Title

Atypical Antipsychotic Haloperidol Disrupts Prepulse Inhibition of Acoustic Startle Reflex in Larval Zebrafish

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A thesis submitted in partial satisfaction

of the requirements for the degree Master of Science

in Physiological Science

by

Ronny Choe

2016

ABSTRACT OF THE THESIS

Atypical Antipsychotic Haloperidol Disrupts Prepulse Inhibition

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Ronny Choe

Master of Science in Physiological Science

University of California, Los Angeles, 2016

Professor David L. Glanzman, Chair

Prepulse inhibition (PPI) in larval zebrafish can be utilized as a behavioral marker for neural dysfunction. In this study, we show that application of pharmacological agents to affect the Mauthner Cell mediated startle movement in response to acoustic stimuli can be used to quantify dysfunction. By establishing a robust protocol, we were able to confirm the effects of numerous pharmacological agents on PPI. We were also able to establish that haloperidol had a negative effect on PPI. This parallels some recent mammalian PPI studies that indicate that haloperidol has mixed effect on PPI. Dose dependent effects, timing, and even the nature of the reductionist zebrafish model may be responsible for this difference. However, the large sample sizes and simple neural circuitry makes the larval zebrafish and excellent model for high throughput screens of psychotropic therapeutic agents.

The thesis of Ronny Choe is approved.

Alan D. Grinnell

Barnett Schlinger

Stephanie Ann White

David L. Glanzman, Committee Chair

University of California, Los Angeles

2016

Dedication Page

Ronny Choe would like to thank all of the colleagues, staff, faculty, family, and friends who have helped in the completion of this thesis. This work was inspired by a desire to contribute to the field of Neurobiology and hopefully towards the discovery of a cure for Schizophrenia.

This one is for you, James.

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Acknowledgements

Some of the figures used in this paper was reprinted with permission from the authors of the paper, “Habituation of the C-start response in larval zebrafish exhibits several distinct phases and sensitivity to NMDA receptor blockade” published to PLOS ONE in 2011 by Adam C. Roberts and others from the David L. Glanzman Laboratory.

Introduction

Many protective reflexes have been studied in neuroscience, including the gill withdrawal reflex in *Aplysia californica* by David Glanzman at UCLA (Glanzman 2009 – Cheshire cat of neurobiology; Kandel, 1976). The startle response is a type of defensive reflex observed in many animals, including crayfish (Edwards et al., 1999), squid (Otis and Gilly, 1990), teleost fish (Eaton et al., 1977), and mammals (Lingenhohl and Friauf, 1994). Typically, the startle response allows rapid escape from a potentially threatening stimulus through a rapid, extensive activation of muscles (Medan and Preuss, 2014). This vital behavior allows avoidance of predation through an initial detection of the approaching predator as well as subsequent decision making to initiate the correct trajectory for escape.

In teleost fish, including the zebrafish of the present study, the Mauthner-cell controls the startle response (Korn and Faber, 1996). These cells are located in the hindbrain of the zebrafish as a pair of bilateral cells, which can integrate sensory information – primarily auditory and mechanical – to produce startle behavior (Valsamis, 2011). Given its function as a gateway for sensory information to be integrated into motor activity, this cell has a sensorimotor integrating function which can be modified through plasticity of the cell. Startle behavior can be habituated or enhanced (Valsamis, 2011; Ganser 2013).

The Mauthner cell startle follows a stereotyped motor sequence which results in a stereotypes escape reaction (Oda, 2008). There is a massive unilateral

contraction of trunk muscles and the fish will assume a tightly coiled C-shape body position. It is followed by a second return flip where the return stroke with help to straighten the body and propel the animal away from the aversive stimulus (Eaton, 1977).

The startle response is controlled by a single action potential from the M-cells. As a result, the observation of a C-start response is an accurate readout that the M-cell fired an action potential- there is no electrophysiological measure necessary for confirmation of this fact. This makes the larval zebrafish an exciting animal model to use for its ease of data collection. We can make cellular observations by simply observing behavior. Depending on the direction of the startle response, we can also determine which of the two M-cells fired (contralateral activity) (Faber, 2005).

Of primary interest in this study was the inhibition that is observed at this reflex. Feedforward inhibition establishes a threshold for the incoming stimulus to regulate excitability of the M-cell. In this way, the M-cell initiates a startle response only when appropriate (Faber and Korn, 1978). This has important implications for the survival of the zebrafish as proper startle is necessary to avoid predation but also making sure to not be so easily excited that it cannot ignore unimportant stimuli. Thus, the M-cell can be considered a cellular decision maker for the startle reflex.

Prepulse inhibition (PPI) is observed as the reduction of the c-start rate in response to an auditory stimulus (Granato, 2007). This reduction occurs due to the

presentation of a smaller prepulse which precedes the stimulus. This time between the prepulse and the stimulus is known as the interstimulus interval (ISI).

Preuss (2011) was able to show that there can be dopaminergic modulation of PPI. Application of apomorphine disrupted normal PPI about 25% of the time when the ISI was held to 50 msec. Their experiments were able to show that this deficit in PPI could be restored by haloperidol. This design was convenient because we saw that a loss of function could be recovered. We used the findings from this study to inspire our own set of experiments with pharmacological agents. Those include Dizocilpine (MK-801), APV (AP5), D-Serine, Haloperidol, Prozac, and Serotonin. MK-801 is a uncompetitive NMDA receptor antagonist, AP5 is a competitive NMDA receptor antagonist, D-Serine is a NMDA receptor coagonist, Haloperidol is a dopamine antagonist, Prozac is a serotonin reuptake inhibitor, and serotonin is a facilitator of synaptic plasticity.

Patients with schizophrenia are reported to have deficits in PPI (Duncan, 2006). Some attribute this deficit to hypoactivity of the NMDA receptor. Given that the larval zebrafish m-cell mediated escape response is mediated by glutamatergic transmission through the NMDA receptor, we concluded that it was an ideal behavior to study for observing the effects of pharmacological agents on PPI. PPI, used as a proxy for schizophrenia-like dysfunction, can be observed in the larval zebrafish, which is an ideal model to use for its high fecundity, transparent body, resilience, and ease of drug delivery. The high fecundity allows for large sample sizes in a relatively short period of time. The transparent body allows us to visually evaluate for poor health and developmental abnormalities.

This allows us to exclude animals that are unhealthy from the experiments. The resilience is important because we are able to use strong drugs at high concentrations, while also manipulating the animals physically without hurting them. Despite their small size, they are easily transferred between locations without any damage being done to their neural system. Lastly, because they are marine animals, their gill system allows agents dissolved in the water to be directly absorbed into their blood stream. This makes drug delivery extremely effective, safe, convenient, and rapid. Team members simply dissolve the necessary compounds in the water, and the animal will have absorbed the drug into their blood stream within minutes.

In this study, we will determine the ideal conditions to produce PPI in larval zebrafish and also determine the effects of neurobiologically significant drugs on PPI.

Materials and Methods

Animal housing

Zebrafish used in our experiments were obtained from the Tupfel long fin (TL) line which are homozygous for leo^{t1} and lof^{dt2} (Zfin.org). The TL line was obtained from the UCLA zebrafish core facility, run by Shuo Lin. The animals were housed at breeding facilities in the zebrafish core facility and maintained by Yuan Linda Dong and her team of undergraduate work study assistants. Zebrafish were kept in zebrafish housing racks designed by Aquaneering Inc. The system features steel racks with a continuous water delivery and drainage system. The water was monitored regularly and controlled for particulates, debris, salinity, temperature, oxygen, water flow rate, waste, and pH. Water is decontaminated by an organic paper filter and UV light. Light was cycled throughout the facility daily for 14 hours of light and 10 hours of dark (08:00AM-10:00PM, 10:00PM-8:00AM, respectively).

Zebrafish tanks were self-cleaning tanks which are made of translucent polycarbonate. There is a removable polycarbonate lid that allowed easy access to the animals. Additionally, a removable baffle acted as a filter, preventing animals from escaping the chambers. The zebrafish core uses three different sized tanks to house the animals in the racks: 1.4L, 2.8L, and 9.5L tanks (small, medium, large, respectively). Small tanks were limited to two adults maximum, medium tanks were limited to six adults maximum, and large tanks were limited to twenty-five adults maximum. Animals that were between 5-35 days post fertilization (dpf), were stored in medium tanks at with up to 200 animals. Instead of the normal

baffle, we used a fry screen which included an extra thin mesh filter that would prevent the animals from escaping into the drainage system.

Water flow into the housing chambers of adult animals (15 dpf and older) arrived from two small branching hoses and the velocity is adjusted so that the water in the chamber is circulating in two concentric radial currents. This allows for proper cycling of water for filtration and oxygenation. Younger animals (5 dpf to 15 dpf) are given low water drips to allow for oxygenation but no currents are created within the chamber water. This allows the relatively feeble younger animals to thrive.

Larvae (0-5 dpf)

Zebrafish larvae were not kept in the zebrafish core facility. They were stored in the Glanzman laboratory in a stationary incubator locked at 28.6° C. Larvae were collected after they were bred in the core facilities, and manually carrying the eggs to be hatched and relocated to the Glanzman laboratory incubator.

Husbandry

Adults were bred in the UCLA Zebrafish core facility. Mating pairs were carefully selected and transferred to zebrafish breeding tanks made by Carolina biological supply company. Depending on the age and size of the adults, two to five adults were placed in the breeding tanks to breed. If the animals were larger, then breeding pairs were isolated. If the animals were smaller, up to five adults were placed in the chamber. The animals were placed in the breeding chambers

and filled about three quarters of the way with the same system water that the adults had been in before.

Zebrafish rigidly follow a fixed diurnal cycle. They are only able to breed in the first few hours of daylight, between 8:00AM to 12:00PM. They also are very sensitive to disturbance, which will usually prevent any possibly of spawning. Disturbances include loud noises, mechanical turbulence of their breeding chamber, interruption of their normal diurnal cycle, feeding, density of tanks, cleanliness of tanks, water quality and temperature, and even mate selection. It was very important to address all of these needs as the adults would not spawn if any one of these conditions were not met. The following rules were adhered to strictly whenever breeding:

1. Mating pairs were matched off one day before the intended spawn date of the embryos after the adult animals were fed (between the hours of 3:00PM and 8PM).
2. Breeding chambers were reused and shared with several other labs who utilized the core facilities to house their animals. Chambers were labelled clearly with waterproof labelling tape. Chambers were rinsed with tap water and then distilled water after every use. There were monthly thorough cleanings by the core facility with warm water and soap.
3. Breeding chambers were stored on rolling carts that allowed the stacking of multiple breeding chambers throughout the night. The carts were rolled to quiet corners of the zebrafish core facility to minimize potential human disturbance.

4. Sometimes, dividers are placed in the breeding chamber to separate the males and females. By doing so, the animals are not be able to breed until a team member removes the barrier. This was important for ensuring the age of the embryos and larvae that were being used for experiments. For some experiments, we needed to control spawn times within very narrow windows, such as in the case with some *shocked* mutants.
5. The animals were allowed to then acclimate to the chamber overnight. Between 8:00AM and 9:00AM, any breeding chamber dividers that needed to be removed were removed by a team member. No other disturbance is made to the animals for the following few hours until embryo collection.
6. At 1:00PM, a team member removed the adults from the breeding chamber and returned them to their holding tanks. This timing was important so that the animals could be fed again by 2:00PM. Maintaining the daily nutrition for the animal is essential for a quick refractory period before the adults can be paired off for breeding again. Males could be bred again in three days. Females can be bred a subsequent time as early as seven days.
7. After the adults are returned, the eggs are collect by running the breeding chamber water through a plastic kitchen sieve, brand kitchen craft 12 cm. For thorough collection, a second rinse and filtration through the sieve was utilized.
8. The eggs that are trapped in the plastic sieve are transferred to 150mL VWR brand disposable petri dishes, which were 150 x 15mm in size. They are

washed out with system water and carried by a team member back to Gonda building 2524, the Glanzman laboratory.

9. Using a microscope for better visibility, any debris and unfertilized eggs are removed with a polyethylene 3mL bulb draw transfer pipette from Sigma Aldrich. The eggs are washed three times and then transferred into embryo medium (E3 solution: 5mM NaCl, 0.33mM $MgCl_2$, 0.33 mM $CaCl_2$, 0.17 mM KCl, 10-5% methylene blue, pH 7.2) as detailed by Westerfield in The Zebrafish Book (Westerfield 1995).
10. The embryos are then stored in a darkened incubator which is held to 28.6° C. They are cleaned daily by removing dead animals and debris and replacing the embryo medium with a transfer pipette.
11. The animals are not fed during the 5 days that they are held in the incubator.
12. At the end of 5 dpf, the animals are transferred to the larvae racks in the core facility.

Feeding

Adult animals (15 dpf and older) were fed brine shrimp, INVE brand. Brine shrimp are raised within the core from artemia cysts. The cysts are placed in a polycarbonate transparent hatching cone and filled with water that is adjusted to 25 parts per thousand in salinity. Cysts are added at a density of 1 gram per liter and aerated by a motorized water pump. The brine shrimp hatch approximately 24-36 hours later and are harvested by undergraduate assistants who feed the adults with approximately ~10mL of a concentrated solution of adult brine shrimp

for small tanks, ~15mL for medium tanks, and ~20mL for large tanks. The animals are fed promptly at 2:00PM every day. Younger animals (5 dpf to 15 dpf) were fed freeze dried brine shrimp, by Omega One.

Testing

5 dpf zebrafish larvae were loaded into 24-well Corning Costar cell culture plates from Sigma-Aldrich. Each well was filled up to the brim, which varied between 3-4mL. Each animal was loaded individually to each cell culture well. Either the embryo medium or the experimental drug does old insulation was used to fill the well. All animals were selected for good health and motor activity to ensure that underdeveloped or sick larvae were not used for our experiments. The selection was done by light microscopy and use of a transfer pipette.

All testing was done in an isolated room in Gonda Neuroscience building. The room was kept separate to isolate all outside sound. After transferring larvae to the cell culture plates, the animals were given a minimum of one hour to acclimate to the testing room's new environment. This allows the animals to get acclimated to the ambient sound levels, temperature, and lightbox. The lightbox is a stage with a fluorescent light source that illuminates the tray for the camera above.

Camera setup

The cell culture plates with the animals are placed onto a light box (Gagne Inc., Johnson City, NY) in the isolated sound room. It is atop a table and placed

directly in front of a speaker, Pyle Pro PSTUDIO5 5.25” 2-way active studio monitor speaker, which was used to deliver auditory pulses of various intensities and frequencies. Above the light box directly is a high-speed camera, TroubleShooter TS100MS, which is capable of recording at 1000 frames per second. The camera and the speaker are both triggered by a computer, Apple Imac 2009. Additionally, the computer also triggered a small LED light which was used for video analysis purposes. All three peripheral devices are connected to the computer through a Master-8 Pulse stimulator. This set up aloud simultaneous triggering of the camera, speaker, and LED light. The camera automatically captured three seconds of video total, 1.5 seconds preceding the polls and 1.5 seconds following the pulse. It team member was then able to select an appropriate portion of the three seconds to download the file onto a SanDisk SD memory card for transfer to a computer. See figure 2a for a schematic of the set up.

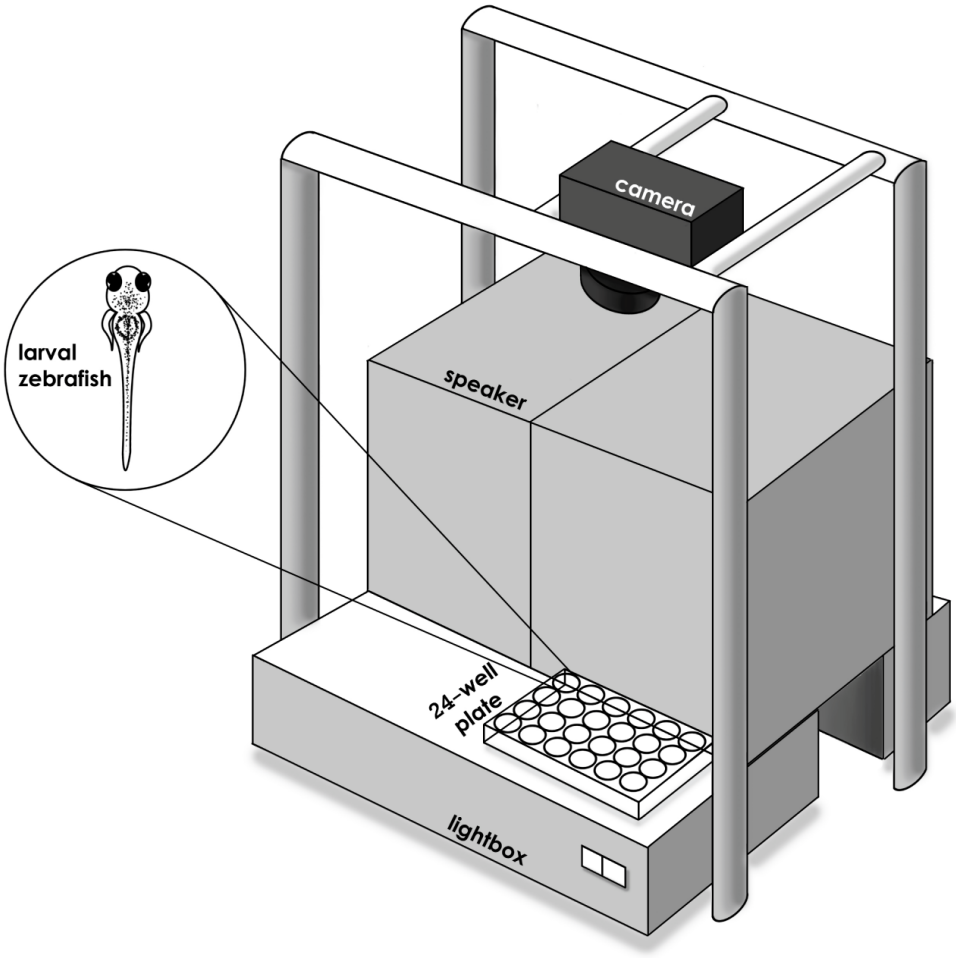


Figure 2a – The schematic shows the camera setup. Larval zebrafish are loaded into individual wells in a 24-well cell culture plate. The speaker is place just behind the light box and additionally supported using blocks. A camera is placed overhead to capture any movement of the larval zebrafish. (Figure from Roberts et al, 2011)

Software

The software program Audacity, a free multi-track audio editor and recorder, was used to design the timing and properties of the waveforms delivered as the auditory stimulus. The audacity software allowed the specific control of the waveform, sound intensity, duration, and sound frequency.

ImageJ was used to analyze the videos recorded by the camera.

Measuring startle

Kohashi (2012) determined that fast response startles in larval zebrafish occur with a latency of 15 msec on average. In conjuction with a 1% error in the timing of the camera set up, we determined that a 30 msec window before and after the auditory pulse would adequately record acoustic startle responses. C-starts we counted manually by a team member in an all or none fashion. A score of 1 was given for every instance of a c-start, and a score of 0 was given for anything else. C-starts were indicated by the stereotyped curved body formation as seen in figure 2b. Any body formations or movements that were not exactly congruent to the c-start formation were not considered c-starts.

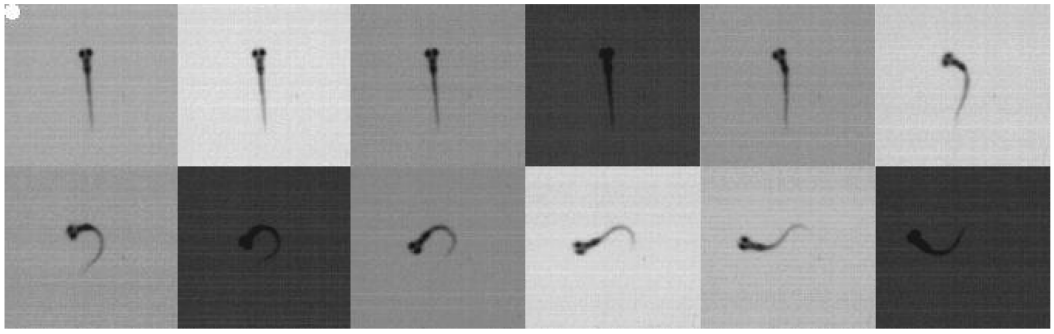


Figure 2b – this figure shows the frames of a recording of the body formations of a 5dpf larval zebrafish immediately following the delivery of an acoustic startle sound. Each frame of the recording shows the rapid, stereotyped, dramatic body curvature (figure from Roberts, et al., 2012).

All recordings of startle were spaced by either an acclimation period or a minimum of five minutes to eliminate the effects of habituation of the c-start response (Roberts, et al., 2012).

Measuring Prepulse Inhibition

To measure degree to which an animal's startle rate was inhibited by the presentation of a prepulse, we recorded 3 trials of only a startle and 3 trials of a prepulse followed by a startle for each experiment. To quantify the degree of inhibition, we used the following calculation:

PPI% = (Number of startles during the prepulse and startle pulse presentation – Number of startles during just the startle pulse presentation) / total number of startles

The rationale for dividing by the total number of startles is to standardize the values to the overall reactivity of the larval zebrafish. There is variability in the sensitivity to startle across animals, so we divide by the total number of startles to account for this variability. PPI% ranges from a value from -1 to +1. A PPI% score of -1 indicates that the animal startled more frequently to the presence

of a prepulse than if there were no prepulse. A PPI% score of +1 indicates that the animal does not startle at when there is a prepulse.

Preparation of pharmacological agents

Several pharmacological agents were used in this project. Dizocilpine (MK801), 2-amino-5-phosphonopentanoic acid (APV), D-serine, Haloperidol, Prozac, and serotonin were acquired from Sigma-Aldrich. Each drug was dissolved in the appropriate solvent and diluted into E3 for the trials.

Results and Discussion

Designing optimal waveform

Before conducting trials, we had to optimize the waveform properties to maximize the number of startles. A non-ideal waveform would not cause enough startles. The characteristics that we tested for were pulse duration, pulse amplitude, and prepulse interval. We tried various different volumes to see which would produce the most c-starts. We sampled 48 larval zebrafish at 5 dpf. The wells were filled with normal E3. Using the 500 Hz 2msec waveform as the startle sound (Roberts, et al., 2012), we ran these wildtype TL zebrafish through 3 trials and calculated the percentage of startles out of 3 throughout the different volumes. We interspersed the volumes to control for habituation. The results are seen in Figure 3a.

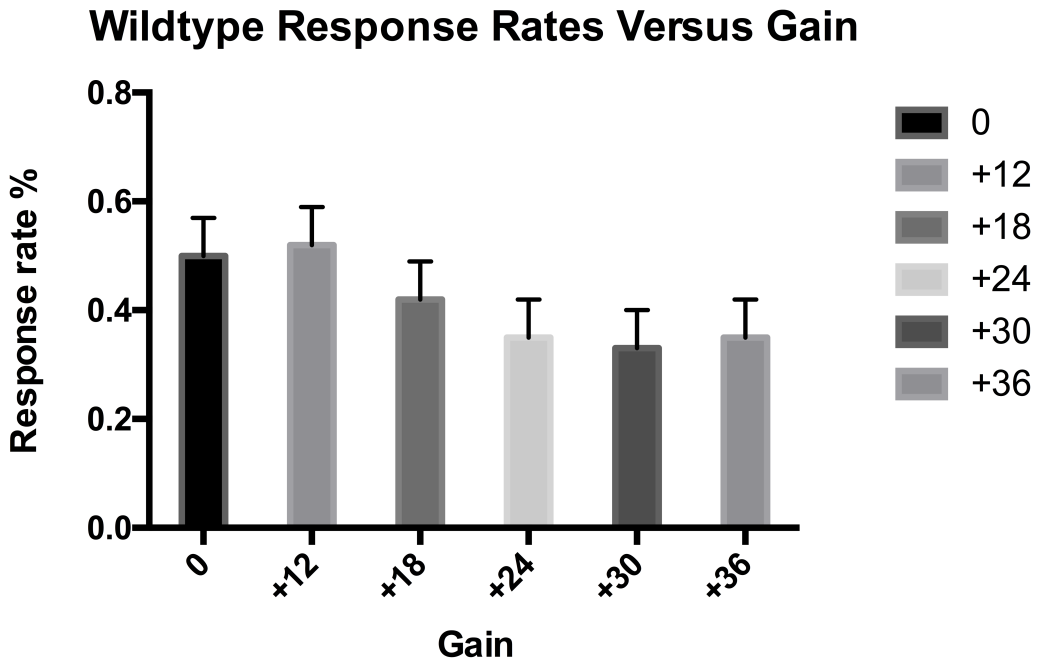


Figure 3a – Wildtype responses versus Gain. TL wildtype, 5 dpf. We ran 48 larval zebrafish through three trials of

each volume setting. Using 500 Hz pulses of 2 msec, we tried 6 different volume settings ranging from 98 dB (0 gain) up to 134 dB (+36 gain). 110 dB (+12 dB) had the highest response rate with a mean of 52%.

With the highest response rate being at +12 gain, or 110 dB, we decided to use this volume setting for all future experiments. With higher startle rates, we expect to be able to see more pronounced effects due to pharmacological agents to allow us better.

Now that we had the ideal startle sound, we had to figure out the ideal prepulse conditions. We need to find out which prepulse interval (also known as interstimulus interval, ISI) and prepulse duration combination was ideal for maximizing prepulse inhibition. ISI is the amount of time elapsed between the presentation of the prepulse and the startle sounds. Prepulse duration is the length of the prepulse sound.

We ran 144 larval zebrafish, aged 5 dpf through a combination of durations (2, 20, 100, 1000 msec) and interstimulus intervals (ISI) (2, 10, 100, 1000 msec). Each group of 48 larvae went through 3 trials of startles and 3 trials prepulse plus startles for the 4 different ISIs with the prepulse duration was held constant for each group. Each of the larvae were spawned from the same TL wildtype parents at the same time. This was to minimize variation across breedings. All of the groups were populated at random from the same population of healthy larvae from the same parents. See figure 3b for the results.

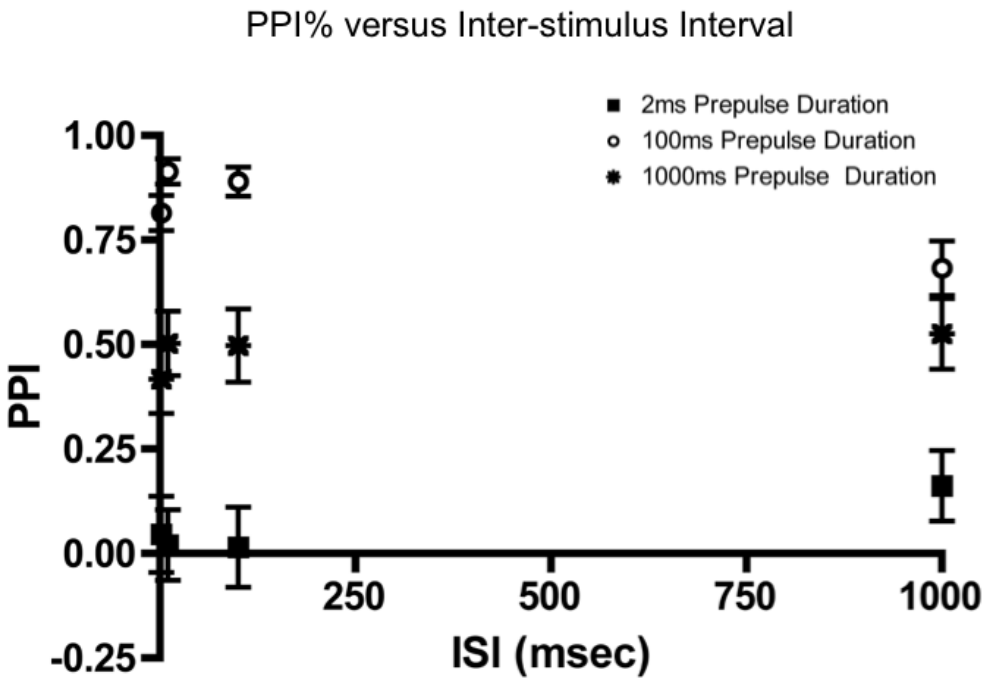


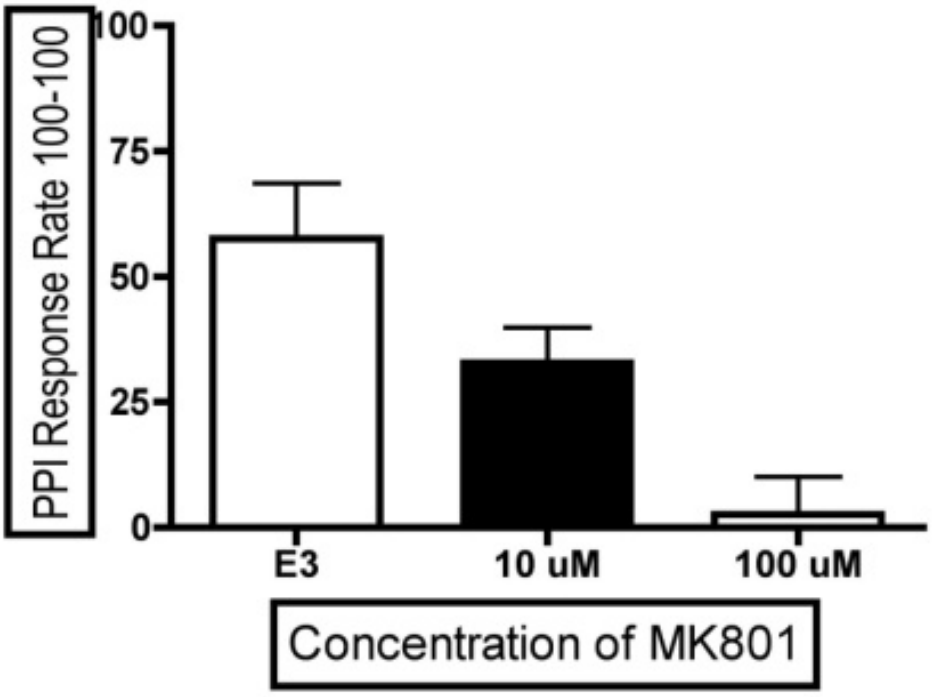
Figure 3b – PPI% at different ISIs and Prepulse durations in TL Wildtype larval zebrafish aged 5dpf. Groups of 48 animals were run through different ISIs with prepulse durations held constant. Each group is indicated by different symbols (open circle, asterisk, and solid square). PPI% was calculated using the formula described in the materials and methods of this paper. There is a statistically significant different beween the different prepulse durations, with 100 msec prepulse duration being the most ideal. There is also a statistically significant difference between the ISIs, with 20 and 100msec ISI being the most effective.

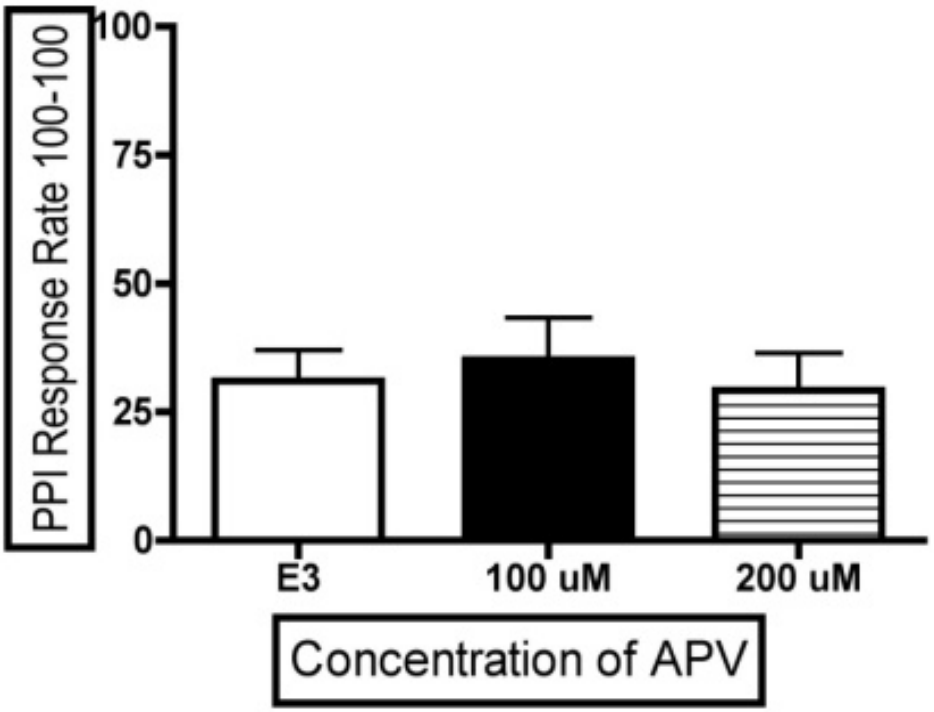
The results of this experiment showed us that the prepulse duration of 100msec was best able to inhibit c-starts. We also found that an interstimulus interval of 20msec and 100msec were the most effective at causing prepulse inhibition. Given the results of this experiment and the previous experiment, we had a decision to make for all future PPI experiments: do we use 100 msec ISI or 20 msec ISI? The answer came down to technical limitations. Given that we gave a 30 msec margin of error for our camera equipment to correctly capture a c-start escape in response to an auditory stimulus, an ISI of 20msec would not give us a comfortable buffer period between the prepulse and the beginning of the c-start recording. Given that 100 msec ISI was equally effective in prepulse inhibition, we chose the 100msec ISI. Couple with the 100 msec prepulse duration, we expected to see nearly 90% PPI with this protocol.

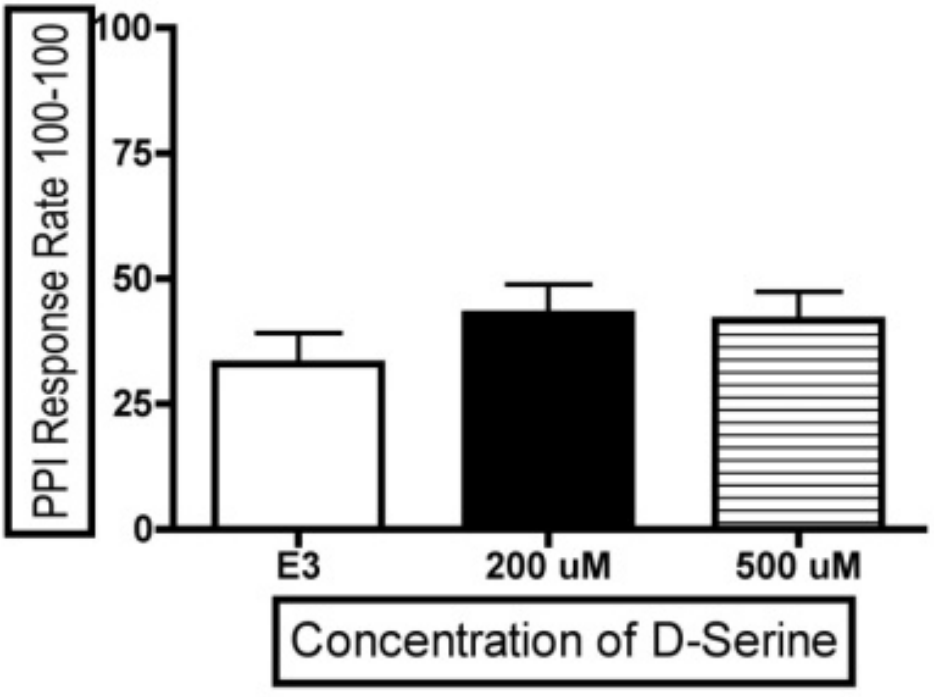
Finding correct pharmacological agent concentrations

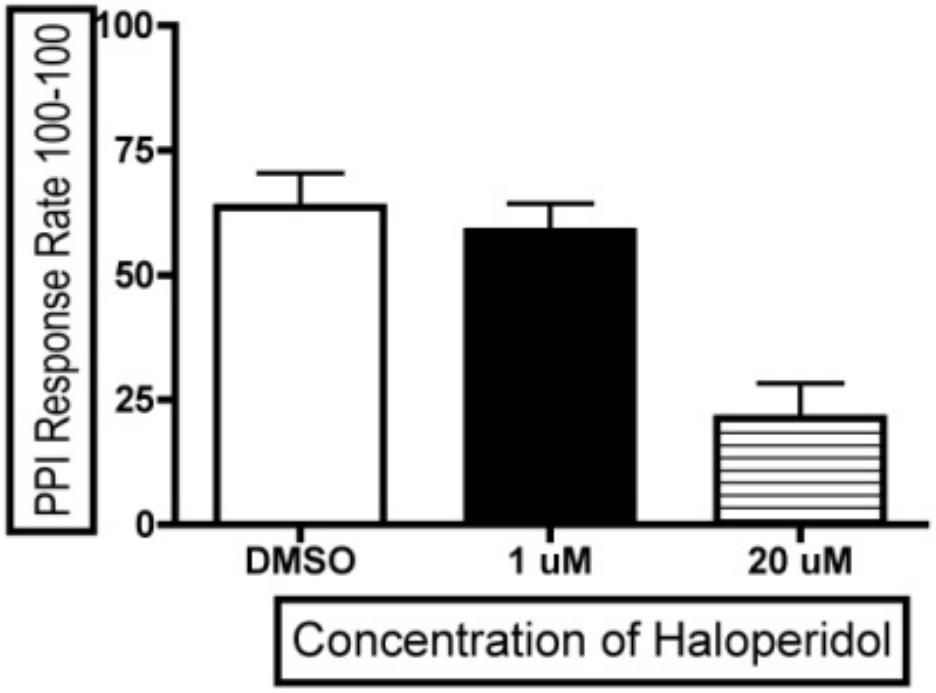
We then wanted to find out the effect of several neurobiological pharmacological agents on PPI% in larval zebrafish. Although we determined the optimal parameters for PPI, we did not know the optimal concentrations of the drugs to use to maximize PPI. We did a pilot trial to determine the optimal concentrations. For MK-801, we tried 10 uM and 100 uM in E3. For APV, we tried 100 uM and 200 uM in E3. For D-Serine, we tried 200 uM and 500 uM. For Haloperidol, we tried 1uM and 20uM in Dimethyl sulfoxode (DMSO). We used DMSO because haloperidol requires an organic solvent. We used 0.1% DMSO to reduce toxicity to the animals. For Prozac, we used 10 uM and 50 uM in E3. For

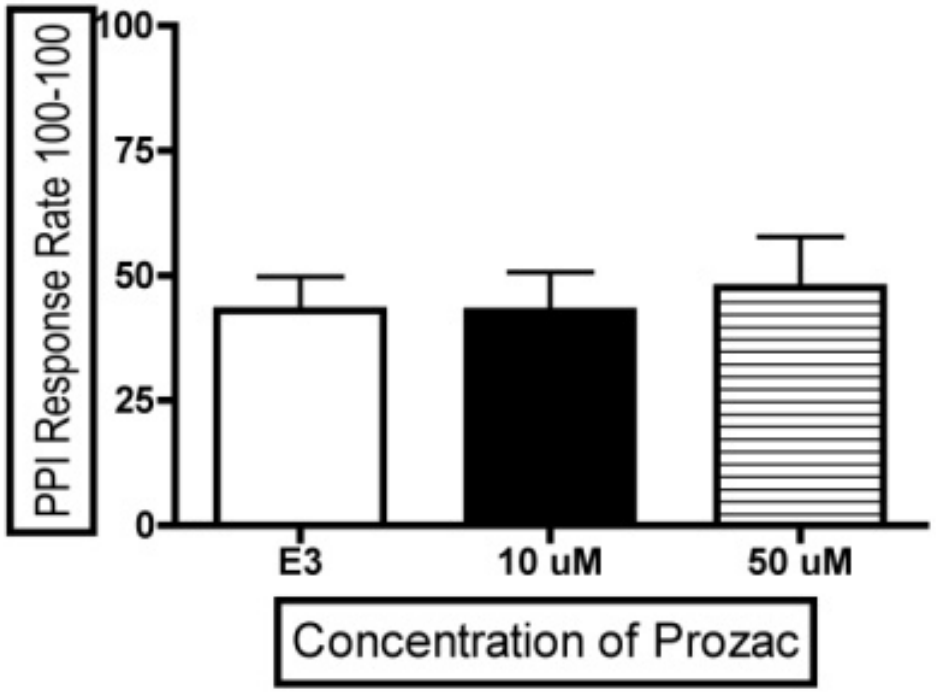
5-HT, we used 1 uM and 100 uM in E3. The results for each drug trial is summarized in Figure 4a. PPI% is calculated as discussed in the materials and methods section.




PPI Response Rate 100-100
100
75
50
25
0
E3
100 uM
200 uM
Concentration of APV


PPI Response Rate 100-100
100
75
50
25
0
E3
200 uM
500 uM
Concentration of D-Serine


PPI Response Rate 100-100
100
75
50
25
0
DMSO
1 uM
20 uM
Concentration of Haloperidol


PPI Response Rate 100-100
100
75
50
25
0
E3
10 uM
50 uM
Concentration of Prozac

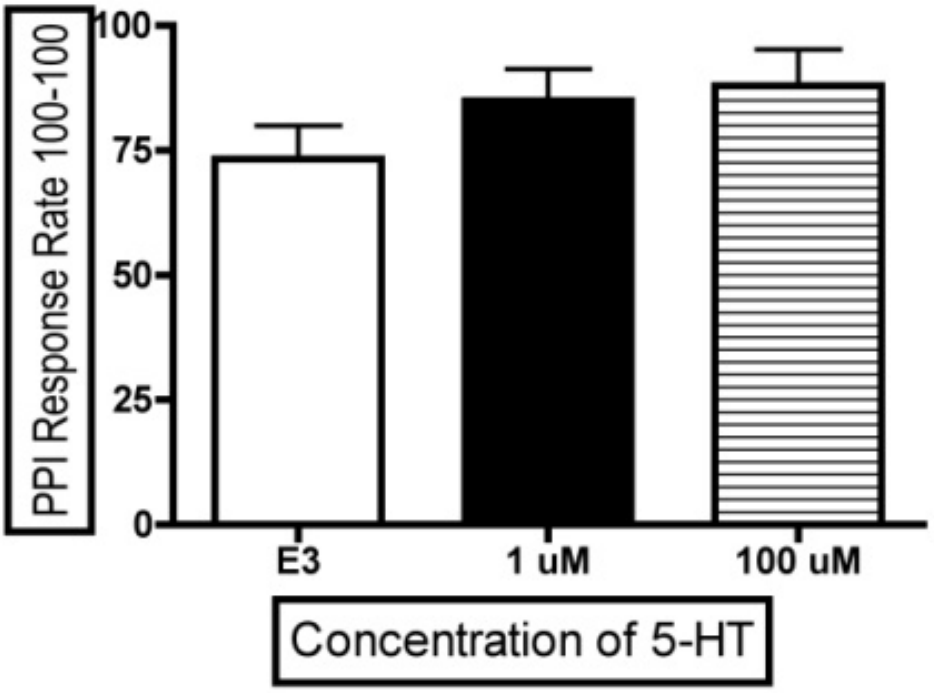
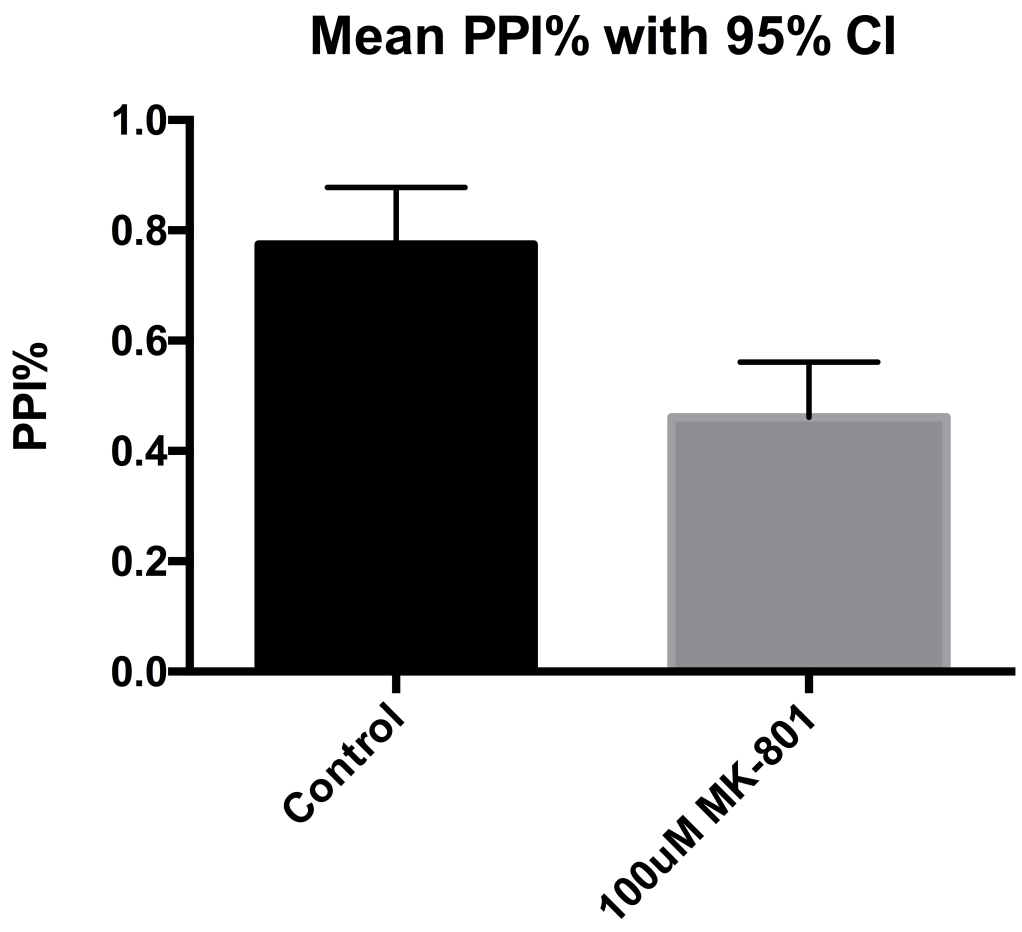


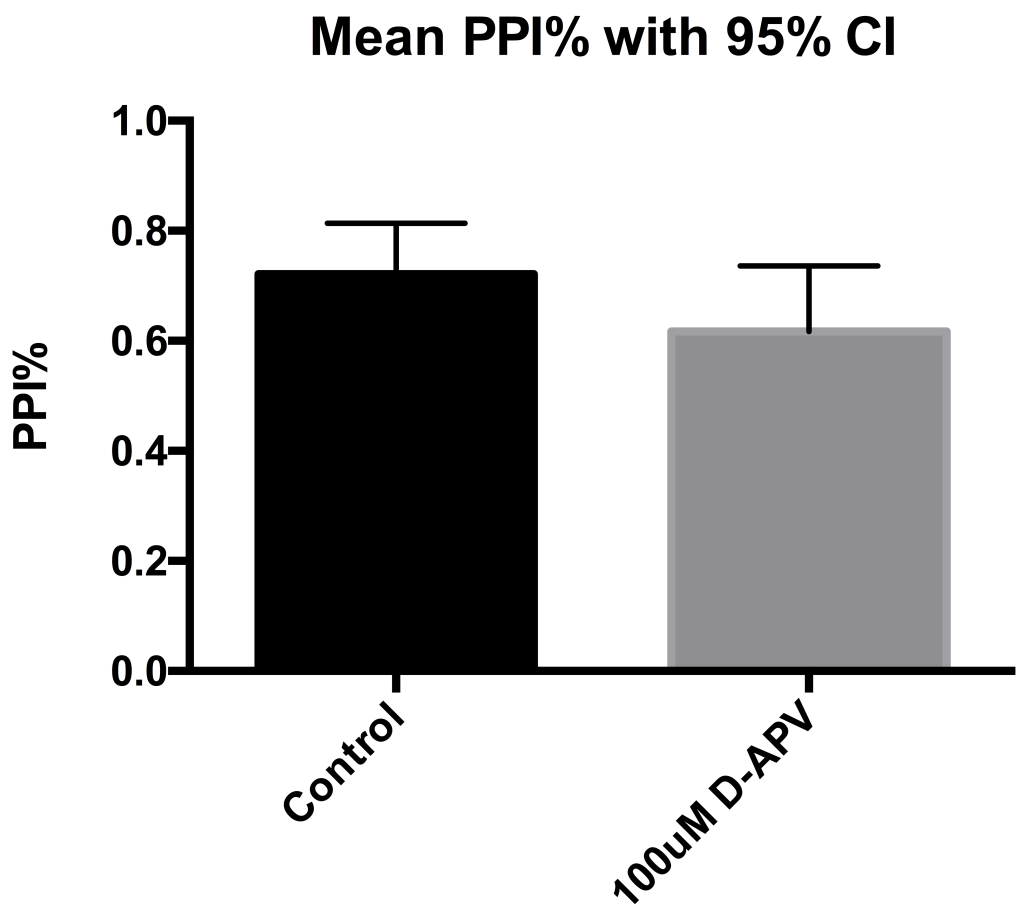
Figure 4a. PPI% of larval zebrafish at 5 dpf when exposed to various pharmacological agents. Groups for 48 larvae were tested for prepulse duration 100 msec and ISI 100 msec with 500 Hz 2msec startle duration at 110 dB. MK-801 shows moderate PPI at 10 uM and marked PPI at 100 uM. APV showed minimal effects on PPI. D-Serine showed no change in PPI at both concentrations. Haloperidol showed significantly impaired PPI with only 20 uM. Prozac showed no change in PPI at both concentrations. 5-HT showed a significant effect on increasing PPI.

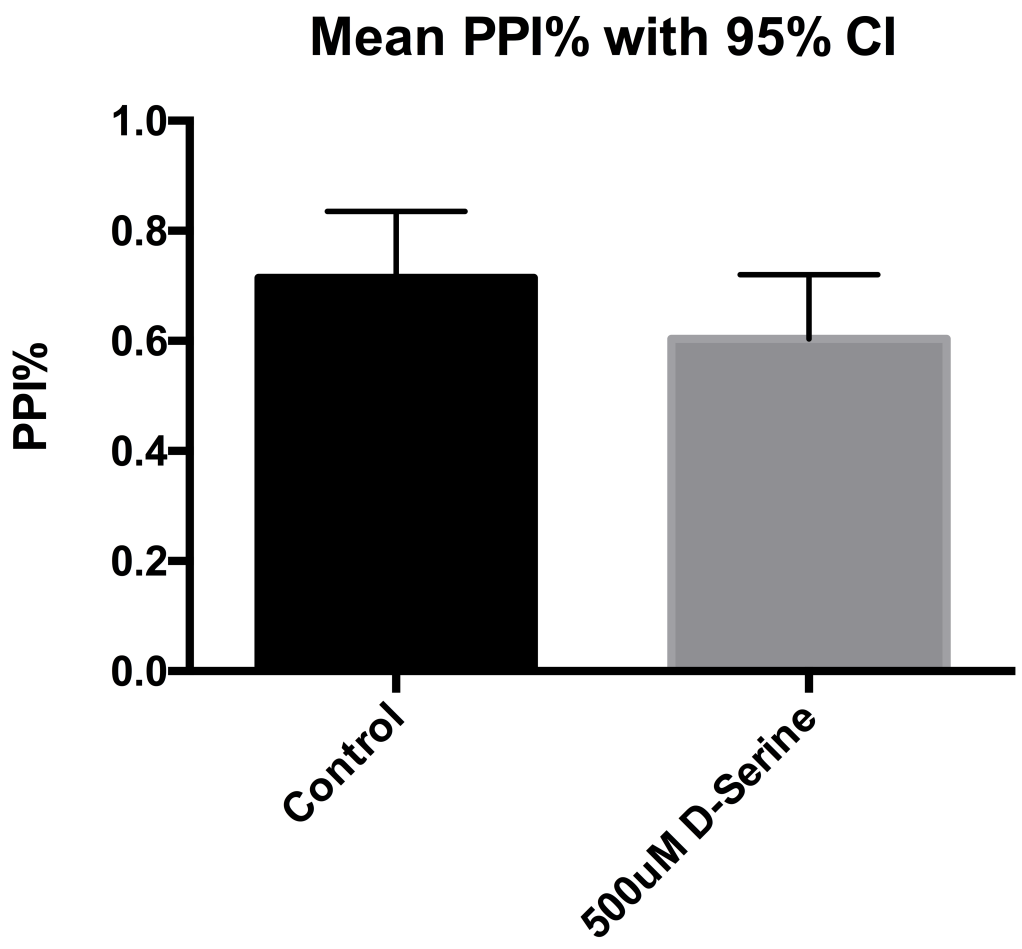
The results show that only MK-801 (down), Haloperidol (down), and 5-HT (up) affected PPI. The effect of MK-801 is expected, as the startle response is already known to be modulated by NMDA receptors (Roberts, et al., 2012). We also expected that serotonin would facilitate PPI because of its effect at the synapse (Glanzman, 2015). However, the more interesting findings were that Haloperidol decreased PPI and that Prozac did not affect PPI. This finding went against the findings of previous papers (Bhandiwad, 2013; Braff 1978). Thus, we had to ensure that our data were correct.

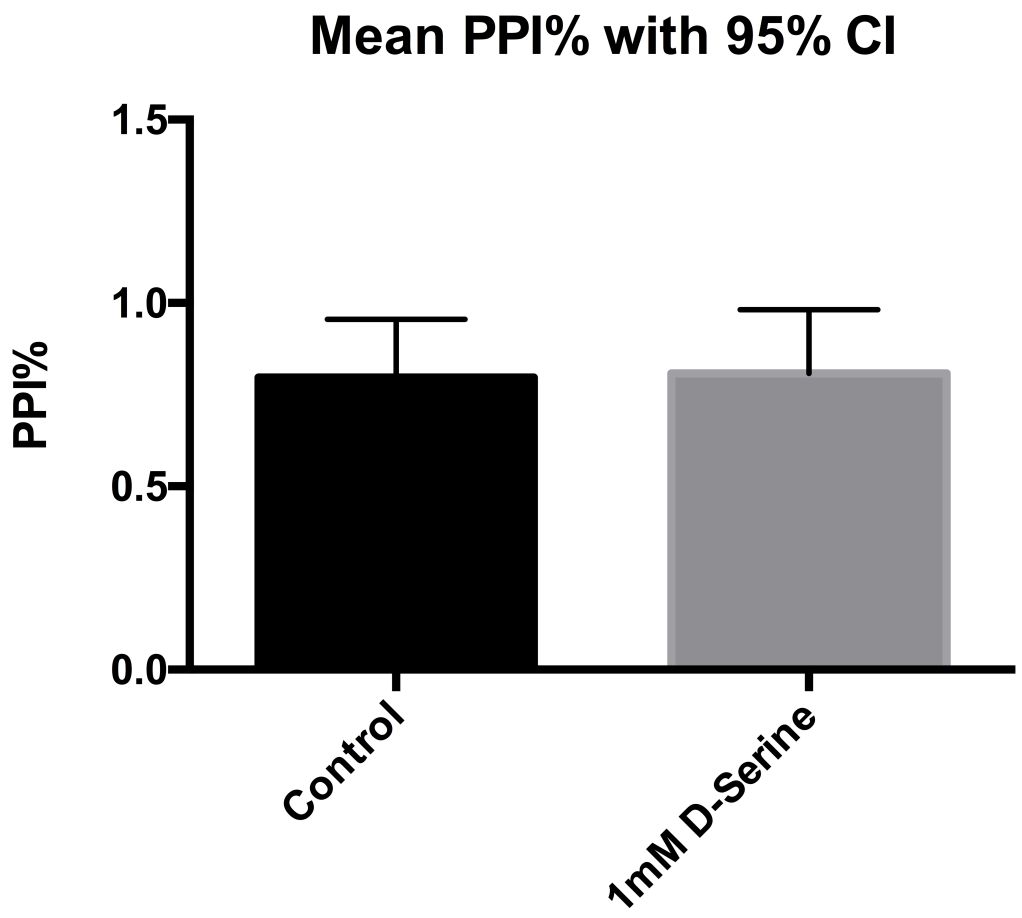
Replication of PPI% with pharmacological agents

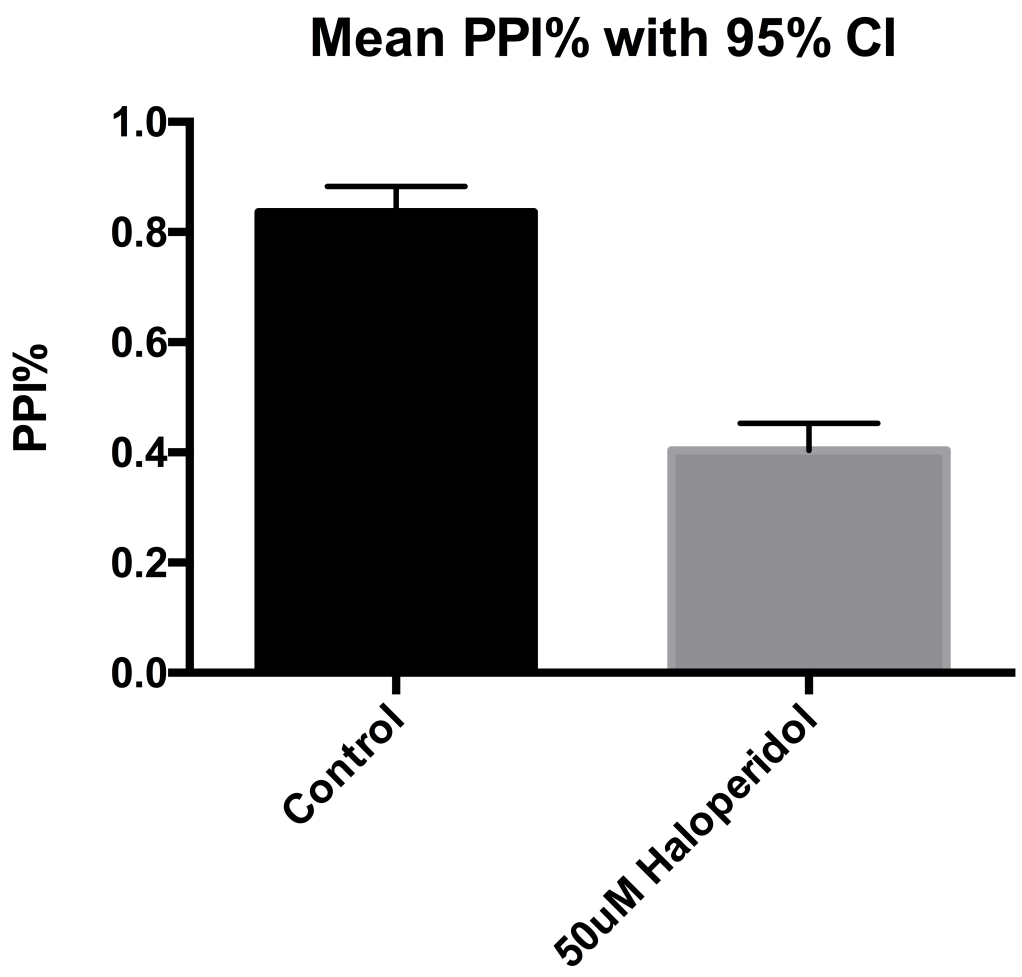
We replicated the pharmacological experiments to confirm our findings. This time, we increased sample sizes significantly to have more statistical power. The results are seen in Figure 4b. We chose the most optimal concentrations found in the previous experiment as our starting point and in some cases tried a second higher dosage to reveal any drug interactions. We wanted to know for certain if our findings were repeatable.


Mean PPI% with 95% CI
PPI%
1.0
0.8
0.6
0.4
0.2
0.0
Control
100uM MK-801


Mean PPI% with 95% CI
PPI%
1.0
0.8
0.6
0.4
0.2
0.0
Control
100uM D-APV


Mean PPI% with 95% CI
PPI%
1.0
0.8
0.6
0.4
0.2
0.0
Control
500uM D-Serine


Mean PPI% with 95% CI
PPI%
1.5
1.0
0.5
0.0
Control
1mM D-Serine


Mean PPI% with 95% CI
PPI%
1.0
0.8
0.6
0.4
0.2
0.0
Control
50uM Haloperidol

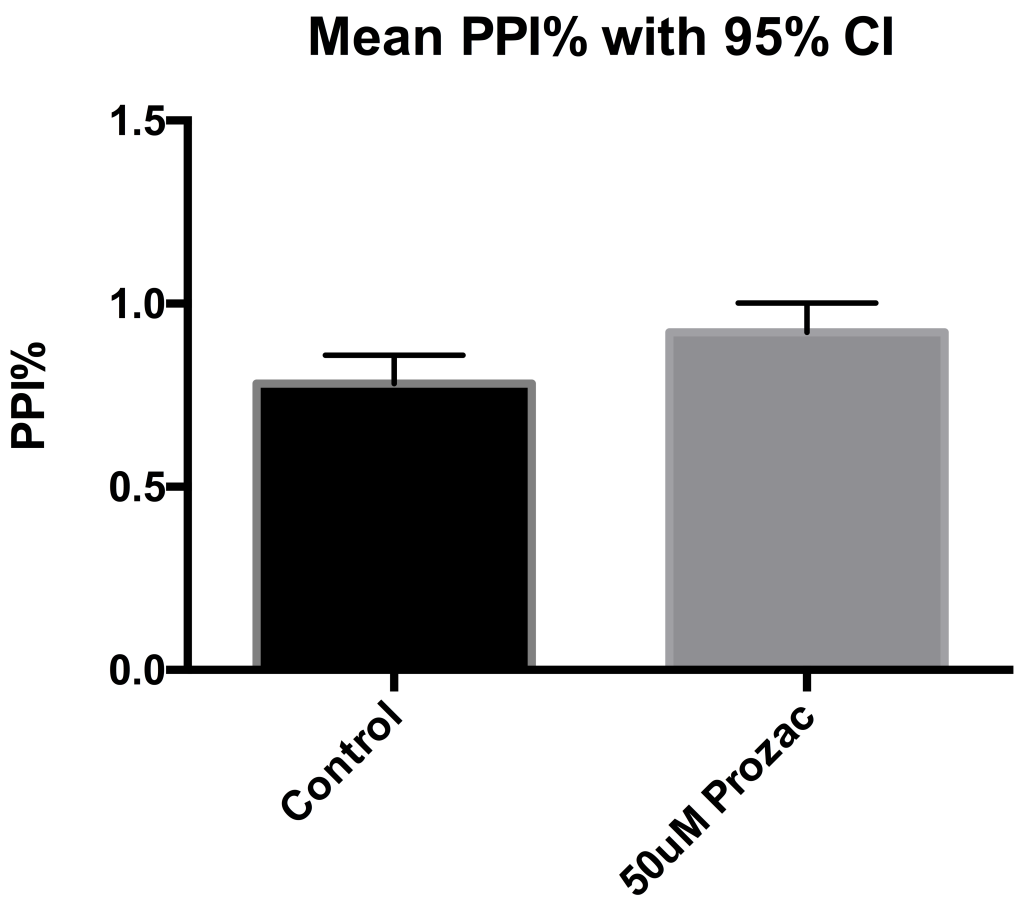


Figure 4b – Replication of PPI experiments using pharmacological agents. PPI% of larval zebrafish at 5 dpf when exposed to various pharmacological agents. Multiple Groups for 48 larvae were tested for prepulse duration 100 msec and ISI 100 msec with 500 Hz 2msec startle duration at 110 dB. MK-801 100 uM indeed confirmed a statistically significant decrease in PPI% (N=78 control, N=80 MK-801). 100 uM D-APV confirmed that there was no effect on PPI% (N=78 Control, N=81 D-APV). 500 uM D-serine confirmed that there was no effect on PPI% (N=60 Control,

N=65 D-serine 500uM). 1 mM D-serine confirmed that there was no effect on PPI (N=22 control, N=20 D-serine 1 mM). 20 uM Haloperidol revealed that there was actually no effect at 20 uM on PPI% (N=140 control, N=151 Haloperidol 20 uM). However, at 50 uM Haloperidol, there was a significant decrease in PPI% (N=149 control, N=168 Haloperidol 50 uM). At 50 uM Prozac, there was actually a significant increase in PPI% (N= 110 control, N=75 Prozac).

The replication revealed two significant things for us: first that high concentrations of Haloperidol actually does decrease PPI% and that Prozac will actually facilitate PPI, which is what is expected.

The reason for these conflicting findings can be explained by sample size. Given that our replications had far higher sample sizes, our confidence in our replication studies are high. Because the startle response rates (Figure 3a) were hovering in the 50-60% range anyway, we knew that a replication study would be ideal and time consuming. Our pilot study with Prozac showed a small increase in PPI at 50 uM. By increasing sample size, we were able to tease apart the true effect.

The findings of this study are important because we PPI is proposed as a potential proxy for many neurological diseases. This behavioral assay is used for high throughput screens of pharmacological agents that are being investigated for their therapeutic benefits ultimately in humans with mental illnesses. It is

interesting to see that Prozac, a drug commonly used to treat depression, is effective in restoring PPI which is seen as a normal function in healthy people. This leads me to believe that this drug has further evidence to support its use to treat some of the catatonic symptoms of depression.

Similarly, the contradictory findings of Haloperidol had us concerned. The replication showed us that in fact, this drug does decrease PPI. The finding is not too surprising given the fact that many patients report severe side effects from atypical antipsychotic medications, included Haloperidol. Excitability, irritiability, and impaired PPI are reported in patients with mental illnesses, including schizophrenia. Efficacy is limited and there is no cure. This parallels the finding that Haloperidol may cause some unwanted, severe side effects.

One of the theories for the explanation of PPI dysfunction in schizophrenia is an NMDA receptor hypoactivity theory (Burgess, Granato, 2007). The finding that MK-801, an NMDA receptor antagonist, would disrupt PPI is expected. Our data agrees with this model. Given that hypofunction of NMDA receptors can be a result of increased dopamine levels which inhibit glutamate transmission (Duncan 2006). Our data also agrees with this statement, given that our experiments also showed that Haloperidol, which is a dopamine agonist, was able to disrupt PPI at higher concentrations.

One interesting finding was that APV, another NMDA receptor antagonist, had no effect on PPI. Given that dopamine is responsible for the hypoactive NMDA receptor activity, it is an odd finding which leads us to believe

that there is more to the picture than simply a dopaminergic component to schizophrenia.

Lastly, an interesting finding was that serotonin facilitated PPI. Our first finding was not able to reveal the positive effect of Prozac, but our replication was able to show that Prozac, a serotonin reuptake inhibitor, showed a significant increase in PPI.

These findings suggest that Prozac may also have potential as a therapeutic for schizophrenia and that haloperidol may not be as good as we originally thought. PPI was used as a marker for neural dysfunction, so it is impossible to say with certainty how it would affect the actual symptoms in schizophrenic patients.

We found in this study the optimal conditions that reliably produce PPI behavior and can tease apart differences in pharmacological agents. Our data coincides with most of the literature on schizophrenia, but also highlights an important distinction with the side effects haloperidol. The NMDA hypofunction theory is supported by our data as a component of the etiology of schizophrenia.

We also conclude that the larval zebrafish is an appropriate model for high throughput drug screening. It is very important that we have a reliable behavioral assay that can be used for preclinical trials of pharmacological agents. In the future, this system can be used to test thousands of animals at once. With automation, it can become a very efficient process whereby many possible agents can be identified as potentially therapeutic or inhibitory for PPI. Both findings can lend more insight into the neurobiological cause of schizophrenia.

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