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The Effect of Dopamine Blockade on Sensorimotor Gating in Social Phenotypes of the African Cichlid Fish Astatotilapia burtoni

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The Effect of Dopamine Blockade on Sensorimotor Gating in Social Phenotypes of the African Cichlid Fish *Astatotilapia burtoni*

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Abstract

It is thought that chronic social defeat contributes to increased dopamine transmission and deficits of sensorimotor gating, which may account for fundamental symptomatology in schizophrenia. Challenges in measuring social defeat in humans persist due to the complexity of human behavior and the bias of self-reporting. We asked if blocking dopamine could improve implicit sensory gating deficits in a socially-defeated fish of a naturally-agonistic species. This study investigated the effects of dopamine D2R receptor antagonist metoclopramide on sensorimotor gating using a prepulse inhibition (PPI) paradigm in social phenotypes of male *A. burtoni* African cichlid fish. Males (N = 53) were divided based on their engagement in agonistic behavior in a social community into low, medium, or high conflict groups. Individuals injected with either metoclopramide or saline were tested for anxiety-related behavior (open field) and PPI, the latter with prepulse/pulse interstimulus intervals of 50 and 200 ms. Results revealed low conflict males exposed to the drug entered the center of the arena significantly less often than low conflict males exposed to saline (p = .03). In analyzing PPI trials for 200 ms interstimulus intervals, fish exposed to drug startled more often than saline-exposed controls of the medium conflict group (p = .011), while fish exposed to drug startled less than saline controls of the high conflict group (p = .011). Treatment differences were not significant for the low conflict group at ISI 200 ms. *Post hoc* analysis revealed that the treatment difference in mean startle ratio in the medium conflict group is driven solely by the subordinates. Results suggest that blocking D2R compromises the resiliency of the medium conflict group, but improves resiliency of the high conflict group, reinforcing physiological and behavioral traits of both phenotypes by modulating dopaminergic transmission.

Keywords: cichlid, dopamine, PPI, startle, open field

Introduction

Prepulse inhibition (PPI) is a sensorimotor gating mechanism that regulates the inflow of sensory information in order to protect early-stage information processing (Graham, 1975). PPI is measured as an attenuation of the startle behavior evoked by a sensory stimulus (prepulse). The startle behavior can be assessed across species, including the human eye-blink reflex (Hoffman & Ison, 1980), the whole-body flinch in rodents (Valsamis & Schmid, 2011), the C-start in fish (Burgess & Granato, 2007), and the *Tritonia* mollusk escape swim response (Mongeluzi, Hoppe, & Frost, 1998).

In teleost fish, startle escape behavior is controlled by an opposing pair of multimodal reticulospinal neurons called Mauthner cells. An action potential firing in one cell triggers contralateral motor networks in the spinal cord, causing a rapid (<19 ms) body bend, or C-start, typically away from the stimulus (Weiss, Zottoli, Do, Faber, & Preuss, 2006; Zottoli, 1977). The C-start is therefore an all-or-none response that can be correlated to the cell that triggered the behavior (Zottoli, Newman, Rieff, & Winters, 1999; Liu & Fetcho, 1999).

PPI is sensitive to changes in neurotransmitter levels and physiological state, such as anxiety, with deficits implicated in such notable diseases related to information processing as schizophrenia (Swerdlow et al., 2006), Tourette syndrome (Swerdlow, 2012), and autism (Cheng, Chan, Hsu, & Liu, 2018). These deficits have been previously elicited cross-species through exogenous pharmacological manipulation in ways that shed new light on neuropsychiatric disorders and the improvement of their treatments (Braff, Geyer, & Swerdlow, 2001). Dopamine (DA) has been shown to regulate PPI in the auditory startle network of goldfish, whereby D2R antagonist haloperidol reverses PPI deficits induced by D2R agonist apomorphine administration (Medan & Preuss, 2011). In rats, increasing DA transmission with

D2R agonist quinpirole has been shown to decrease PPI, increasing startle behavior, where blocking DA transmission with D2R antagonist raclopride has been shown to enhance PPI, decreasing startle behavior (Zhang, Forkstam, Engel, & Svensson, 2000). One meta-analysis by Geyer, Krebs-Thomson, Braff, and Swerdlow (2001) on pharmacological studies of PPI models found strong support for decreasing PPI through DA agonists, and increasing PPI through DA antagonists in rats.

The stress of social defeat has been connected to information-processing deficits in other species. In two studies using mice, the stressors of systematic social defeat were associated with increased deficits in PPI (Adamcio, Havemann-Reinecke, & Ehrenreich, 2009; Brzózka, Fischer, Falkai, & Havemann-Reinecke, 2011).

Chronic social defeat has been hypothesized as one of many risk factors for developing schizophrenia (Selten & Cantor-Graae, 2005), but issues related to the quantification of human agonistic interactions, the bias of self-report, and the inference of causality have led the original investigators to cast doubts on this connection in humans (Selten, van Os & Cantor-Graae, 2016).

The complex and multi-faceted role of DA in schizophrenia symptomatology, namely the psychosis aspect, has become widely-accepted as advancing research consistently breaks ground towards new models. Presently, it is thought that presynaptic striatal D2R hyperactivity contributes to the incorrect appraisal of neutral stimuli at the root of psychosis (Howes & Kapur, 2009). In research by Tidey and Miczek, socially-defeated rats were found to have extracellular levels of DA at 160% of baseline in the nucleus accumbens and prefrontal cortex upon in vivo microdialysis (1996). Moreover, the ventral tegmental area (VTA), the origin point of the

mesocorticolimbic DA system, has been shown to upregulate the neurotransmitter in response to chronic social defeat in mice (Greenberg, Steinman, Doig, Hao, & Trainor, 2015).

The incorrect appraisal of neutral stimuli, hypothesized as an origin of the psychotic state and termed aberrant salience, has been connected to the attentional gating concept implicit in prepulse inhibition. Unmedicated first-episode schizophrenics have shown PPI deficits and thus startle more frequently than healthy controls (Barkus et al., 2014; Ludewig, Geyer, & Vollenweider, 2003).

Previous research in rodents supports a relationship between social defeat stress and an increase in DA in the substantia nigra, and while clinical studies have shown PPI deficits in psychotic patients, a clearer link between social defeat, dopamine dysfunction, and PPI deficit has not yet been elucidated. The complexity of human behavior has been highlighted as an obstacle to quantifying defeat in humans, as mentioned above.

The African cichlid fish *Astatotilapia burtoni* displays a naturally-occurring dominance/subordinacy hierarchy, whereby the social environment influences behavior, endocrinology and gene expression (Fernald, 1977; Hofmann & Fernald, 2001). Males compete for territory used as breeding grounds in agonistic interactions and by their wins and losses in conflict, become dominant or subordinate, and can transition from one state to the other as dynamics change. This hierarchy is associated with both body pigmentation and reproductive ability, where dominant fish (DOMs) are brightly colored blue or yellow, reproductively active, and in possession of a territory, and subordinates (SUBs) are pale and unable to reproduce (Fernald & Hirata, 1977). Threat displays by DOM males toward subordinate males, as well as losses in agonistic confrontations on the part of the subordinates, encourage social defeat in subordinate males (Fox, White, Kao, & Fernald, 1997).

Mammalian homologues in the African cichlid fish have been identified. The cichlid preoptic area (POA) has been associated with its counterpart in mammals, and the posterior tuberculum (TPp) has been compared to the substantia nigra pars compacta and ventral tegmental area (SNc/VTA) with significant similarity in gene expression indicating the presence of dopaminergic neurons (O'Connell, Fontenot, & Hofmann, 2013). The presence of homologues helps to strengthen comparative conceptual connections between mammals and teleosts.

The simple quantification of the socially-dynamic hierarchy central to *A. burtoni* makes this species a prime model for the study of social defeat. Socially-reactive, defeated SUB males have demonstrated PPI deficits compared to DOM conspecifics (Neumeister et al., 2017). It is thought that this socially-reactive subtype would also show significant increases in DA based on the implications of previously-mentioned studies in rodent models (Greenberg, Steinman, Doig, Hao, & Trainor, 2015; Tidey & Miczek, 1996). Moreover, reactive subordinate cichlids also show differential anxiety-related behaviors in an open field test, whereby they spent less time in the center of the arena (Neumeister et al., 2017).

Increased anxiety has been connected to PPI deficits in humans (Franklin, Bowker, & Blumenthal, 2009). In rats genetically-modified to lack the Adcyap1 gene, which increases hyperactivity, amphetamine administration was associated with both thigmotaxic behavior in open field and deficits in PPI (Tanaka et al., 2006).

By this logic, PPI deficits of the socially-reactive subordinate group in cichlids can be associated with open field measures of anxiety. Dopamine has also been found to play a role in thigmotaxis: it has been found that increased DA transmission is anxiogenic, leading to an

increase in anxiety-related behaviors in an open field test in mice (Simon, Dupuis, & Costentin, 1994).

To recapitulate, elevations in DA after chronic social defeat have been observed in rodents (Tidey & Miczek, 1996; Greenberg, Steinman, Doig, Hao, & Trainor, 2015), just as DA increases have been associated with the disruption of PPI in both rodents and fish (Zhang, Forkstam, Engel, & Svensson, 2000; Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001; Medan & Preuss, 2011). Previous research has yet to investigate the effect of DA blockade on PPI and anxiety-related behavior through the lens of social defeat in a hierarchical teleost model system.

In this present study, we have employed the D2R antagonist metoclopramide to test the effect of dopamine blockade on prepulse inhibition and anxiety-related behaviors as related to social status and agonistic activity level. We hypothesized that the drug would have differential effects on PPI and anxiety in subordinate and dominant fish.

Method

Subjects

Adult male *Astatotilapia burtoni* African cichlids were housed in five separate communities of 10 to 12 males each, with an equivalent number of females, in acrylic tanks measuring 30 cm x 30 cm x 60 cm. Crushed coral gravel was provided as substrate, faux plants provided shelter, and four to six terracotta pots served as territories. Standard water conditions (27 ±1 °C, pH 8.5 ± 0.5) were maintained by a flow-through system. Overhead lighting was set on a 12-hour light/dark cycle. Fish were fed daily using a standard cichlid diet.

One community of males (n = 8) came from a strain originating from another lab (Fernald), whose fish (Body length [cm]: M = 7.08 ± 0.63 SD; body weight [g]: M = 12.25 ± 2.80 SD) were larger than the fish of the home strain (n = 45; body length [cm]: M = 5.18 ± 0.51 SD;

body weight [g]: $M = 3.96 \pm 0.97$ *SD*), that is to say a body length mean difference of 1.9 cm and a body weight mean difference of 8.29 g. Their inclusion did not demonstrably affect the outcome of any measure tested.

All animals were treated in conditions concordant with the norms and guidelines set forth by the Hunter College Institute of Animal Care and Use Committee.

Materials and Measures

Dominance index. In order to quantify social behaviors, a traditional focal observation ethogram was employed, modelled after original observations by Fernald (1977). Each male in each community was observed for 10 minutes, twice per week, for eight weeks. At the end of each observation session a composite score was computed called a Dominance Index (DI), calculated as the sum of the tallies of all behaviors indicative of dominance, such as chasing, biting, threat display, carousel fighting, and courting, minus the tally of all behaviors indicative of submission, chiefly fleeing. A mean score was taken from the last four weeks before the subject's experiment date. A positive mean was considered characteristic of DOM status and a negative mean characteristic of SUB status.

Conflict index. On the same ethogram, an alternative composite score, the Conflict Index (CI), was computed, which is based on the sum of agonistic interactions of an individual within a four-week observation period (Fulmer, 2017; Neumeister et al., 2017). A hierarchical cluster analysis was performed in SPSS (IBM, version 25) to separate fish into three distinct CI groups: low ($n = 18$), medium ($n = 21$), and high ($n = 14$).

The division into low, medium, and high groups helped to distinguish between DOMs (generally found in the high CI group), proactive SUBs (generally found in the medium CI

group) who engage in fights but lose them, and reactive SUBs (generally found in the low CI group) that typically avoid conflict altogether (Neumeister et al., 2017).

Procedure

Fifty-three *A. burtoni* males were randomly selected from one of five tanks for same-day experimentation, and received an IP injection of either 1 μg/g body weight dopamine D2R antagonist metoclopramide hydrochloride (n = 25; DOM = 12, SUB = 13; low CI = 8, medium CI = 10, high CI = 7) or 1 μg/g body weight saline solution (n = 28; DOM = 12, SUB = 16; low CI = 10, medium CI = 11, high CI = 7), the drug dosage as suggested by Weitekamp, Nguyen, and Hofmann (2017). The injected fish was returned to its home tank for 45 min to allow the drug to reach its estimated peak level in the brain during open field (Baker, Florczynski, Beninger, 2015). After the wait period, all fish were removed from the community in question to preserve the social hierarchy without the injected fish, who was transferred to a circular acrylic experimental tank (76 cm in diameter) and placed inside a circular mesh arena (27 cm in diameter). The experimental tank was situated on an anti-vibration table in order to eliminate external vibrations, and was surrounded by black curtain to minimize visual cues. Footage of the single fish swimming in the arena was captured from below by a high-speed video camera (30 fps, resolution 800 × 600 px; I-Speed 2 Olympus, Tokyo, Japan). The tank was filled with aerated water matching the community home tank in pH and temperature. After the conclusion of each experiment, the water was discarded, and the tank was cleaned with a 50% ethanol solution.

Experiment I: open field. A 20-minute open field test was conducted to assess the effect of the drug on anxiety-related behaviors as related also to conflict group and status. Each fish swam freely and was recorded on video. The 20 min video clip was then trimmed to the last 15

minutes to exclude a five-minute habituation period as per previous experiments' methodology (Avidemux, Version 2.7). Trimmed videos were processed through tracking software (BIOBSERVE, demo version 3.0.1.255). A derived center of the arena was calculated specific to each fish as one body length from the wall. Variables used for analysis were number of visits to the center, total time spent in the center in seconds, and total distance travelled adjusted to body length. Three animals were excluded from open field analysis due to corrupted video files (n = 3).

Experiment II: prepulse inhibition. After the conclusion of open field, prepulse inhibition trials began with no interruption. To elicit a startle escape response, or C-start, an acoustic pulse was delivered at random by one of two opposite-facing underwater loudspeakers (UW30, University Sound, Buchanan, MI), triggered by a Mac running Igor Pro (WaveMetrics, Portland, OR). Acoustic stimulation consisted of three conditions presented at random in 18 trials, each occurring six times during an experiment: a single pulse (pulse-only), and two different prepulse/pulse stimulation conditions: one with the prepulse preceding the pulse with an interstimulus interval (ISI) of 50 ms, and another with an ISI of 200 ms. Interstimulus intervals between 50 ms and 200 ms typically evoke maximum prepulse inhibition, as observed in goldfish (Neumeister, Szabo, & Preuss, 2008). Pulse intensity varied at random at either 166 or 172 dB (200 Hz single sine wave, in water re. 1 μPa), whereas prepulse intensity varied at random at either 150 or 152 dB (200 Hz single sine wave, in water re. 1 μPa). Acoustic trials were recorded at 1000 fps with the same video camera mentioned above and stored digitally for reference. Time between trials varied randomly from three minutes to six minutes. Including the 20-minute open field period, the experiment lasted typically for two hours, after which the fish

was returned with its community into the community home tank. Each fish was used in the experiment only once.

Statistics

Open field. Analyses were conducted in SPSS using results values from BIOBSERVE Viewer. The data set was tested for normality, and if normality was not found, we analyzed treatment effects in Mann-Whitney-Wilcoxon tests. Distinct analyses were conducted for each social status (DOM, SUB) and each CI group (low, medium, and high). If normality was found, we ran two-way ANOVAs, first with treatment and social status as independent factors and then with treatment and conflict group as independent factors. Benjamini-Hochberg corrections were performed on the resulting *p*-values for all multiple comparisons to control for the expected proportion of false discoveries (Benjamini & Hochberg, 1995). The *r* effect size ($r = Z/\sqrt{N}$) was calculated for relevant Wilcoxon tests in order to determine the magnitude of significant treatment differences as proposed by Rosenthal (1991).

Standard box-whisker plots were generated in SPSS showing the four quartiles and the medians, and outliers were drawn as individual dots following the 1.5 x IQR rule. For normally-distributed variables, mean values ± standard deviation (*SD*) are reported in the figure legend.

Startle and prepulse inhibition. For each fish, startle responsiveness to pulse-only and prepulse/pulse stimuli (with interstimulus intervals of 50 and 200 ms) was calculated as the ratio between the number of evoked startles and the number of possible startles for a given stimulus paradigm. In this case, higher startle ratios correspond to less PPI (Neumeister et al., 2017). Repeated measures generalized estimating equations (GEEs) were run in SPSS (IBM, version 25) to test the effect of drug treatment on startle ratio using binary logistic regression models with a logit link function, considering status and conflict group separately. Hypotheses

concerning model effects in the GEE were tested using Wald chi-square statistics. ISI conditions were analyzed in distinct tests. The GEE method does not require assumptions regarding normality and it considers the dependence and hierarchical structure of the raw dataset. Benjamini-Hochberg corrections were performed on the resulting *p*-values for all multiple comparisons to control for the expected proportion of false discoveries (Benjamini & Hochberg, 1995). Results in the text are reported as mean startle ratios (*M*) and standard error (*SE*), and number of animals per subgroup (*n*).

Results

As noted in the Method section, we analyzed treatment effects separately for social status (DOM and SUB) and CI groups (low, medium, and high).

Open Field Behavior

We first tested the effect of the D2R antagonist on anxiety-related behaviors and motor behavior using males divided into two cohorts (drug $n = 24$, saline $n = 26$). Results showed no effect of drug on time spent in the center (thigmotaxis) when using status (*i.e.* DOM, SUB) as an independent factor. However, subdividing into CI groups showed an decrease in time spent in the center in the low CI group for drug-exposed animals (Fig. 1; $W = 49$, $p = .027$, $p = .01$, corrected; $r = .54$, large effect). No drug effect was found in the medium CI group or the high CI group.

A related measure of thigmotaxis is center visit frequency, in which we found a significant reduction in center visits for the low CI drug group (Fig 2; $W = 47$, $p = .016$, $p = .006$ corrected; $r = .59$, large effect). Using social status as a factor, there was no effect of treatment on number of center visits in SUBs or DOMs. There was also no overall effect of treatment on

number of visits to the center and no treatment effect in the medium CI group or the high CI group.

Total distance traveled was analyzed using a two-way ANOVA with treatment and CI group as factors. We found no overall treatment difference in total distance traveled between drug and saline cohorts, and no interaction between treatment and social status (DOM, SUB), or between treatment and CI group (Fig 3).

Prepulse Inhibition

We tested the effect of treatment on startle ratio in distinct analyses separated by ISI condition for both social and conflict group.

Social status. Overall, baseline startle ratio was not affected by treatment, $\chi^2(1) = .001$, $p = .971$. The interaction of status and treatment did not significantly predict startle response ratio in the pulse-only condition, $\chi^2(1) = 2.610$, $p = .106$.

At ISI 50 ms, startle ratio was not affected by treatment, $\chi^2(1) = .001$, $p = .975$. The interaction of status and treatment did not significantly predict startle response in this ISI condition, $\chi^2(1) = .143$, $p = .705$.

At ISI 200 ms, startle ratio was not affected by treatment overall, $\chi^2(1) = .491$, $p = .483$. The interaction of status and treatment did not significantly predict startle response ratio in this ISI condition.

Conflict group. Overall, baseline startle ratio was not affected by treatment, $\chi^2(1) = .003$, $p = .959$. The interaction of CI group and treatment did not significantly predict startle response ratio at the pulse-only level, $\chi^2(2) = .741$, $p = .690$.

At ISI 50 ms, startle ratio was not affected by treatment, $\chi^2(1) = .00003$, $p = .855$. The interaction of CI group and treatment did not significantly predict startle response ratio at this ISI level, $\chi^2(2) = .112$, $p = .946$.

At ISI 200 ms, startle ratio was not affected by treatment overall, $\chi^2(1) = .510$, $p = .475$. The interaction of CI group and treatment significantly predicted startle response ratio at ISI 200 ms, $\chi^2(2) = 6.997$, $p = .030$. In the low CI group, fish exposed to drug ($M = .52 \pm .08$ *SE*) did not differ significantly in startle rate from fish exposed to saline ($M = .48 \pm .05$ *SE*). In the medium CI group, fish exposed to the drug ($M = .47 \pm .08$ *SE*) had a significantly higher startle ratio than fish exposed to saline ($M = .25 \pm .08$ *SE*, $p = .049$). In the high CI group, fish exposed to drug ($M = .21 \pm .06$ *SE*) had a significantly lower startle ratio than fish exposed to saline ($M = .33 \pm .00$ *SE*, $p = .032$). After correcting for multiple comparisons, both of these significant treatment differences were retained (medium CI $p = .016$, high CI $p = .012$; corrected).

Mean startle ratios comparing fish exposed to the drug to saline controls as divided by conflict group for all ISI models are displayed (Fig 4).

Medium CI group *post hoc*. The composition of the medium CI group, as a more heterogeneous mixture of social status phenotypes (DOM $n = 7$, SUB $n = 14$), compared to the SUB-prevalent low CI group (DOM $n = 4$, SUB $n = 14$), and the DOM-prevalent high CI group (DOM $n = 13$, SUB $n = 1$). Thus, we asked if the treatment effects in the medium CI group at ISI 200 ms may be influenced by social status composition. The results indicate that SUBs may be driving the increase in startle in the medium CI group, although it only approaches significance (Fig 5; GEE; $p = .054$, corrected).

Discussion

We asked if the blockade of dopamine D2 receptors would affect PPI in *A. burtoni* males experiencing social defeat, a state that previously has been shown to reduce PPI, particularly in socially-reactive (low CI) individuals (Neumeister et al., 2017). The results show no effect of the drug in the low CI group, which maintained a low level of PPI. However, we found that the drug diminished PPI in socially-defeated males who show medium levels of social activity (medium CI). In contrast, dominant males, which are by far the most active fish (high CI), showed an increase in PPI with the drug. As discussed below, the drug produced a differential effect, indicating that dopamine may modulate PPI in a social context.

Differential Effects of Dopamine on PPI in Social Phenotypes

Our results showed that the drug effects on PPI in the medium CI group at ISI 200 ms are mostly carried by SUBs. It has previously been shown that socially-ascending SUBs showing a relatively high level of engagement in fights (*i.e.* med CI males; Fulmer, 2017). In addition, medium CI males also show resilience to the otherwise disrupting effects of social defeat on PPI in fish and rodents (Neumeister et al., 2017; Adelman, Chen, Aberg, Neumeister, & Preuss, 2019; Adamcio, Havemann-Reinecke, & Ehrenreich, 2009; Brzózka, Fischer, Falkai, & Havemann-Reinecke, 2011). As such, our results suggest that blocking D2 receptors might compromise this resilience. Furthermore, the fact that the drug further increases PPI in high CI DOMs implies that the drug increases the social phenotype-dependent differences in PPI.

The fact that PPI did not change in the low CI group appears to indicate a ceiling effect, whereby the drug cannot worsen inhibition, *i.e.* raise the startle response ratio, in the low CI group due to neurobiological limitations.

Differential Effects of Dopamine on PPI in Different ISI Trials

The treatment effects found in 200 ms ISI trials were not observed in 50 ms ISI trials. Rather than suggest that a 50 ms interval between prepulse and pulse stimuli is insufficient to detect significant treatment differences, previous research points to ISI-dependent differences in DA modulation by other neurotransmitters. A study using both mice and rats found that the $GABA_B$ antagonist phaclofen reduced PPI only at long ISIs of 100-500 ms compared to baseline PPI (Yeomans et al., 2010). Furthermore, inverse $GABA_B$ modulation of D2 receptors has been observed in the nucleus accumbens of mice, such that a $GABA_B$ agonist suppressed DA release at D2 receptors (Pitman, Puil, & Borgland, 2014). In this way, a multi-mechanistic interaction of $GABA_B$ and D2 receptors may contribute to the significant treatment differences in 200 ms ISI trials that are not found in 50 ms ISI trials. However, it is important to note that in the study by Yeomans et al. (2010), $GABA_A$ antagonist bicuculline significantly reduced PPI at ISI trials of 20-300 ms, an interval that includes our 50 ms interval. Though previous research suggests some tentative interaction and cross-talk between $GABA_A$ receptors and D2 receptors (Shrivastava, Triller, & Sieghart, 2011), our lack of significant results in 50 ms ISI trials do not fit this potential connection.

In fact, research in goldfish has shown that haloperidol administration post-apomorphine treatment restored PPI at only 50 ms ISI trials, and not at 150 or 300 ms trials (Medan & Preuss, 2011), which also hints at some underlying post-synaptic mechanism regulating PPI at different ISI lengths. Conversely, in larval zebrafish, increased PPI was observed only at trials with 50 ms and 300 ms intervals, though no drug was administered when testing the 50-300 ms ISI range (Burgess & Granato, 2007). Instead, this research by Burgess and Granato suggests a glutamate

and DA interaction on PPI, through NMDA antagonist ketamine application, though this was found at 500 ms, beyond our tested ISI lengths.

The interaction between GABA and DA that regulates PPI, at $GABA_B$ and D2 receptor sites, appears to be supported by the results found in these studies. This points to GABA as a primary focus of future research in other neurotransmitters that have an effect on PPI, though the role of glutamate as hinted above cannot be ruled out.

The Predatory Threat Context Implicit in Open Field

Saline controls of the low CI group interacted with the center significantly more than drug-exposed fish, displaying less anxiety-related behaviors than the fish exposed to the drug. While some previous research posits that increased DA transmission through D2R activity is anxiogenic (Simon, Dupuis, & Costentin, 1994), our results suggest that a D2R antagonist can increase anxiety-related behaviors.

In one study using rats, D2R antagonist sulpride decreased time spent on the open arms of an elevated plus maze (EPM), a test classified as a measure of anxiety (Pellow, Chopin, File, & Briley, 1985). Sulpride decreased conditioned fear in an aversive-appetitive context, which consisted of a chamber pairing sucrose with an electric shock, a conflicting paradigm (Nguyen, Alushaj, Erb, & Ito, 2019).

This contradiction of previous findings is best interpreted when the open field test is viewed as an anxiogenic context for prey fish such as the African cichlid, comparable to an EPM for rats. Thigmotaxis is an anxiety-related response to the center area, which in nature constitutes an exposure to predation for prey fish. The blockade of DA can be connected to increased anxiety-related thigmotaxis and so, the understanding of open field testing as an anxiogenic paradigm

recontextualizes the role of DA transmission as related to measures of anxiety in light of the above-mentioned role of sulpride in an EPM with rats.

Alternative Pharmacological Agents to Metoclopramide

An increase in PPI was not observed in drug-exposed low CI animals, nor did the drug appear to affect PPI at 50 ms ISI trials, as might be expected with a D2 antagonist given the previous research of Medan and Preuss (2011). In order to draw clearer connections between the social defeat hypothesis, dopamine, interstimulus interval, and PPI disruption in future studies of this kind, a change in drug may benefit the design. Implicit elevations of dopamine may not have been reduced as expected due to the nature of metoclopramide as a D2R antagonist distinct from the antipsychotic class.

In a study on genetically-modified hypertensive rats, metoclopramide had no effect on contextual fear conditioning compared to conditioned fear deficits that were improved by the neuroleptics haloperidol, ziprasidone, risperidone, amisulpride, and clozapine (Calzavara et al., 2009). In addition, metoclopramide had no effect on PPI deficits in genetically-modified hypertensive rats compared to the restorative effects of clozapine, a first-generation antipsychotic for treatment-resistant schizophrenia (Levin et al., 2011). Both of these findings offer a wide variety of alternative dopaminergic drugs of the antipsychotic type for future studies.

In previous research with cichlids, metoclopramide inhibited intruder-directed aggression in a reproductive context of a resident-intruder paradigm, and increased aggression in a neutral context (Weitekamp, Nguyen, & Hofmann, 2017). However, the lack of consistent support for the drug in designs investigating PPI when compared to neuroleptics raises doubts about the ultimate usefulness of this drug in influencing this sensorimotor gating mechanism.

Future studies might endeavor to use antipsychotics specifically with this design to attempt to tackle the social defeat hypothesis and its complex relationship to schizophrenia. As described above, the use of haloperidol in goldfish restored deficits in PPI introduced by apomorphine administration (Medan & Preuss, 2011). With regards to social defeat, haloperidol has been used to block dopamine in cynomolgus monkeys, who act in a social hierarchy where the reduction of DA is observed in subordinate animals through differing responses to prolactin (Shively, 1998). The use of haloperidol in a social defeat-related study is particularly suggestive of promise in investigating the hypothesized social defeat-dopamine-PPI connection in African cichlids.

These studies suggest that haloperidol administration as opposed to metoclopramide administration might help to shed more light on the complex relationship between social defeat, dopamine transmission, and prepulse inhibition deficits.

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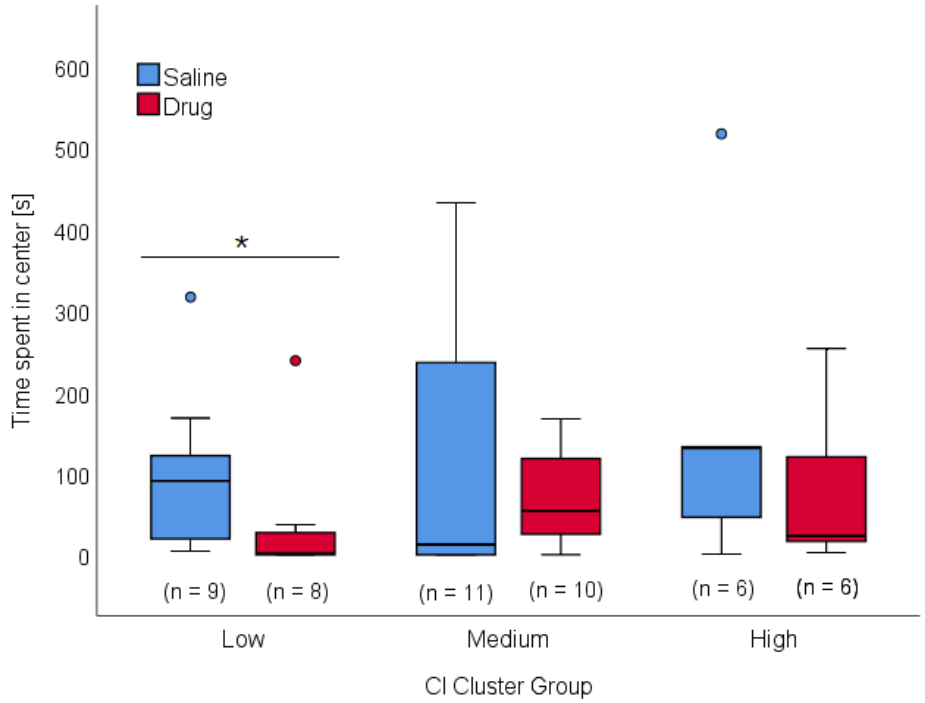

Figure 1. Effect of metoclopramide on time spent in the center during open field swimming. Drug-exposed males with low agonistic activity level (low CI) spent less time in the center as compared to saline controls (drug *Mdn* = 1.1, saline *Mdn* = 90.8, **p* = .01, corrected).

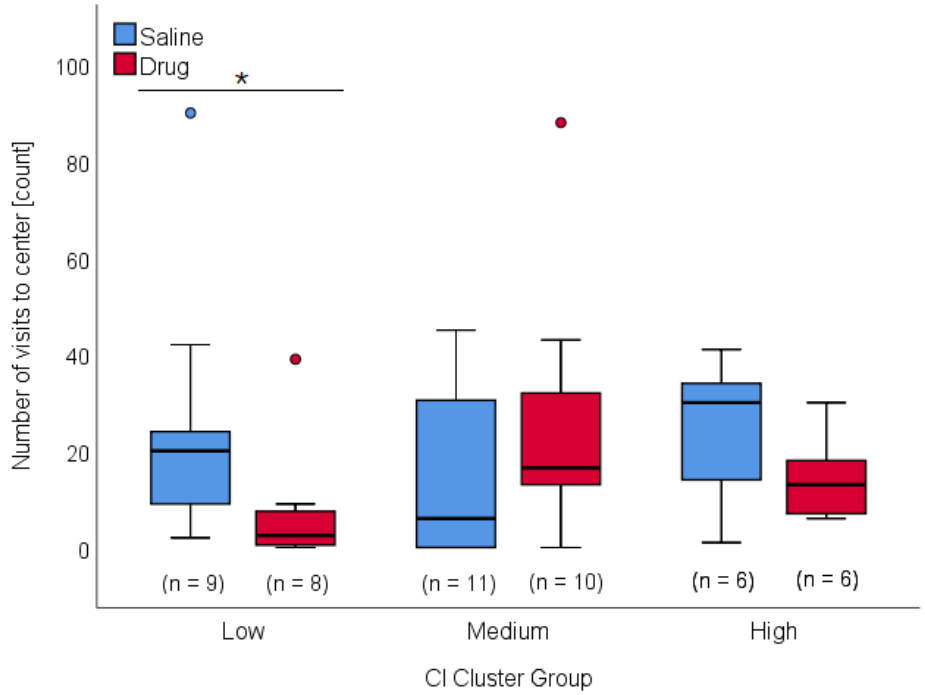

Figure 2. Effect of metoclopramide on number of visits to the center during open field swimming. Drug-exposed males with low agonistic activity level (low CI) entered the center significantly less often compared to saline controls (drug *Mdn* = 2.5, saline *Mdn* = 20.0, **p* = .006, corrected).

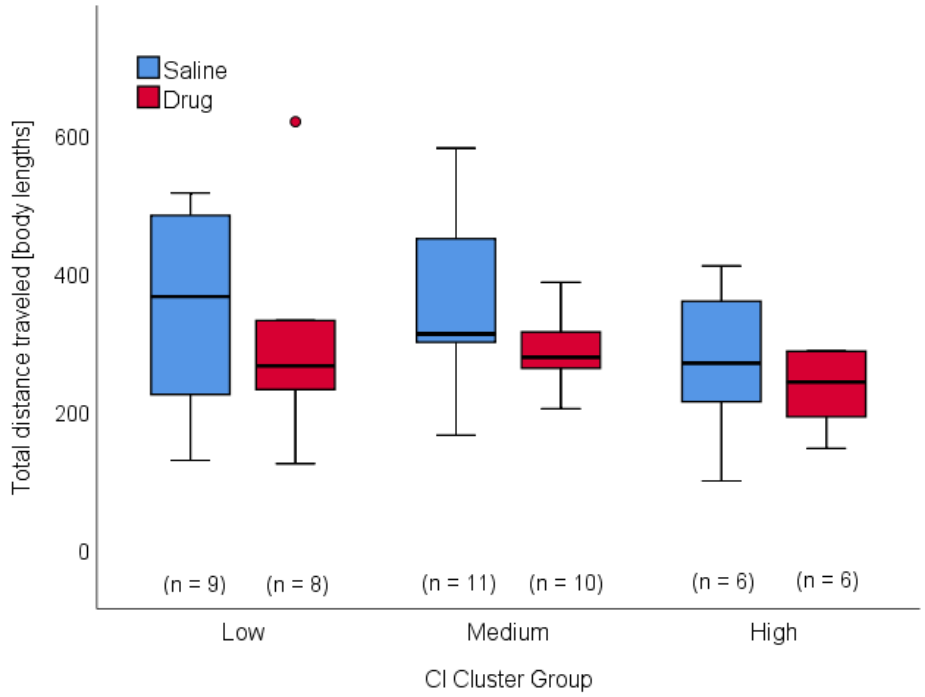

Figure 3. Effect of metoclopramide on total distance traveled, adjusted by body length, during open field swimming. Drug-exposed males did not differ from saline controls in total distance traveled within any conflict group. (Low CI group: drug M = 299.16 ± 145.32 *SD*, saline M = 342.01 ± 149.46 *SD*; medium CI group: drug M = 282.73 ± 57.24 *SD*, saline M = 363.19 ± 128.98 *SD*; high CI group: drug M = 231.82 ± 55.44 *SD*, saline M = 269.43 ± 115.31 *SD*)

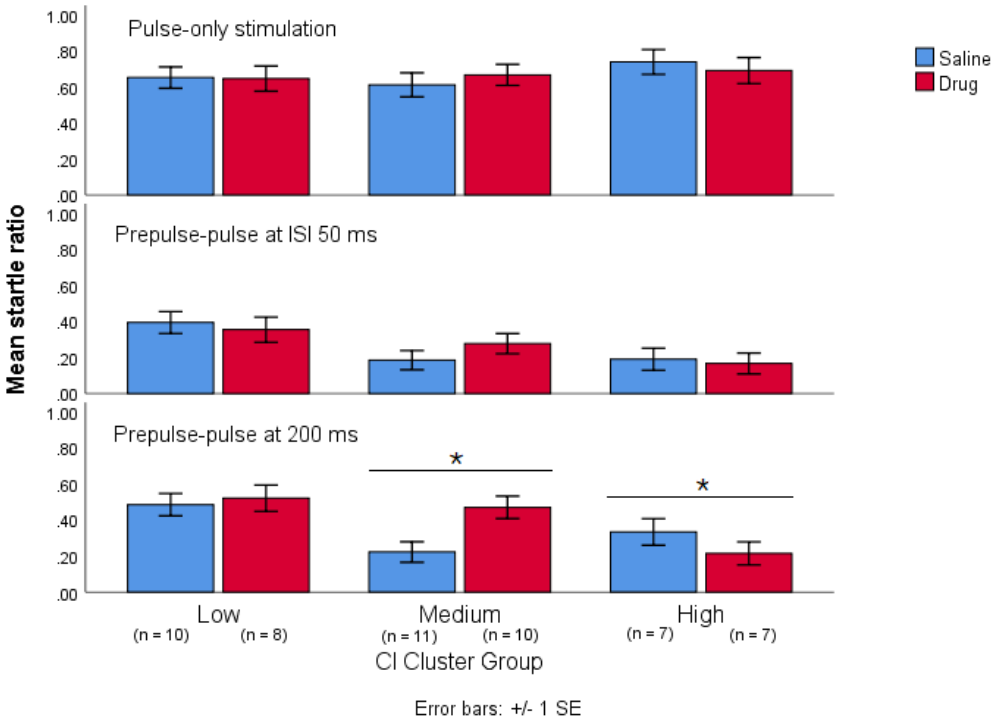

Figure 4. Effect of metoclopramide on mean startle ratio in each conflict group for pulse-only stimulation trials, prepulse-pulse stimulation trials at ISI 50 ms, and prepulse-pulse stimulation trials at ISI 200 ms. In prepulse-pulse stimulation trials at ISI 200 ms, drug-treated fish of the medium CI group had a significantly higher mean startle ratio than saline controls. In the high CI group, drug-treated fish had a significantly lower mean startle ratio compared to saline controls (medium CI p = .016, high CI p = .012, corrected).

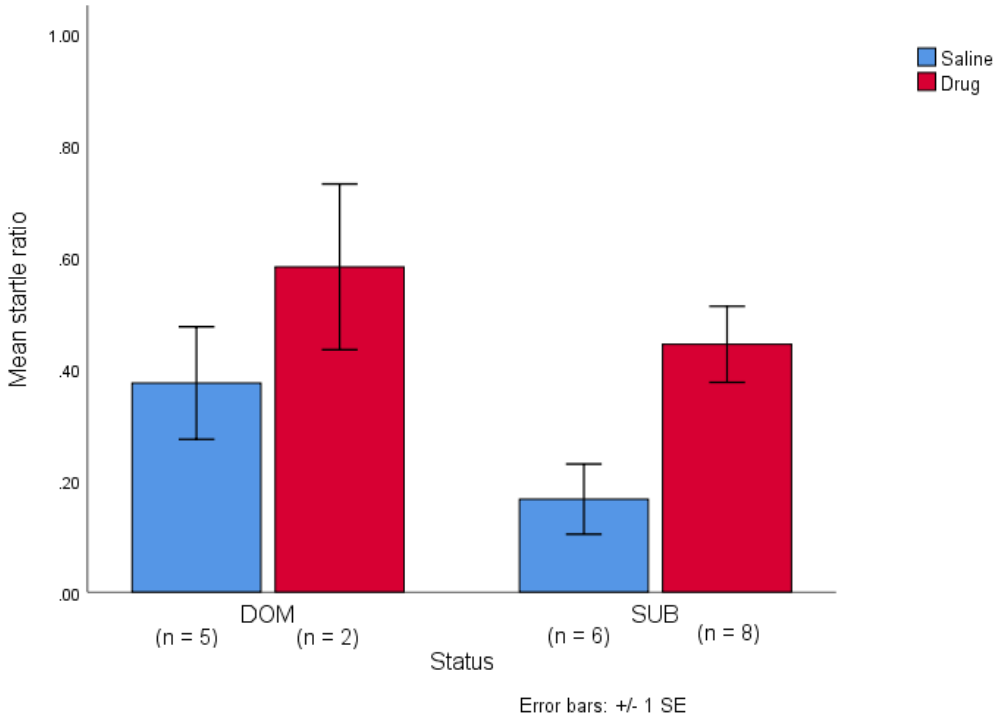

Figure 5. Effect of metoclopramide on mean startle ratio in dominant and subordinate social status of the medium conflict group for prepulse-pulse stimulation trials at ISI 200 ms. *Note*: There is a trend approaching significance in SUBs, whereby those exposed to the drug had a higher mean startle ratio compared to SUBs exposed to saline (p = .054).