). Increased dopamine transporter function as a mechanism for dopamine hypoactivity in the adult infralimbic medial prefrontal cortex following adolescent social stress. *Neuropharmacology*, 97, 194–200. <https://doi.org/10.1016/j.neuropharm.2015.05.032>.
- O'Connell, L. A., & Hofmann, H. A. (2011). The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *The Journal of comparative neurology*, 519(18), 3599–3639. <https://doi.org/10.1002/cne.22735>.
- Oestreicher, E., Arnold, W., Ehrenberger, K., & Felix, D. (1997). Dopamine regulates the glutamatergic inner hair cell activity in guinea pigs. *Hearing research*, 107(1-2), 46–52.
- Ogino, K., Yamada, K., Nishioka, T., Oda, Y., Kaibuchi, K., & Hirata, H. (2019). Phosphorylation of Gephyrin in Zebrafish Mauthner Cells Governs Glycine Receptor Clustering and Behavioral Desensitization to Sound. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 39(45), 8988–8997. <https://doi.org/10.1523/JNEUROSCI.1315-19.2019>.
- Okada, Y., Sasaki, K., Miyazaki, S., Shimoji, H., Tsuji, K., & Miura, T. (2015). Social dominance and reproductive differentiation mediated by dopaminergic signaling in a queenless ant. *The Journal of experimental biology*, 218(Pt 7), 1091–1098. <https://doi.org/10.1242/jeb.118414>.
- Oliveira R.F., Silva F.J. and Simões J.M. (2011). Fighting zebrafish: characterization of aggressive behavior and winner-loser effects. *Zebrafish*, 8, 73–81.

- Øverli Ø., Harris C.A., Winberg S. (1999). Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain, Behav, and Evolution*, 54(5), 263-275.
- Owesson-White, C.A., Ariansen, J., Stuber, G.D., Cleaveland, N.A., Cheer, J.F., Wightman, R.M., and Carelli, R.M. (2009). Neural encoding of cocaine seeking behavior is coincident with phasic dopamine release in the accumbens core and shell. *Eur. J. Neurosci.*, 30, 1117–1127.
- Pagel, M., and M. S. Dawkins. 1997. Peck orders and group size in laying hens: “futures contracts” for non-aggression. *Behav. Process*, 40: 13–25.
- Pantoja C., Hoagland A., Carroll E.C., Karalis V., Conner A., Isacoff E.Y. (2016). Neuromodulatory Regulation of Behavioral Individuality in Zebrafish. *Neuron*, 91, 587-601.
- Park, C., Clements, K. N., Issa, F. A., & Ahn, S. (2018). Effects of Social Experience on the Habituation Rate of Zebrafish Startle Escape Response: Empirical and Computational Analyses. *Frontiers in neural circuits*, 12, 7. <https://doi.org/10.3389/fncir.2018.00007>.
- Parker, M. O., Brock, A. J., Walton, R. T. & Brennan, C. H. (2013). The role of zebrafish (*Danio rerio*) in dissecting the genetics and neural circuits of executive function. *Front. Neural Circuits*, 7, 63.
- Pavlidis, M., Sundvik, M., Chen, Y. C., & Panula, P. (2011). Adaptive changes in zebrafish brain in dominant-subordinate behavioral context. *Behavioural brain research*, 225(2), 529–537. <https://doi.org/10.1016/j.bbr.2011.08.022>.
- Pereda, A. E., Bell, T. D., & Faber, D. S. (1995). Retrograde synaptic communication via gap junctions coupling auditory afferents to the Mauthner cell. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 15(9), 5943–5955. <https://doi.org/10.1523/JNEUROSCI.15-09-05943.1995>.
- Pereda, A., Triller, A., Korn, H., & Faber, D. S. (1992). Dopamine enhances both electrotonic coupling and chemical excitatory postsynaptic potentials at mixed synapses. *Proceedings of the National Academy of Sciences of the United States of America*, 89(24), 12088–12092. <https://doi.org/10.1073/pnas.89.24.12088>.
- Perelmuter, J. T., Wilson, A. B., Sisneros, J. A., & Forlano, P. M. (2019). Forebrain dopamine system regulates inner ear auditory sensitivity to socially relevant acoustic signals. *Current Biology*, 29(13), 2190-2198.
- Petrelli, F., Dallérac, G., Pucci, L., Calì, C., Zehnder, T., Sultan, S., Lecca, S., Chicca, A., Ivanov, A., Asensio, C. S., Gundersen, V., Toni, N., Knott, G. W., Magara, F., Gertsch, J., Kirchhoff, F., Déglon, N., Giros, B., Edwards, R. H., Mothet, J. P., ... Bezzi, P. (2020). Dysfunction of homeostatic control of dopamine by astrocytes in the developing prefrontal cortex leads to cognitive impairments. *Molecular psychiatry*, 25(4), 732–749. <https://doi.org/10.1038/s41380-018-0226-y>.
- Petrov, T., Seitanidou, T., Triller, A., Korn, H. (1991). Differential distribution of GABA and serotonin-containing afferents on an identified central neuron. *Brain Res.*, 559, 75–81.
- Phelps, E. A., Delgado, M. R., Nearing, K. I., & LeDoux, J. E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron*, 43(6), 897–905. <https://doi.org/10.1016/j.neuron.2004.08.042>.

- Pizzo, A. B., Karam, C. S., Zhang, Y., Yano, H., Freyberg, R. J., Karam, D. S., Freyberg, Z., Yamamoto, A., McCabe, B. D., & Javitch, J. A. (2013). The membrane raft protein Flotillin-1 is essential in dopamine neurons for amphetamine-induced behavior in *Drosophila*. *Molecular psychiatry*, 18(7), 824–833. <https://doi.org/10.1038/mp.2012.82>.
- Plavén-Sigra, P., Gustavsson, P., Farde, L., Borg, J., Stenkrona, P., Nyberg, L., ... & Cervenka, S. (2014). Dopamine D1 receptor availability is related to social behavior: a positron emission tomography study. *NeuroImage*, 102, 590-595.
- Pogarell, O., Tatsch, K., Juckel, G., Hamann, C., Mulert, C., Pöpperl, G., ... & Hegerl, U. (2004). Serotonin and dopamine transporter availabilities correlate with the loudness dependence of auditory evoked potentials in patients with obsessive-compulsive disorder. *Neuropsychopharmacology*, 29(10), 1910-1917.
- Porter, B. A., & Mueller, T. (2020). The Zebrafish Amygdaloid Complex - Functional Ground Plan, Molecular Delineation, and Everted Topology. *Frontiers in neuroscience*, 14, 608. <https://doi.org/10.3389/fnins.2020.00608>
- Prugh J.I., Kimmel C.B. and Metcalfe W.K. (1982). Noninvasive recording of the Mauthner neuron action potential in larval zebrafish. *J. Exp. Biol.*, 101, 83-92.
- Qu, C., Ligneul, R., Van der Henst, J. B., & Dreher, J. C. (2017). An integrative interdisciplinary perspective on social dominance hierarchies. *Trends in cognitive sciences*, 21(11), 893-908.
- Quickel, K. E., Jr, Feldman, J. M., & Lebovitz, H. E. (1971). Inhibition of insulin secretion by serotonin and dopamine: species variation. *Endocrinology*, 89(5), 1295–1302. <https://doi.org/10.1210/endo-89-5-1295>.
- Rankin, M. L., Marinec, P. S., Cabrera, D. M., Wang, Z., Jose, P. A., & Sibley, D. R. (2006). The D1 dopamine receptor is constitutively phosphorylated by G protein-coupled receptor kinase 4. *Molecular pharmacology*, 69(3), 759–769. <https://doi.org/10.1124/mol.105.019901>.
- Razzoli, M., Sanghez, V., & Bartolomucci, A. (2015). Chronic subordination stress induces hyperphagia and disrupts eating behavior in mice modeling binge-eating-like disorder. *Frontiers in nutrition*, 1(30), 30. <https://doi.org/10.3389/fnut.2014.00030>.
- Reinig, S., Driever, W., & Arrenberg, A. B. (2017). The Descending Diencephalic Dopamine System Is Tuned to Sensory Stimuli. *Current biology: CB*, 27(3), 318–333. <https://doi.org/10.1016/j.cub.2016.11.059>.
- Rial, D., Puighermanal, E., Chazalon, M., Valjent, E., Schiffmann, S. N., & de Kerchove d'Exaerde, A. (2020). Mammalian target of rapamycin-RhoA signaling impairments in direct striatal projection neurons induce altered behaviors and striatal physiology in mice. *Biological psychiatry*, 88(12), 945-954.
- Rice, M. E., & Cragg, S. J. (2008). Dopamine spillover after quantal release: rethinking dopamine transmission in the nigrostriatal pathway. *Brain research reviews*, 58(2), 303-313.
- Rice, M. E., Patel, J. C., & Cragg, S. J. (2011). Dopamine release in the basal ganglia. *Neuroscience*, 198, 112–137. <https://doi.org/10.1016/j.neuroscience.2011.08.066>.
- Richter-Levin, G., & Akirav, I. (2000). Amygdala-hippocampus dynamic interaction in relation to memory. *Molecular neurobiology*, 22(1-3), 11–20. <https://doi.org/10.1385/MN:22:1-3:011>.

- Roberts, A. C., Pearce, K. C., Choe, R. C., Alzagatiti, J. B., Yeung, A. K., Bill, B. R., & Glanzman, D. L. (2016). Long-term habituation of the C-start escape response in zebrafish larvae. *Neurobiology of learning and memory*, 134(Pt B), 360–368. <https://doi.org/10.1016/j.nlm.2016.08.014>.
- Rodgers, W. L., Melzack, R., & Segal, J. R. (1963). "Tail flip response" in goldfish. *Journal of Comparative and Physiological Psychology*, 56(5), 917–923. <https://doi.org/10.1037/h0039700>.
- Rodriguez-Santiago, M., Jordan, A., & Hofmann, H. A. (2022). Neural activity patterns differ between learning contexts in a social fish. *Proceedings. Biological sciences*, 289(1974), 20220135. <https://doi.org/10.1098/rspb.2022.0135>.
- Rodriguez, R. M., Chu, R., Caron, M. G., & Wetsel, W. C. (2004). Aberrant responses in social interaction of dopamine transporter knockout mice. *Behavioural brain research*, 148(1-2), 185-198.
- Roseberry T. K., Lee A. M., Lalive A. L., Wilbrecht L., Bonci A., Kreitzer A. C. (2016). Cell-type-specific control of brainstem locomotor circuits by basal ganglia. *Cell*, 164, 526–537. [10.1016/j.cell.2015.12.037](https://doi.org/10.1016/j.cell.2015.12.037).
- Rosvold, H. E., Mirsky, A. F., & Pribram, K. H. (1954). Influence of amygdectomy on social behavior in monkeys. *Journal of Comparative and Physiological Psychology*, 47(3), 173–178. <https://doi.org/10.1037/h0058870>.
- RStudio Team. (2011). RStudio: Integrated Development Environment for R; RStudio PBC: Boston, MA, USA.
- Rubí, B., & Maechler, P. (2010). Minireview: new roles for peripheral dopamine on metabolic control and tumor growth: let's seek the balance. *Endocrinology*, 151(12), 5570–5581. <https://doi.org/10.1210/en.2010-0745>.
- Ruel, J., Nouvian, R., d'Aldin, C. G., Pujol, R., Eybalin, M., & Puel, J. L. (2001). Dopamine inhibition of auditory nerve activity in the adult mammalian cochlea. *European Journal of Neuroscience*, 14(6), 977-986.
- Ruel, J., Wang, J., Demêmes, D., Gobaille, S., Puel, J. L., & Rebillard, G. (2006). Dopamine transporter is essential for the maintenance of spontaneous activity of auditory nerve neurons and their responsiveness to sound stimulation. *Journal of neurochemistry*, 97(1), 190-200.
- Ryczko, D., Gratsch, S., Auclair, F., Dube, C., Bergeron, S., Alpert, M. H., et al. (2013). Forebrain dopamine neurons project down to a brainstem region controlling locomotion. *Proc. Natl. Acad. Sci. U.S.A.*, 110, E3235–E3242. doi: 10.1073/pnas.1301125110.
- Saltiel, H., & Thomopoulos, P. (1981). Dopamine et hormones antéhypophysaires. Physiologie et pathologie [Dopamine and pituitary hormones. Physiology and pathology (author's transl)]. *Annales d'endocrinologie*, 42(6), 497–516.
- Sampio A.S., Hounie A.G...Miguel E.C. (2015). COMT and MAO-A Polymorphisms and obsessive compulsive disorder: a family-based association study. *PloS one*, doi: <https://doi.org/10.1371/journal.pone.0119592>.

- San-Jose, L.M., & Roulin, A. (2020). On the Potential Role of the Neural Crest Cells in Integrating Pigmentation Into Behavioral and Physiological Syndromes. *Front. Ecol. Evol.*, <https://doi.org/10.3389/fevo.2020.00278>.
- Sankrithi N.S. and O'Malley D.M. (2010). Activation of a Multisensory, Multifunctional Nucleus in the Zebrafish Midbrain during Diverse Locomotor Behaviors. *J. Neurol.*, 166, 970-993.
- Sapolsky R.M. (2006). Social cultures among nonhuman primates. *Current Anthropology*, 47(4):641-656.
- Sasaki, K., & Harada, M. (2020). Dopamine production in the brain is associated with caste-specific morphology and behavior in an artificial intermediate honey bee caste. *PloS one*, 15(12), e0244140. <https://doi.org/10.1371/journal.pone.0244140>.
- Saverino, C., & Gerlai, R. (2008). The social zebrafish: behavioral responses to conspecific, heterospecific, and computer animated fish. *Behavioural brain research*, 191(1), 77-87. <https://doi.org/10.1016/j.bbr.2008.03.013>.
- Schwartz, J. J., & Melloni, R. H., Jr (2010). Dopamine activity in the lateral anterior hypothalamus modulates AAS-induced aggression through D2 but not D5 receptors. *Behavioral neuroscience*, 124(5), 645-655. <https://doi.org/10.1037/a0020899>.
- Senar, J. C., Hill, G. E., & McGraw, K. J. (2006). Color displays as intrasexual signals of aggression and dominance. *Bird coloration*, 2, 87-136.
- Shan, Q., Hu, Y., Chen, S., & Tian, Y. (2022). Nucleus accumbens dichotomically controls social dominance in male mice. *Neuropsychopharmacology*, 47(3), 776-787. <https://doi.org/10.1038/s41386-021-01220-1>.
- Sheehan, T. P., Chambers, R. A., & Russell, D. S. (2004). Regulation of affect by the lateral septum: implications for neuropsychiatry. *Brain Research Reviews*, 46(1), 71-117.
- Shinohara, R., Taniguchi, M., Ehrlich, A. T., Yokogawa, K., Deguchi, Y., Cherasse, Y., Lazarus, M., Urade, Y., Ogawa, A., Kitaoka, S., Sawa, A., Narumiya, S., & Furuyashiki, T. (2018). Dopamine D1 receptor subtype mediates acute stress-induced dendritic growth in excitatory neurons of the medial prefrontal cortex and contributes to suppression of stress susceptibility in mice. *Molecular psychiatry*, 23(8), 1717-1730. <https://doi.org/10.1038/mp.2017.177>.
- Shively, C. A. (1998). Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys. *Biological Psychiatry*, 44(9), 882-891. [https://doi.org/10.1016/S0006-3223\(97\)00437-X](https://doi.org/10.1016/S0006-3223(97)00437-X).
- Sholomenko G. N., Funk G. D., Steeves J. D. (1991). Avian locomotion activated by brainstem infusion of neurotransmitter agonists and antagonists. II. gamma-Aminobutyric acid. *Exp. Brain Res.*, 85, 674-681. 10.1007/BF00231753.
- Singh A.P., Nüsslein-Volhard C. (2015). Zebrafish stripes as a model for vertebrate colour pattern formation. *Curr. Biol.*, 25, R81-92.
- Sirota M. G., Di Prisco G. V., Dubuc R. (2000). Stimulation of the mesencephalic locomotor region elicits controlled swimming in semi-intact lampreys. *Eur. J. Neurosci.*, 12, 4081-4092. 10.1046/j.1460-9568.2000.00301.x.

- Slavík, O., Horký, P., & Wackermannová, M. (2016). How does agonistic behaviour differ in albino and pigmented fish? *PeerJ*, 4, e1937. <https://doi.org/10.7717/peerj.1937>.
- Snekser J.L., McRobert S.P., Murphy C.E., Clotfelter E.D. (2006). Aggregation Behavior in Wildtype and Transgenic Zebrafish. *Ethology*, 112, 181-187.
- Sowers, J. R., Crane, P. D., Beck, F. W., McClanahan, M., King, M. E., & Mohanty, P. K. (1984). Relationship between urinary dopamine production and natriuresis after acute intravascular volume expansion with sodium chloride in dogs. *Endocrinology*, 115(6), 2085–2090. <https://doi.org/10.1210/endo-115-6-2085>.
- Speedie, N., & Gerlai, R. (2008). Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). *Behavioural brain research*, 188(1), 168–177. <https://doi.org/10.1016/j.bbr.2007.10.031>.
- Stednitz, S. J., McDermott, E. M., Ncube, D., Tallafuss, A., Eisen, J. S., & Washbourne, P. (2018). Forebrain control of behaviorally driven social orienting in zebrafish. *Current Biology*, 28(15), 2445-2451.
- Stein, R. B., Gossen, E. R., & Jones, K. E. (2005). Neuronal variability: noise or part of the signal? *Nature Reviews Neuroscience*, 6(5), 389-397.
- Stouffer, M. A., Ali, S., Reith, M. E., Patel, J. C., Sarti, F., Carr, K. D., & Rice, M. E. (2011). SKF-83566, a D1-dopamine receptor antagonist, inhibits the dopamine transporter. *Journal of neurochemistry*, 118(5), 714–720. <https://doi.org/10.1111/j.1471-4159.2011.07357.x>.
- Sukhodolskaya, E. M., Fehretdinova, D. I., Shibalev, D. V., Lazebny, O. E., Mabulla, A., Butovskaya, M. L., Ryskov, A. P., & Vasilyev, V. V. (2018). Polymorphisms of dopamine receptor genes DRD2 and DRD4 in African populations of Hadza and Datoga differing in the level of culturally permitted aggression. *Annals of human genetics*, 82(6), 407–414. <https://doi.org/10.1111/ahg.12263>.
- Sullivan, G. M., Apergis, J., Gorman, J. M., & LeDoux, J. E. (2003). Rodent doxapram model of panic: behavioral effects and c-Fos immunoreactivity in the amygdala. *Biological psychiatry*, 53(10), 863-870.
- Sun, W., & Salvi, R. J. (2001). Dopamine modulates sodium currents in cochlear spiral ganglion neurons. *Neuroreport*, 12(4), 803-807.
- Sur, C., Korn, H., & Triller, A. (1994). Colocalization of somatostatin with GABA or glutamate in distinct afferent terminals presynaptic to the Mauthner cell. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 14(2), 576–589. <https://doi.org/10.1523/JNEUROSCI.14-02-00576.1994>.
- Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., Nairn, A. C., & Greengard, P. (2004). DARPP-32: an integrator of neurotransmission. *Annual review of pharmacology and toxicology*, 44, 269–296. <https://doi.org/10.1146/annurev.pharmtox.44.101802.121415>.
- Tea, J., Alderman, S. L., & Gilmour, K. M. (2019). Social stress increases plasma cortisol and reduces forebrain cell proliferation in subordinate male zebrafish (*Danio rerio*). *The Journal of experimental biology*, 222(Pt 2), jeb194894. <https://doi.org/10.1242/jeb.194894>.
- Teles, M. C., & Oliveira, R. F. (2016). Quantifying Aggressive Behavior in Zebrafish. *Methods in molecular biology*, 1451, 293–305. https://doi.org/10.1007/978-1-4939-3771-4_20.

- Teles, M. C., Cardoso, S. D., & Oliveira, R. F. (2016a). Social plasticity relies on different neuroplasticity mechanisms across the brain social decision-making network in zebrafish. *Frontiers in behavioral neuroscience*, 10, 16.
- Teles, M. C., Dahlbom, S. J., Winberg, S., & Oliveira, R. F. (2013). Social modulation of brain monoamine levels in zebrafish. *Behavioural brain research*, 253, 17-24.
- Teles, M. C., Gozdowska, M., Kalamarz-Kubiak, H., Kulczykowska, E., & Oliveira, R. F. (2016b). Agonistic interactions elicit rapid changes in brain nonapeptide levels in zebrafish. *Hormones and behavior*, 84, 57–63.
- Toro, C., Trapani, J. G., Pacentine, I., Maeda, R., Sheets, L., Mo, W., & Nicolson, T. (2015). Dopamine Modulates the Activity of Sensory Hair Cells. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 35(50), 16494–16503. <https://doi.org/10.1523/JNEUROSCI.1691-15.2015>.
- Trumpp-Kallmeyer, S., Hoflack, J., Bruinvels, A., Hibert, M. (1992). Modeling of G-Protein Coupled Receptors: Application to dopamine, adrenaline, serotonin, acetylcholine, and mammalian opsin receptors. *J. Med. Chem.* 25:3448-3462.
- Van Erp A.M.M., & Miczek K.A. (2000). Aggressive behavior, increased accumbal dopamine, and decreased serotonin in rats. *Journal of Neuroscience*. 20 (24) 9320-9325.
- Venables W.N. and Ripley B.D. (2002). Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.
- Vignet, C., Bégout, M. L., Péan, S., Lyphout, L., Leguay, D., & Cousin, X. (2013). Systematic screening of behavioral responses in two zebrafish strains. *Zebrafish*, 10(3), 365-375.
- Vijayanathan, Y., Lim, F. T., Lim, S. M., Long, C. M., Tan, M. P., Majeed, A., & Ramasamy, K. (2017). 6-OHDA-Lesioned Adult Zebrafish as a Useful Parkinson's Disease Model for Dopaminergic Neuroregeneration. *Neurotoxicity research*, 32(3), 496–508. <https://doi.org/10.1007/s12640-017-9778-x>.
- Wang D., Zhang J., Zhang Z. (2012). Effect of testosterone and melatonin on social dominance and agonistic behavior in male *Tscheskia triton*. *Behav Processes*. 89(3):271-277.
- Wang G, Zhang G, Li Z, Fawcett CH, Coble M, Sosa MX, Tsai T, Malesky K, Thibodeaux SJ, Zhu P, Glass DJ, Fishman MC. (2019). Abnormal Behavior of Zebrafish Mutant in Dopamine Transporter Is Rescued by Clozapine. *iScience*. doi: 10.1016/j.isci.2019.06.039.
- Wang, X. H., Souders II, C. L., Xavier, P., Li, X. Y., Yan, B., & Martyniuk, C. J. (2020). The pyrethroid esfenvalerate induces hypoactivity and decreases dopamine transporter expression in embryonic/larval zebrafish (*Danio rerio*). *Chemosphere*, 243, 125416.
- Watanabe, N., & Yamamoto, M. (2015). Neural mechanisms of social dominance. *Frontiers in neuroscience*, 9, 154. <https://doi.org/10.3389/fnins.2015.00154>.
- Whitaker, K. W., Neumeister, H., Huffman, L. S., Kidd, C. E., Preuss, T., & Hofmann, H. A. (2011). Serotonergic modulation of startle-escape plasticity in an African cichlid fish: a single-cell molecular and physiological analysis of a vital neural circuit. *Journal of neurophysiology*, 106(1), 127–137. <https://doi.org/10.1152/jn.01126.2010>.

- White, S. A., & Fernald, R. D. (1993). Gonadotropin-releasing hormone-containing neurons change size with reproductive state in female *Haplochromis burtoni*. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 13(2), 434–441. <https://doi.org/10.1523/JNEUROSCI.13-02-00434.1993>.
- Wilson, Edward O. (1975). *Sociobiology: the New Synthesis*. Cambridge, Mass.: Belknap Press of Harvard University Press.
- Winberg, S., & Lepage, O. (1998). Elevation of brain 5-HT activity, POMC expression, and plasma cortisol in socially subordinate rainbow trout. *The American journal of physiology*, 274(3), R645–R654. <https://doi.org/10.1152/ajpregu.1998.274.3.R645>.
- Wise, R.A. (2008). Dopamine and reward: the anhedonia hypothesis 30 years on. *Neurotox. Res.* 14, 169–183.
- Wolf, M. E., Mangiavacchi, S., & Sun, X. (2003). Mechanisms by which dopamine receptors may influence synaptic plasticity. *Annals of the New York Academy of Sciences*, 1003(1), 241–249.
- Wolman, M. A., Jain, R. A., Marsden, K. C., Bell, H., Skinner, J., Hayer, K. E., Hogenesch, J. B., & Granato, M. (2015). A genome-wide screen identifies PAPP-AA-mediated IGFR signaling as a novel regulator of habituation learning. *Neuron*, 85(6), 1200–1211. <https://doi.org/10.1016/j.neuron.2015.02.025>.
- Wolszon, L. R., Pereda, A. E., & Faber, D. S. (1997). A fast synaptic potential mediated by NMDA and non-NMDA receptors. *Journal of neurophysiology*, 78(5), 2693–2706. <https://doi.org/10.1152/jn.1997.78.5.2693>.
- Xi, Y., Yu, M., Godoy, R., Hatch, G., Poitras, L., & Ekker, M. (2011). Transgenic zebrafish expressing green fluorescent protein in dopaminergic neurons of the ventral diencephalon. *Developmental dynamics: an official publication of the American Association of Anatomists*, 240(11), 2539–2547. <https://doi.org/10.1002/dvdy.22742>.
- Xing, B., Mack, N. R., Zhang, Y. X., McEachern, E. P., & Gao, W. J. (2022). Distinct Roles for Prefrontal Dopamine D1 and D2 Neurons in Social Hierarchy. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 42(2), 313–324. <https://doi.org/10.1523/JNEUROSCI.0741-21.2021>.
- Xu, X., Zhang, Z., Guo, H., Qin, J., & Zhang, X. (2020). Changes in Aggressive Behavior, Cortisol and Brain Monoamines during the Formation of Social Hierarchy in Black Rockfish (*Sebastes schlegelii*). *Animals: an open access journal from MDPI*, 10(12), 2357. <https://doi.org/10.3390/ani10122357>.
- Yamaguchi, M., Yoshimoto, E., & Kondo, S. (2007). Pattern regulation in the stripe of zebrafish suggests an underlying dynamic and autonomous mechanism. *Proceedings of the National Academy of Sciences of the United States of America*, 104(12), 4790–4793. <https://doi.org/10.1073/pnas.0607790104>.
- Yamaguchi, Y., Lee, Y. A., Kato, A., & Goto, Y. (2017). The Roles of Dopamine D1 Receptor on the Social Hierarchy of Rodents and Nonhuman Primates. *The international journal of neuropsychopharmacology*, 20(4), 324–335. <https://doi.org/10.1093/ijnp/pyw106>.

- Yao Y., Li X., Zhang B., Yin C., Liu Y., Chen W., Zeng S. and Du J. (2016). Visual Cue-Discriminative Dopaminergic Control of Visuomotor Transformation and Behavior Selection. *Neuron*, 89, 598-612.
- Yeh, S. R., Fricke, R. A., & Edwards, D. H. (1996). The effect of social experience on serotonergic modulation of the escape circuit of crayfish. *Science*, 271(5247), 366–369. <https://doi.org/10.1126/science.271.5247.366>.
- Yeomans, J. S., Li, L., Scott, B. W., & Frankland, P. W. (2002). Tactile, acoustic and vestibular systems sum to elicit the startle reflex. *Neuroscience and biobehavioral reviews*, 26(1), 1–11. [https://doi.org/10.1016/s0149-7634\(01\)00057-4](https://doi.org/10.1016/s0149-7634(01)00057-4).
- Zempo, B., Tanaka, N., Daikoku, E. et al. (2021). High-speed camera recordings uncover previously unidentified elements of zebrafish mating behaviors integral to successful fertilization. *Sci Rep*, 11, 20228 <https://doi.org/10.1038/s41598-021-99638-6>.
- Zhang, J., Forkstam, C., Engel, J. A., & Svensson, L. (2000). Role of dopamine in prepulse inhibition of acoustic startle. *Psychopharmacology*, 149(2), 181–188. <https://doi.org/10.1007/s002130000369>.
- Zhao, T., Gao, X., & Huang, G. B. (2021). Effects of Chronic Social Defeat Stress on Behavior and Dopamine Receptors in Adolescent Mice With 6-Hydroxydopamine Lesions of the Medial Prefrontal Cortex. *Frontiers in behavioral neuroscience*, 15, 731373. <https://doi.org/10.3389/fnbeh.2021.731373>.
- Zink, C. F., Tong, Y., Chen, Q., Bassett, D. S., Stein, J. L., and Meyer-Lindenberg, A. (2008). Know your place: neural processing of social hierarchy in humans. *Neuron*, 58, 273–283 doi: 10.1016/j.neuron.2008.01.025.
- Zottoli S. J. (1978). Comparison of Mauthner cell size in teleosts. *The Journal of comparative neurology*, 178(4), 741–757. <https://doi.org/10.1002/cne.901780409>.
- Zottoli, S. J., & Faber, D. S. (1979). Properties and distribution of anterior VIIIth nerve excitatory inputs to the goldfish Mauthner cell. *Brain research*, 174(2), 319–323. [https://doi.org/10.1016/0006-8993\(79\)90854-0](https://doi.org/10.1016/0006-8993(79)90854-0).
- Zottoli, S. J., Hordes, A. R., & Faber, D. S. (1987). Localization of optic tectal input to the ventral dendrite of the goldfish Mauthner cell. *Brain research*, 401(1), 113–121. [https://doi.org/10.1016/0006-8993\(87\)91170-x](https://doi.org/10.1016/0006-8993(87)91170-x).

APPENDIX A: ANIMAL USE PROTOCOL APPROVAL LETTER



**Animal Care and
Use Committee**

212 Ed Warren Life
Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

December 1, 2014

Fadi Issa, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Issa:

Your Animal Use Protocol entitled, "Developmental and Functional Effects of Spinocerebellar Ataxia Type-13 on Zebrafish Cerebellum and the Effects of Social Experience on Zebrafish Startle Escape Response" (AUP #D320) was reviewed by this institution's Animal Care and Use Committee on 12/1/14. The following action was taken by the Committee:

"Approved as submitted"

Please contact Dale Aycock at 744-2997 prior to hazard use

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP and are familiar with its contents.**

Sincerely yours,

A handwritten signature in black ink, appearing to read 'S. B. McRae'.

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/jd

Enclosure

APPENDIX B: ANIMAL USE AND CARE COMMITTEE APPROVAL LETTER



**Animal Care and
Use Committee**
212 Ed Warren Life
Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

MEMORANDUM

TO: Katie Clements
Department of Biology

FROM: Dorcas O'Rourke, D.V.M.
University Veterinarian 

SUBJECT: Certificate of Training
Training Date 10/8/14

DATE: October 8, 2014

This letter is provided to certify that you have completed training in humane methods of animal experimentation, proper handling of selected species of research animals, and methods for reporting deficiencies in animal care and treatment. The training was provided in accordance with U.S. Department of Agriculture (9 CFR 2.32) regulations and the Public Health Service Policy.

This training included information on ECU animal care organizational structure, regulatory requirements, IACUC procedures, program for veterinary and animal care, occupational health and safety program, and methods for reporting concerns. Information on biology and care, proper restraint and procedures, and allergies and zoonoses were also provided.

We suggest that you retain this letter in your training file for future reference.

APPENDIX C: SUPPLEMENTAL FIGURE – L-DOPA

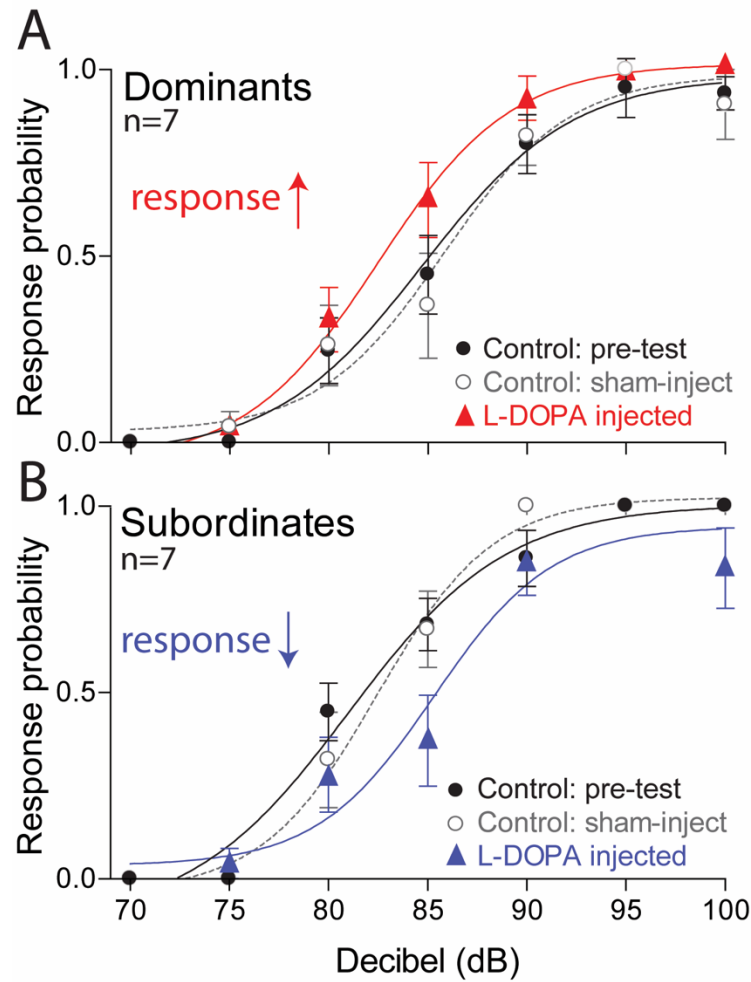


Figure 22. The effect of L-DOPA on startle escape. Startle response probability before (control) and after injection of L-DOPA for dominant (A) and subordinate (B) zebrafish. Control and experimental data are also compared to a second set of control animals that were sham injected with equal volume of RO water. In dominants, L-DOPA leads to moderate increase in startle probability, while in subordinates L-DOPA leads to a decrease in startle.

APPENDIX D: SUPPLEMENTAL FIGURE – ANTAGONIST SWIMMING

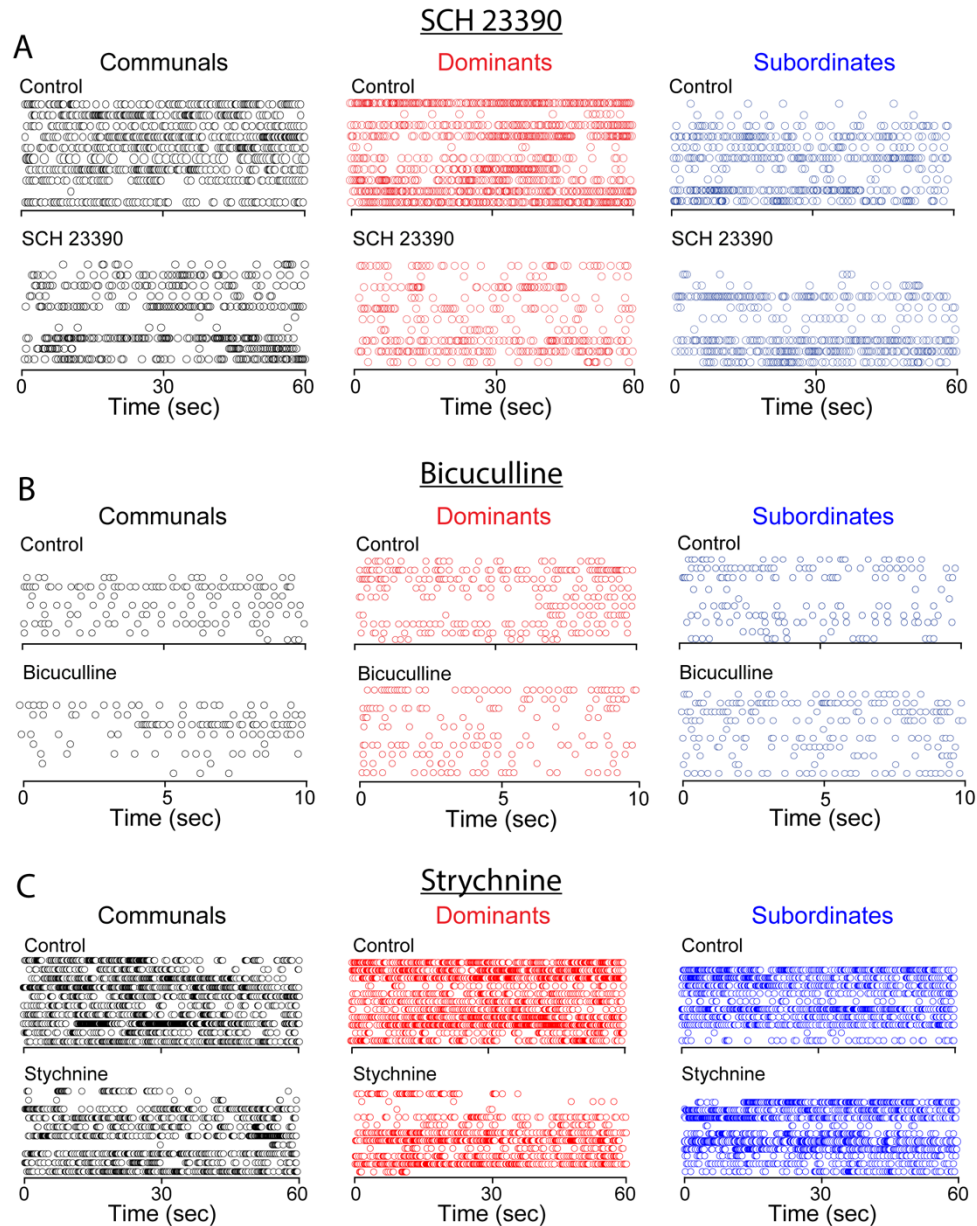


Figure 23. Effects of DRD1, GABA_A and glycine receptor antagonists on swimming. (A) Raster plots of individual group-housed, dominant and subordinate zebrafish before (Control) and after injection of DRD1 antagonist (SCH 23390). (B) Raster plots of individual group-housed, dominant and subordinate zebrafish before (Control) and after injection of GABA_A antagonist (Bicuculline)*. (C) Raster plots of individual group-housed, dominant and subordinate zebrafish before (Control) and after injection of glycine receptor antagonist (Strychnine). Each row represents the swimming activity of individual fish, and each circle represents a swim burst. *Bicuculline swimming was only recorded for 10 seconds rather than 60 seconds.

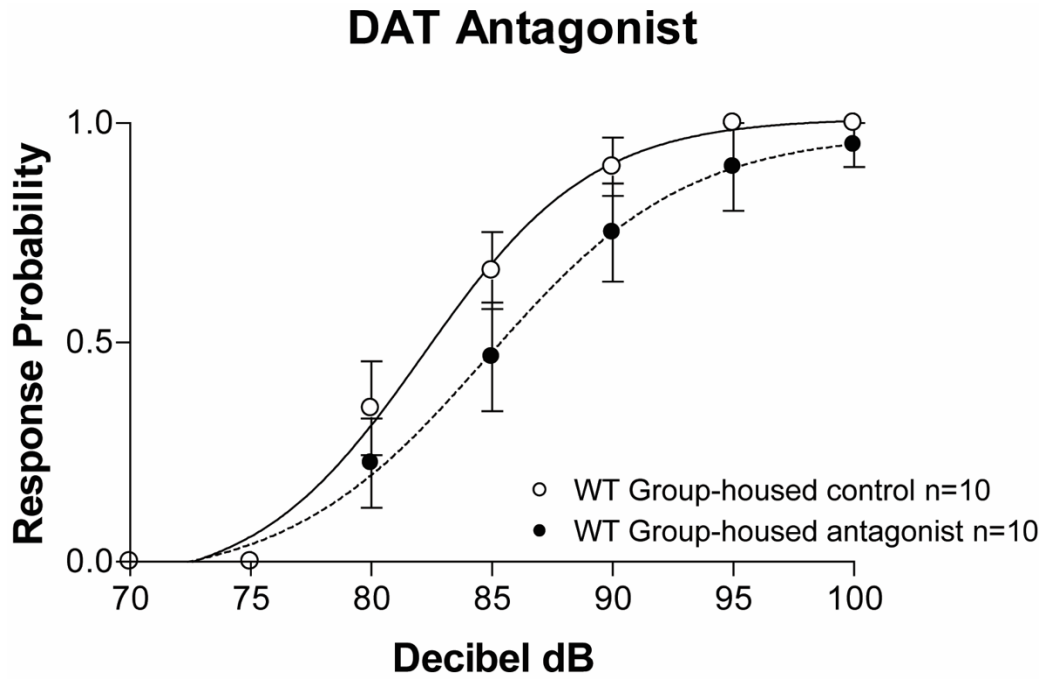


Figure 24. DAT antagonist decreases escape in WT group-housed fish. WT Group-housed animals showed decreased escape probability with administration of a DAT antagonist, as seen in the shift from the solid black line to the dashed black line.

APPENDIX F: SUPPLEMENTAL FIGURE – M-CELL AND DRD1 COEXPRESSION

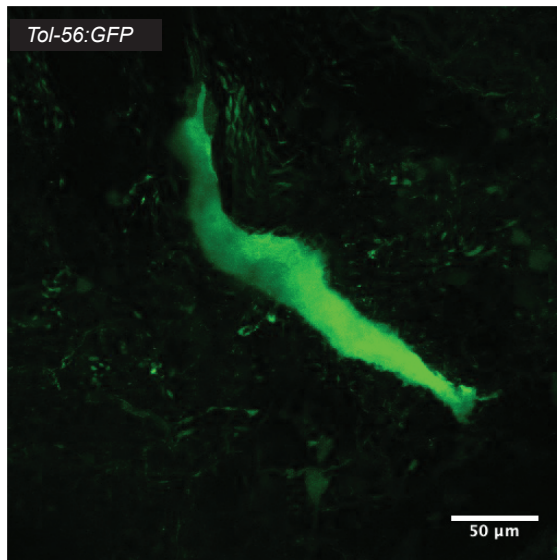
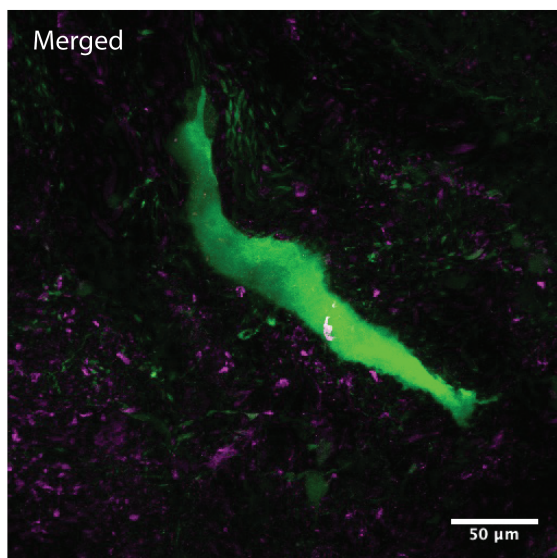
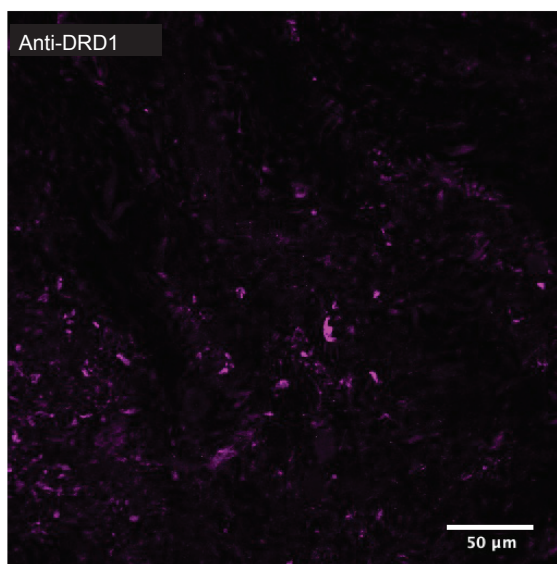


Figure 25. No co-expression of DRD1 on M-cells. (Upper) Transverse section of an M-cell with both dendrites in green. (Middle) DRD1 staining in magenta. (Lower) Merged image of DRD1 staining on the M-cell. Co-expression would appear white.



APPENDIX G: SUPPLEMENTAL FIGURE – GLUTAMATE AND DOPAMINE
COEXPRESSION

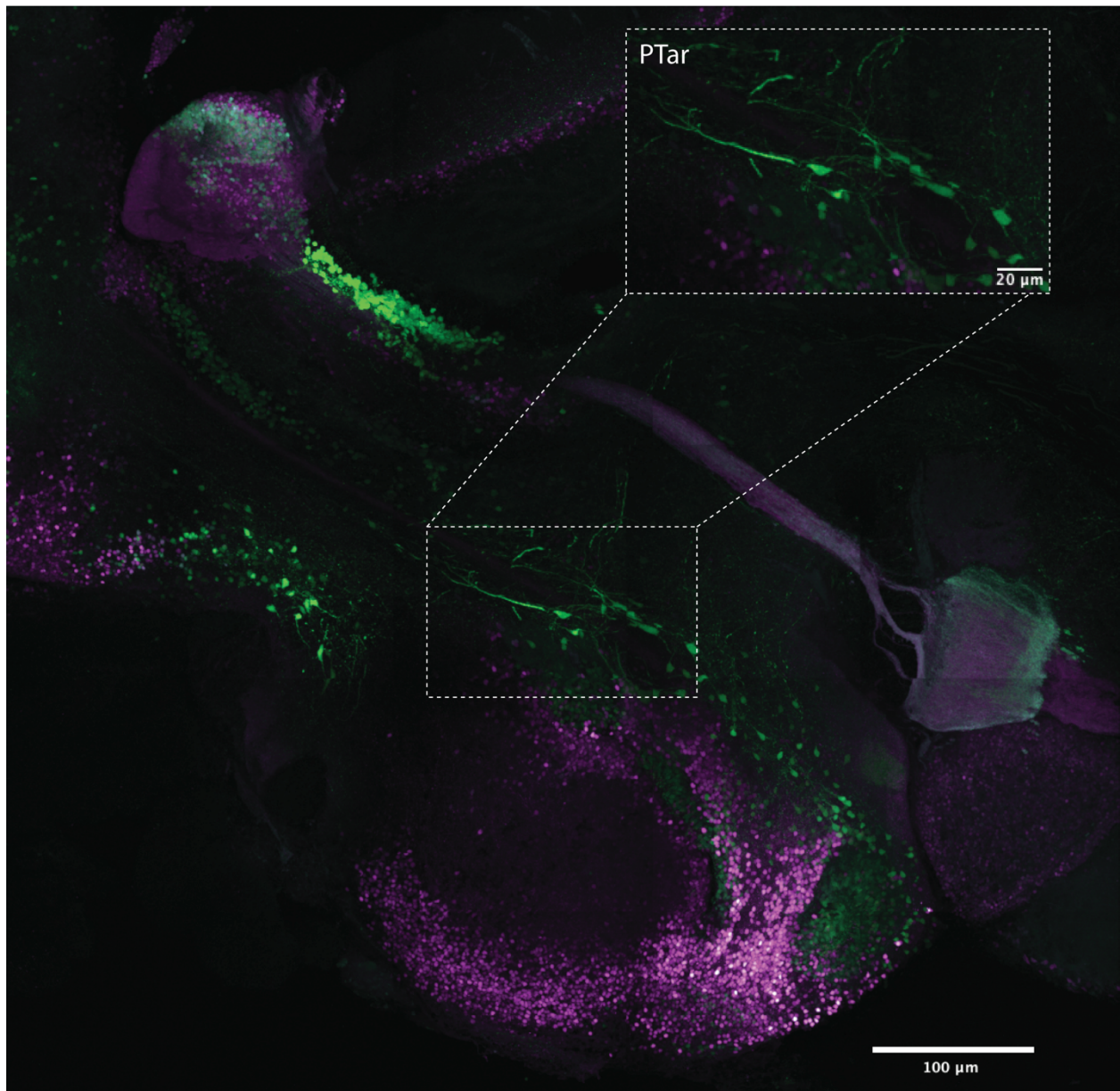


Figure 26. No Glutamate-DA co-expression in PTar. *Tg(VGlut2:RFP),Tg(slc6a3:EGFP)* line labeling glutamate in magenta and dopamine in green, shows distinct expression patterns in the hypothalamus (lower right region) and the habenula (upper left region). Boxed region notates the hypothalamic PTar region and shows only GFP labeled magnocellular neurons. Co-expression would appear white.

