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Acute Administration of Dopaminergic Drugs has Differential Effects on Locomotion in Larval Zebrafish

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Abstract

Altered dopaminergic signaling causes behavioral changes in mammals. In general, dopaminergic receptor agonists increase locomotor activity, while antagonists decrease locomotor activity. In order to determine if zebrafish (a model organism becoming popular in pharmacology and toxicology) respond similarly, the acute effects of drugs known to target dopaminergic receptors in mammals were assessed in zebrafish larvae. Larvae were maintained in 96-well microtiter plates (1 larva/well). Non-lethal concentrations (0.2–50 μM) of dopaminergic agonists (apomorphine, SKF-38393, and quinpirole) and antagonists (butaclamol, SCH-23390, and haloperidol) were administered at 6 days post-fertilization (dpf). An initial experiment identified the time of peak effect of each drug (20–260 minutes post-dosing, depending on the drug). Locomotor activity was then assessed for 70 minutes in alternating light and dark at the time of peak effect for each drug to delineate dose-dependent effects. All drugs altered larval locomotion in a dose-dependent manner. Both the D1- and D2-like selective agonists (SKF-38393 and quinpirole, respectively) increased activity, while the selective antagonists (SCH-23390 and haloperidol, respectively) decreased activity. Both selective antagonists also blunted the response of the larvae to changes in lighting conditions at higher doses. The nonselective drugs had biphasic effects on locomotor activity: apomorphine increased activity at the low dose and at high doses, while butaclamol increased activity at low to intermediate doses, and decreased activity at high doses. This study demonstrates that (1) larval zebrafish locomotion can be altered by dopamine receptor agonists and antagonists, (2) receptor agonists and antagonists generally have opposite effects, and (3) drugs that target dopaminergic receptors in mammals appear, in general, to elicit similar locomotor responses in zebrafish larvae.

Keywords

dopamine; locomotor activity; zebrafish; acute; receptor

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1. Introduction

Dopamine is an essential neurotransmitter involved in the control of many functions of the brain (reviewed in Iversen and Iversen, 2007). With the majority of dopaminergic neurons in mammals innervating the midbrain (Vitalis et al., 2005), dopaminergic signaling modulates a wide array of behaviors (e.g. motor activity, stereotypy; Ungerstedt, 1976, Oberlander et al., 1979). Impaired dopaminergic signaling can lead to behavioral pathologies such as Parkinson's disease, schizophrenia, and attention deficit hyperactivity disorder (Hirsch, 1992, Cortese et al., 2005, Zeiss, 2005, Bowton et al., 2010).

Both pre- and post-synaptic receptors for dopamine are present in the nervous system of vertebrates (reviewed in Missale et al., 1998). These are G-protein coupled receptors that have seven transmembrane domains (Girault and Greengard, 2004). In mammals, these receptors exist in two subfamilies: D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptors. D1-like receptors share extensive homology across their transmembrane domains and are positively coupled to adenylyl cyclase stimulation (Zhuang et al., 2000, Herve et al., 2001). The transmembrane domains of D2-like receptors are also conserved, but their stimulation inhibits adenylyl cyclase activity (Obadiah et al., 1999). Through signaling events mediated by these receptors, dopamine can govern the initiation and execution of movement (Han et al., 2007).

To study the function of dopamine receptors in mammals, the use of agonists and antagonists has been well-established. Apomorphine is a nonselective agonist, acting on all receptor subtypes, but with differing affinities (Millan et al., 2002). The experimental drugs SKF-38393 and quinpirole are D1-like and D2-like selective agonists, respectively (Millan et al., 2002). In mammals, dopamine receptor agonists stimulate locomotor activity (Beninger et al., 1991, Hooks et al., 1994, Archer et al., 2003, Sobrian et al., 2003, Ardayfio et al., 2010). Nonselective dopaminergic antagonists like butaclamol, as well as D1- and D2-like selective blockers (SCH-23390 and haloperidol, respectively) decrease locomotor activity in mammals (Beninger et al., 1991, Sobrian et al., 2003, Choi et al., 2009, Shireen and Haleem, 2011).

Zebrafish are a popular model in studies of behavior (reviewed in Drapeau et al., 2002, Fetcho, 2007, Gerlai, 2010, Norton and Bally-Cuif, 2010). This model provides many benefits over the use of mammals, such as rapid and external development (Kimmel, 1995), small size, and low cost. Zebrafish have a high level of biological homology with mammals (Grillner, 1985, Tropepe and Sive, 2003, Maximino and Herculano, 2010). Specifically, the major dopaminergic pathways in mammals are represented in the zebrafish brain (Panula et al., 2006, Maximino and Herculano, 2010); however, the dopaminergic neurons that control motor activity in the midbrain in mammals correspond to groups located in the forebrain in zebrafish (Rink and Wullimann, 2001, Schweitzer and Driever, 2009, Schweitzer et al, 2012). The system begins to develop in zebrafish at about 15–18 hours post-fertilization (hpf; Boehmler et al., 2004), and by 4 days post fertilization (dpf), all neuronal cell groups, and their projections, are present (Rink and Wullimann, 2002, Tay et al., 2011). Homologous receptors for all the mammalian subtypes have been identified in zebrafish, with the exception of D5, and are detected by 30 hpf in the zebrafish brain (Boehmler et al., 2004, Boehmler et al., 2007, Li et al., 2007).

In sum, the available evidence indicates that the structure of the dopaminergic nervous system in zebrafish resembles that of the mammalian nervous system, but there have been few studies characterizing the pharmacology of this system. In fact, relatively little is known about the behavioral effects of dopaminergic drugs in larval zebrafish. Specifically, altered behaviors as a result of acute exposure to drugs that target dopaminergic receptors have been

reported in only a few studies (Boehmler et al., 2007, Farrell et al., 2011, Giacomini et al., 2006, Kokel et al., 2010, Savio et al., 2011, Seibt et al., 2011, Souza et al., 2011). Of these, only Boehmler and colleagues (2007) investigated a range of drug doses (in this case, quinpirole), while the other studies reported the effects of just one dose. Rihel and colleagues (2010) studied a range of doses of several drugs, including haloperidol, in zebrafish larvae; however, the exposure lasted 3 days, and was, therefore, likely to be developmental (as opposed to acute) in nature. Moreover, no study specifically investigated the time necessary to reach the peak effect of these drugs in zebrafish. Additionally, no study has detailed the behavioral effects of the dopaminergic antagonist butaclamol in zebrafish. Therefore, further investigation into the behavioral profiles of dopaminergic agonists and antagonists in zebrafish is necessary in order to understand how these effects compare to those in mammals.

Our current study was designed to characterize the acute effects of several dopaminergic receptor drugs on larval locomotion. We hypothesized that zebrafish larvae would respond to drugs that target dopaminergic receptors with dose-dependent changes in locomotor activity. It was also expected that these locomotor responses would differ following the administration of receptor agonists vs. antagonists. To test this, the time of peak effect was first identified for each drug, and then detailed dose-response studies were conducted using a behavioral paradigm based on light-stimulated activity (previously described in Irons et al., 2010, MacPhail et al., 2009, Fernandes et al, 2012). The data show that larval zebrafish are sensitive to drugs that target dopaminergic receptors, and that the locomotor responses to each drug are dose-dependent and resemble mammalian responses. Finally, it was determined that stimulation/blockade of either subfamily of dopaminergic receptors results in similar effects; however, stimulation/blockade of all receptor subtypes has complex effects on locomotor activity.

2. Materials and Methods

2.1. Animals and Husbandry

All studies were conducted in accordance with and approved by the Institutional Animal Care and Use Committee at the National Health and Environmental Effects Research Laboratory, US EPA (Guide for the Care and Use of Laboratory Animals, National Research Council, 2011). Wild-type adult zebrafish (*Danio rerio,* outbred) were maintained as breeders in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved animal facility at 28°C on a 14:10 hr light: dark cycle with lights on at 0830 hr. The adult zebrafish were placed in breeding tanks (Aquatic Habitats, Apopka, FL) 1 hr prior to light onset. After 2 hr, embryos were collected and incubated in a water bath at 26°C until the afternoon. The embryos were cleaned using a 0.06% bleach solution for 5 minutes, and then rinsed 3 times using 10% Hanks' balanced salt solution (13.7 mM NaCl, 0.54 mM KCl, 25 μM Na_2HPO_4, 44 μM KH_2PO_4, 130 μM $CaCl_2$, 100 μM $MgSO_4$, 420 μM $NaHCO_3$). This process was repeated once more, then embryos were placed individually into 250 μl of 10% Hanks' solution (changed daily) in the wells of 96-well mesh microtiter plates (Multiscreen™ catalog #MANMN4050, Millipore Corp., Bedford, MA). The embryos/larvae were housed in an incubator at 26 ± 0.2°C on a 14:10 hr light:dark cycle until testing at 6 dpf. At 6 dpf,larvae display functioning sensory and motor systems and are sensitive to a broad range of centrally-acting drugs (Rihel et al., 2010), including some that act on the dopaminergic nervous system (Irons et al., 2010). The dopaminergic system begins to develop in zebrafish at about 15–18 hours postfertilization (hpf; Boehmler et al., 2004), and by 4 dpf, all neuronal cell groups are present (Rink and Wullimann, 2002), and receptors for dopamine are detected in the brain (Boehmler et al., 2004, Boehmler et al., 2007, Li et al., 2007).

2.2. Drugs

All drugs were purchased from Sigma-Aldrich (St. Louis, MO). Dopamine receptor agonists included apomorphine hydrochloride hemi-hydrate (non-selective), (±)SKF-38393 hydrochloride (D1-like receptors), and (−)quinpirole hydrochloride (D2-like receptors). Dopamine receptor antagonists included (+)butaclamol hydrochloride (non-selective), R-(+)-SCH-23390 hydrochloride (D1-like receptors), and haloperidol (D2-like receptors). All drug stock solutions were made by dissolving the compounds in deionized water (with the exception of haloperidol). Solutions were serially diluted further with 10% Hanks' solution to reach final concentrations of 0.2–50 μM. Haloperidol was dissolved in dimethyl sulfoxide (DMSO), and the stock solution was also serially diluted with DMSO to make concentrated solutions for dosing (further diluted in buffer trays with 10% Hanks' to reach final concentrations of 0.2–50 μM, each in 0.4% DMSO). The final concentration of DMSO used in these experiments does not alter locomotor activity in 6 day old larval zebrafish (see Figure A.1 in Appendix A). The range of drug concentrations was kept consistent for all experiments because for most compounds little to no information was available on effective doses or internal concentrations in larval zebrafish.

2.3. Time of Peak Effect Experiments

The time of peak effect was first determined for each drug. Larvae were changed into fresh 10% Hanks' on the morning of day 6, and placed in a light-tight drawer in a darkened testing room (kept at 26°C). That afternoon, larvae were placed into 250 μl of the broad range of concentrations of one of the drugs (evenly distributed across the 96-well plate to avoid variability due to location). The plate was then placed onto the recording platform (described in detail in MacPhail et al., 2009) in the dark. After allowing 20 minutes of rest, activity was recorded for 26 minutes in alternating dark and light (6 min dark, 10 min light, 10 min dark) using a Noldus behavior system (Leesburg, VA). The level of light was 0.51 lux. The microtiter plate remained on the recording platform, and recording was repeated every hr for the next 4 hrs. One plate was used for each drug (n=12 larvae/dose, 12–24 larvae for vehicle controls). The time of peak effect was defined as the time after administration when maximal effects were seen (via visual inspection) at the most doses.

2.4. Dose-Response Experiments

Next, a dose-response profile for locomotor activity was conducted at the time of peak effect for each drug. On the morning of the test, 6-dpf larvae were changed into fresh 10% Hanks' solution, and placed in the light-tight drawer in the darkened testing room. The larvae were later placed into 250 μl of the broad range of drug concentrations (evenly distributed on the 96-well plate), and returned to the light-tight drawer until the time of peak effect was reached. Larvae were then placed onto the recording apparatus in the dark 10 min prior to recording. Activity was recorded for a total of 70 minutes using the following lighting conditions: 10 minutes of dark (for adaptation), 10 minutes of light, 20 minutes of dark, 10 minutes of light and 20 minutes of dark. Dose-response experiments were conducted on two separate plates for each drug (n=24 larvae/dose, 24–48 larvae for vehicle controls).

2.5. Analysis of Locomotor Activity

Locomotor activity was analyzed as previously described (Irons et al, 2010, MacPhail et al., 2009). Briefly, videos of larval behavior were tracked at a rate of 5 samples/sec using Ethovision software Version 3.1 [Noldus Information Technology, Leesburg, VA (www.noldus.com)], with a tracking rate of 5 image samples/sec. Locomotor activity was calculated as distance moved (cm) per 2 min. An input filter of 0.135 cm/200 msec was used to exclude any slight movement that was not considered swimming. Dead or grossly malformed larvae were not used in the analysis.

2.6. Statistics

Activity levels for the two observation periods under each lighting condition were averaged before statistics were calculated (i.e. “dark” = average activity of second and third dark periods per 2 min). All data were collapsed by lighting condition and the mean activity/2 min in each condition was analyzed using Statview© (SAS Institute, Inc., Cary, NC; version 5.0.1). The data for each drug were first assessed using a two-way repeated-measures analysis of variance (ANOVA) with drug treatment (between subject variable) and lighting condition (i.e, light or dark activity; within subject variable) as the independent variables and locomotor activity (distance moved; within subject variable) as the dependent variable. The data under each lighting condition were also assessed using separate one-way ANOVAs, with drug treatment as the independent (between subject) variable and locomotor activity (distance moved) as the dependent (within subject) variable. Significance was set at $p \leq 0.05$. When a treatment effect was obtained, Fisher’s PLSD test was applied post hoc to compare dose groups. Significance was set at $p \leq 0.05$. All data are presented as mean ± standard error of the mean (SEM). Only effects that reached significance are described in Results. The details of each drugs’s statistical analysis are provided in the corresponding figure legend.

3. Results

3.1. Time of Peak Effect Experiments

The time when the maximal effects (in any direction) were reached at the largest number of doses was first determined for all six drugs. As an example, Figure 1 shows the time of peak effect data for the D2-like receptor antagonist haloperidol. Larvae that were given 0.2 and 0.6 μM haloperidol did not display altered locomotion at any time point. Larvae exposed to 1.8 μM haloperidol appeared to be hyperactive in the dark only at 200 min post-administration. Also at 200 min, 5.5 μM caused larvae to maintain a constant level of activity throughout the entire test regardless of lighting condition. Larvae given 16.7 μM were hypoactive in both light and dark beginning at 140 min. Lastly, haloperidol markedly affected locomotion at the highest dose (50 μM) starting at 80 min post-administration, where larvae were hypoactive in both light and dark. The effects at 5.5, 16.7, and 50 μM persisted until the final time point of 260 min post-dosing. Based on these observations, 200 min was chosen as the time of peak effect for haloperidol, since this was when the greatest number of doses (4 out of the possible 6) produced maximal effects.

Applying the same approach for the other drugs, peak effects were reached at 20 min for apomorphine, 140 min for SKF-38393, 80 min for quinpirole, 20 min for butaclamol, and 260 min for SCH-23390. These observations are summarized in Table 1 (see Appendix B for figures for apomorphine, SKF-38393, quinpirole, butaclamol, and SCH-23390).

3.2. Dose-Response Experiments

3.2.1. Apomorphine (nonselective agonist)—When larval locomotion was recorded 20 min after apomorphine administration, biphasic changes in locomotor activity were observed (Figure 2). In the dark, hyperactivity was produced in larvae that were given the lowest and highest concentrations (0.2 and 50 μM; 67% and 89% increases, respectively), while intermediate concentrations did not significantly alter locomotion (Figure 2A and 2C). In the light, no changes in activity reached statistical significance compared to controls (Figure 2B and 2C).

3.2.2. SKF-38393 (D1-like selective agonist)—When larval locomotion was recorded 140 min after SKF-38393 administration, increased activity was observed under certain conditions (Figure 3). In the dark (Fig. 3A and 3C), hyperactivity was noted at only at the

high dose, 50 μM (95%). In the light (Fig. 3B and 3C), the activity of larvae was not significantly altered by SKF-38393 administration.

3.2.3. Quinpirole (D2-like selective agonist)—When larval locomotion was recorded 80 min after quinpirole administration, increased activity was observed at the intermediate doses. As seen in Figure 4, in the dark (Fig. 4A and 4C), this drug increased larval locomotion only at 16.7 μM (by 80%). Increases in activity were also seen in the light (Fig. 4B and 4C) at 5.5 (198%) and 16.7 μM (288%). In both the light and the dark, activity was at control levels at the highest dose (50 μM).

3.2.4 Butaclamol (nonselective antagonist)—When larval locomotion was recorded 20 min after butaclamol was administered, larvae displayed biphasic changes in locomotor activity (Fig. 5). Intermediate concentrations caused hyperactivity: 0.6 μM increased activity by 168% in the dark (Fig. 5A and 5C), and in the light, 0.6 μM increased activity (220%), and 1.8 μM increased activity (96%) (Fig. 5B and 5C). On the other hand, the higher concentrations caused marked hypoactivity: 16.7 μM butaclamol decreased activity (99%) in dark and in light (98%), and 50 μM completely abolished activity in both dark and light (Fig. 5A, 5B, and 5C).

3.2.5. SCH-23390 (D1-like selective antagonist)—When larval locomotion was recorded 260 min after SCH-23390 administration, activity was altered in a different ways in the two lighting conditions (Fig. 6). In the dark (Fig. 6A and 6C), decreased activity was seen at 5.5 (41%) and 50 μM (37%). In the light (Fig. 6B and 6C), however, a seemingly biphasic pattern emerged as activity decreased at a low concentration of 0.6 μM (71%), but increased at the highest concentration, 50 μM (262%). Closer inspection of drug effects throughout the entire session revealed that larvae given 50 μM SCH-23390 were equally active in the dark and light, and did not appear to respond to changes in lighting condition (i.e. the transition from dark to light did not decrease locomotor activity, nor did the transition from light to dark increase activity).

3.2.6. Haloperidol (D2-like selective antagonist)—When larval locomotion was recorded 200 min after haloperidol administration, differing patterns of effects were observed in the two lighting conditions (Fig. 7). In the dark, haloperidol markedly decreased activity at 16.7 μM (81%), and completely abolished activity at 50 μM (Fig. 7A and 7C). In the light, however, biphasic effects occurred, as 5.5 μM haloperidol increased activity (205%), while the highest concentration (50 μM) abolished activity (Fig. 7B and 7C). The mean activity data suggest that changes in the lighting conditions did not affect the levels of activity of larvae given 5.5 or 16.7 μM haloperidol (compare Fig. 7A and 7B), and visual inspection of the data throughout the test session revealed that they had blunted responses to changes in lighting conditions (Fig. 7C), similar to the effect seen with 50 μM SCH-23390 (D1-like antagonist; Fig. 6). At 5.5 μM, larval locomotion throughout the test was comparable to the control levels obtained in dark periods, while 16.7 μM decreased activity to a low level (about the level of activity during light periods) throughout the test.

In summary, all drugs selected for these studies affected larval locomotion over a broad dose range. Additionally, the dopaminergic agonists and antagonists have distinct patterns of effect. All drug effects have been summarized in Table 2 and compared to findings in mammals.

4. Discussion

The present study assessed dose-dependent changes in larval zebrafish locomotor activity following acute exposure to various drugs that target dopaminergic receptors in mammals

(summarized in Table 2). This assay was able to distinguish the actions of receptor agonists from those of antagonists. Behavioral profiles differed for each dopamine receptor drug in terms of alterations in activity and, in some cases, response to changes in lighting conditions, regardless of whether the drug was non-selective or preferentially acted on a specific receptor subtype in mammals.

Previous reports have established that zebrafish larvae display behavior changes following exposure to drugs that are known to act on dopamine receptors in mammals. In our laboratory, we reported that the acute administration of neuroactive drugs that indirectly alter dopaminergic signaling (i.e. ethanol, *d*-amphetamine, or cocaine) caused similar effects on locomotor activity in zebrafish larvae as in mammals (MacPhail et al., 2009, Irons et al., 2010). Also, Boehmler and colleagues (2007) reported that acute quinpirole administration increased locomotor activity only at an intermediate dose, while Giacomini and colleagues (2006) reported decreased locomotor activity after haloperidol exposure. Although most of the previous findings in zebrafish have been consistent with what has been reported in mammals, broad dose ranges were not usually explored, so it is possible that undiscovered differences in the behavioral profiles may exist across species. Additionally, in a study by Rihel et al. (2010), the authors suggested that species differences may exist in the roles dopaminergic receptors play in the control of behavior or in target selectivity of dopamine receptor drugs. Thus, the present study sheds more light on the commonalities and differences in the behavioral profiles of these drugs across species.

Previous studies of acute drug effects in zebrafish did not report whether the testing was conducted at the time of peak effect, nor did they compare the time to reach peak effect among different dopaminergic drugs. Indeed, in the present work, it appears that it is possible to cluster the time to reach peak effect by drug class. For example, the nonselective compounds (apomorphine and butaclamol), which act on both D1- and D2-like receptors, were fast-acting, producing their maximal effects within 20 min of administration. Also, the selective agonists were faster acting than selective antagonists, and the D2-like drugs reached maximal effect faster than the drugs that act preferentially on D1 receptors. Quinpirole (a D2-like selective agonist) and SKF-38393 (a D1-like selective agonist) reached maximal effect at 80 and 140 min, respectively. Likewise, haloperidol (a D2-like selective antagonist) reached its peak effect at 200 min post-administration, while SCH-23390 (a D1-like selective antagonist) administration took 260 min to reach maximum effect. At this time it is not clear whether these differences in time to peak effect represent pharmacodynamic differences or differences in drug uptake into brain.

In general, the selective drugs used in the present study produced effects in larval zebrafish that were consistent with predicted outcomes based on mammalian studies (Table 2). Dopaminergic receptor-selective agonists increased larval locomotion in a dose-dependent manner. Likewise, both SKF-38393 (a D1-like agonist) and quinpirole (a D2-like agonist) have been reported to increase locomotor activity in rodents (Beninger et al., 1991, Sobrian et al., 2003). SCH-23390 and haloperidol, which decreased activity in the present study, have also been shown to decrease activity in rodents (Morato et al., 1989, Beninger et al., 1991, Choi et al., 2009), and haloperidol has been reported to cause a sedative effect in humans (King et al., 1995). Further, the nonselective and D2-like receptor antagonists tested in the present study abolished larval locomotion at high doses, which is consistent with reports of increased catatonia observed in rotarod tests in rats (Morato et al., 1989, Melo et al., 2010). These findings also suggest the induction of similar actions in mammals and zebrafish caused by dopamine receptor drugs.

In some cases in the present study, as well as in the mammalian literature, biphasic dose-response patterns occurred following acute administration of dopaminergic drugs. The

biphasic dose-response profiles of apomorphine and butaclamol might be attributed to the drugs acting on multiple dopaminergic receptors. Interestingly, studies in mammals have established an interdependency between D1-like and D2-like receptor stimulation, such that D1 stimulation is needed to achieve D2-induced behavioral responses, while those following D1 stimulation occur independently of D2 stimulation (Braun and Chase, 1986, Murray and Waddington, 1989). Another factor to consider regarding biphasic patterns is that D3 receptors appear to have opposite effects on locomotion compared to D2 and D4 receptors. That is, the stimulation of D3 receptors has been shown to decrease locomotor activity, while D3 receptor blockade increased activity in mammals (Missale et al., 1998, Kolasiewicz et al., 2008). Additionally, the presence of pre-synaptic D2 receptors may contribute to the biphasic profiles. The stimulation of presynaptic D2 receptors blocks the release of dopamine, thereby inhibiting behavioral activation (Van der Weide et al., 1988), while the stimulation of post-synaptic D2 receptors increases locomotion (Beninger et al., 1991, Missale et al., 1998). Further research is clearly needed to pharmacologically characterize dopaminergic receptors in the zebrafish nervous system.

Quinpirole has a dose-dependent biphasic profile in mice, with decreased activity at low doses and increased activity at high doses (Li et al., 2010). A similar biphasic profile has been noted in adult, but not developing rats (Van Hartesveldt et al., 1994), although this response was time-dependent: activity is decreased early in the test session and increased later. In the present study, quinpirole increased activity only at intermediate doses in larval zebrafish, as larvae given low and high doses displayed control levels of activity. This finding is consistent with what was previously reported in larval zebrafish by Boehmler and colleagues (2007). It is possible that decreases in activity might emerge in zebrafish at lower doses of quinpirole.

The biphasic pattern of activity that resulted from acute butaclamol administration in zebrafish is also different than what is seen in mammals. In mammals, only decreased activity has been reported (Beninger et al., 1991, Bergman et al., 1991, Voith and Herr, 1975). One possible reason for this difference could be that mammalian studies of butaclamol tend to profile dose ranges that exceed doses that are low enough to cause hyperactive behavioral responses.

In addition to effects on the level of locomotor activity, the selective dopaminergic antagonists affected the response of larvae to changes in lighting conditions. The paradigm in the current study included cycles of dark and light that evoke distinct behavior patterns in naïve larvae (i.e., a decrease in activity when transitioned from dark to light, and an increase in activity upon return to dark; see Burgess and Granato, 2007, MacPhail et al., 2009, Emran et al., 2010). In the cases of SCH-23390 and haloperidol in the present study, these patterns were disrupted at intermediate and high doses, resulting in constant levels of activity throughout the test. One possible explanation for these drug-induced alterations is that these behavior patterns are mediated via the retina. The structure and function of the retina is highly conserved in all vertebrate species, including zebrafish (Joselevitch and Kamermans, 2009). Dopamine is the major catecholaminergic neurotransmitter in the vertebrate retina, and dopaminergic receptors are found throughout the structure (reviewed in Nguyen-Legros et al., 1999). In retinal ganglion cells, dopamine functions mainly as a modulator of light adaptation, decreasing the firing of ON retinal ganglion cells, while increasing OFF retinal ganglion cell firing (Ames and Pollen, 1969). Blockade of either D1- or D2-like receptors in retinal ganglion cells could perturb ON and/or OFF cell function and possibly disrupt the locomotor response to the light/dark transitions in the present test. In line with the current findings, acute haloperidol administration has been reported to disrupt visual processing in rats (Dyer et al., 1981), rabbits (Jensen and Daw, 1983) and humans (Lynch et al., 1997). Alternatively, a recent study by Fernandes and colleagues (2012) found that zebrafish larvae

lacking eyes and a pineal gland still exhibited heightened activity following a transition from light to dark. Their results indicated deep brain photoreception in the preoptic region of the hypothalamus ("nonvisual" structures) was likely involved in this behavior pattern. Additional research is clearly needed to determine the relationship between drug effects on the function of the retina, and other regions of the nervous system, and behavioral responses to altered lighting conditions.

While the current findings generally resemble mammalian reports of altered locomotor activity due to drug-induced dopaminergic alterations, additional studies are needed to rule out effects on other signaling pathways. Even though these drugs are known to have higher affinities for their dopaminergic targets than other receptors, none can be considered exclusive dopaminergic receptor ligands. Indeed, studies have shown that these drugs may also bind to adrenergic, cholinergic, histaminergic, and serotonergic receptors, albeit with lower affinities (Hyttel, 1983, Bymaster et al., 1996, Millan et al., 2002). The nominal concentrations (μM range) reported in the present study are higher (in most cases) than the associated inhibitory constants (K_i, reported in nM range) for these off-target receptors in mammals. The relative affinities for these receptors in zebrafish are not known at this time, and need to be determined. Measurements of internal concentrations in the larvae are also needed to remove uncertainties regarding how much drug has crossed the biological membranes and barriers (i.e. skin, bloodbrain barrier, etc.) to gain access to the brain and its receptors.

5. Conclusions

The findings in this study provide behavioral evidence that the dopaminergic nervous system of larval zebrafish may function similarly to that of mammals. In general, the administration of both clinical and experimental drugs that target dopaminergic receptors in humans and other mammals elicits similar effects on locomotor activity in this model. Therefore, this study supports and extends recent efforts to establish zebrafish as a useful model in predicting behavioral effects of acute mammalian exposures to drugs/chemicals suspected to target the nervous system.

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Appendix A – Effects of DMSO on Larval Locomotion

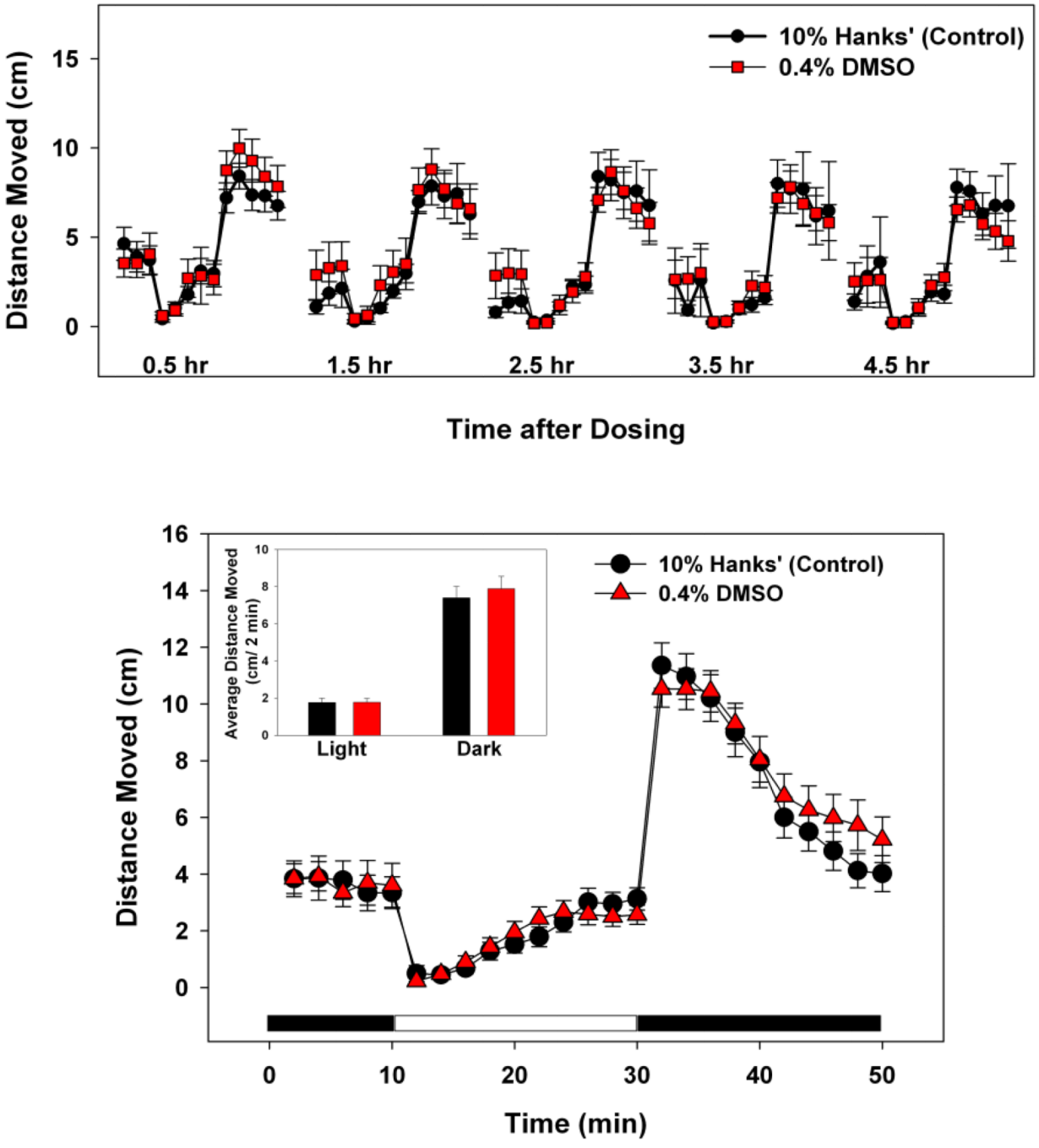

Figure A.1.

DMSO vehicle (0.4%) does not alter larval zebrafish locomotor activity. Upper Panel: The time of peak effect experiments were conducted in the same manner as described in the Methods. On day 6, control zebrafish larvae were administered DMSO such that the final concentration in the well was 0% or 0.4% DMSO in 10% Hanks' buffer. All treatment conditions were on the same plate. The activity of the larvae was then followed over time using an abbreviated dark:light:dark cycle every hour starting 20 minutes after DMSO administration. The presence of 0.4% DMSO did not alter the activity from control levels at

any time after dosing. Lower Panel: A more detailed comparison of locomotor activity was carried out to evaluate the effects of DMSO. Larvae were dosed with DMSO or Hanks' 20 minutes before testing began (n=46 for control and n=45 for 0.4% DMSO). All treatment conditions were on the same plate. An analysis of the activity in light and in the second dark period (inset, lower panel) showed no effect of DMSO. Two-way repeated-measures ANOVA yielded no effect of DMSO treatment ($F_{1,89} = 0.26$, $p = 0.6138$), a significant effect of lighting condition ($F_{1,89} = 156.9$, $p < 0.0001$), and no interaction between DMSO treatment×lighting condition ($F_{1,89} = 0.26$, $p = 0.6140$).

Appendix B – Time of Peak Effect Figures

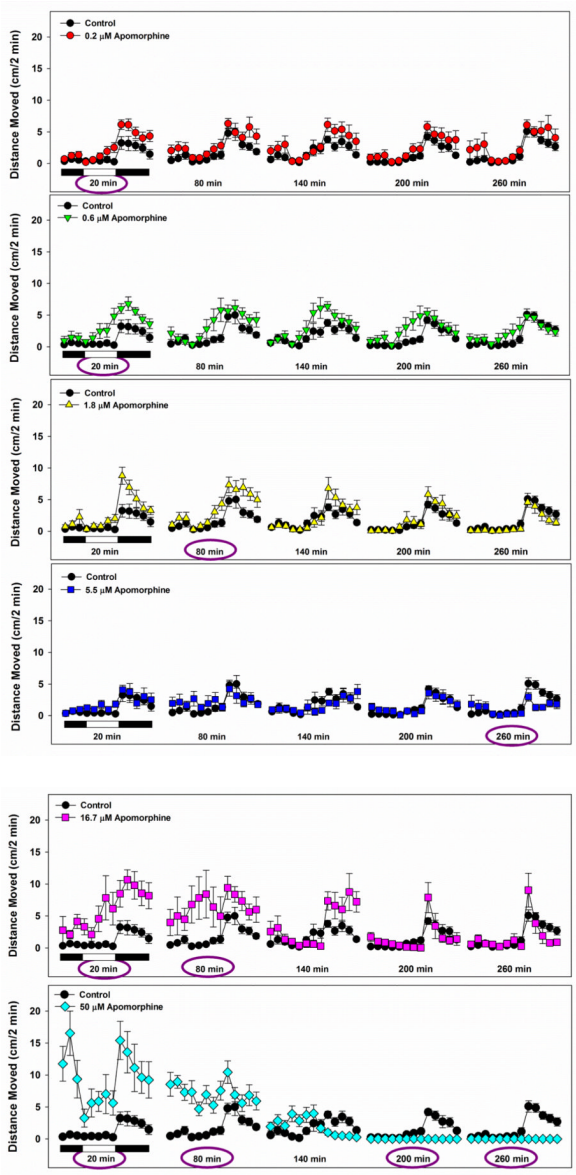

Figure B.1.
Apomorphine time of peak effect. The time of peak effect for apomorphine was determined by identifying the time point at which the most doses produced maximal effects. Data are presented as mean ± SEM. The controls are the same in each panel, and all dose groups were

present on the same experimental plate (n = 12/dose). The black and white bar indicates periods of dark and light, respectively and applies to each recording time point (shown at 20 min for example) in each panel. Time points circled in purple denote maximal effects considered in the determination. Because the greatest number of maximum effects was reached at 20 min and above, 20 min was determined to be the time of peak effect for apomorphine.

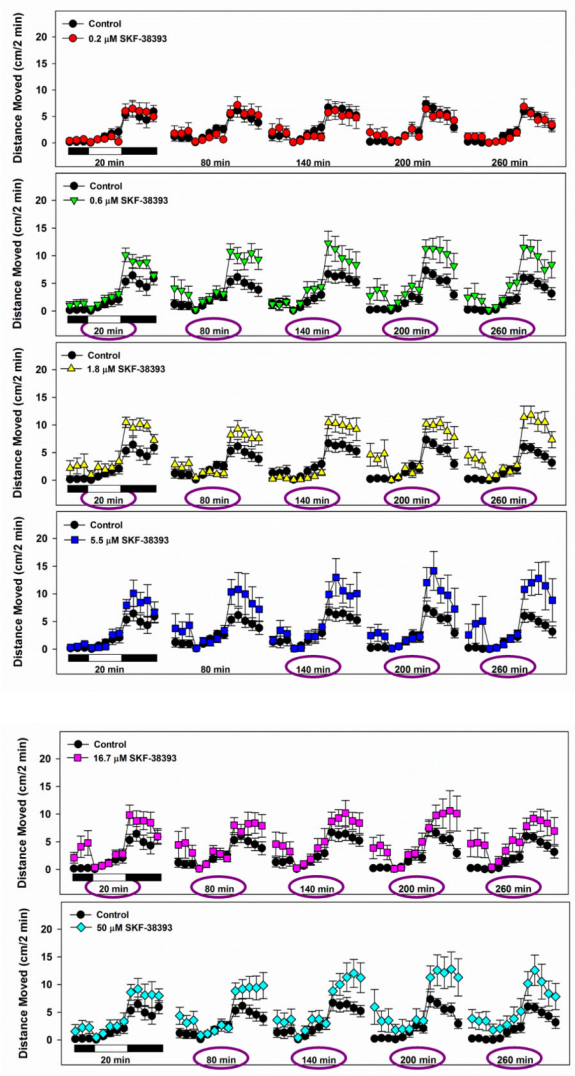

Figure B.2.

SKF-38393 time of peak effect. The time of peak effect for SKF-38393 was determined by identifying the time point at which the most doses produced maximal effects. Data are presented as mean ± SEM. The controls are the same in each panel, and all dose groups were present on the same experimental plate (n = 12/dose). The black and white bar indicates periods of dark and light, respectively and applies to each recording time point (shown at 20 min for example) in each panel. Time points circled in purple denote maximal effects considered in the determination. Because the greatest number of maximum effects was reached at 140 min and above, 140 min was determined to be the time of peak effect for SKF-38393.

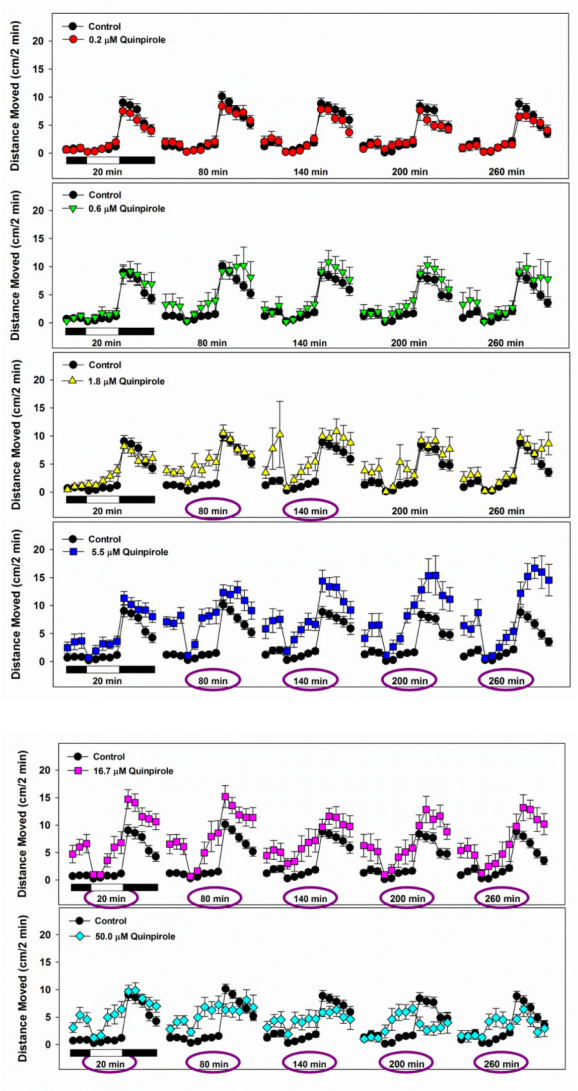

Figure B.3.

Quinpirole time of peak effect. The time of peak effect for quinpirole was determined by identifying the time point at which the most doses produced maximal effects. Data are presented as mean ± SEM. The controls are the same in each panel, and all dose groups were present on the same experimental plate (n = 12/dose). The black and white bar indicates periods of dark and light, respectively and applies to each recording time point (shown at 20 min for example) in each panel. Time points circled in purple denote maximal effects considered in the determination. Because the greatest number of maximum effects was reached at 80 min and above, 80 min was determined to be the time of peak effect for quinpirole.

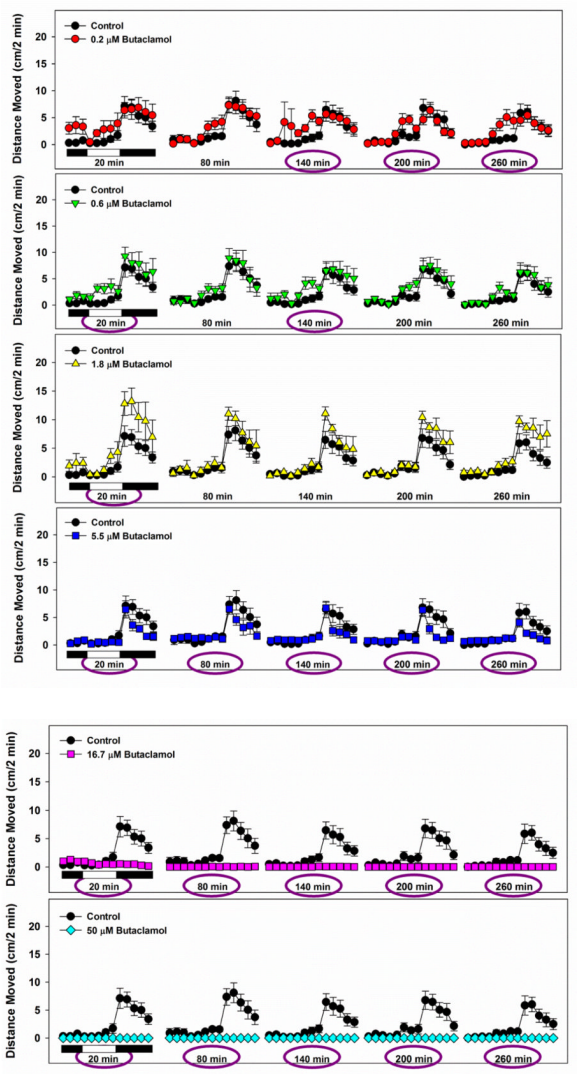

Figure B.4.

Butaclamol time of peak effect. The time of peak effect for butaclamol was determined by identifying the time point at which the most doses produced maximal effects. Data are presented as mean ± SEM. The controls are the same in each panel, and all dose groups were present on the same experimental plate (n = 12/dose). The black and white bar indicates periods of dark and light, respectively and applies to each recording time point (shown at 20 min for example) in each panel. Time points circled in purple denote maximal effects considered in the determination. Because the greatest number of maximum effects was reached at 20 min and above, 20 min was determined to be the time of peak effect for butaclamol.

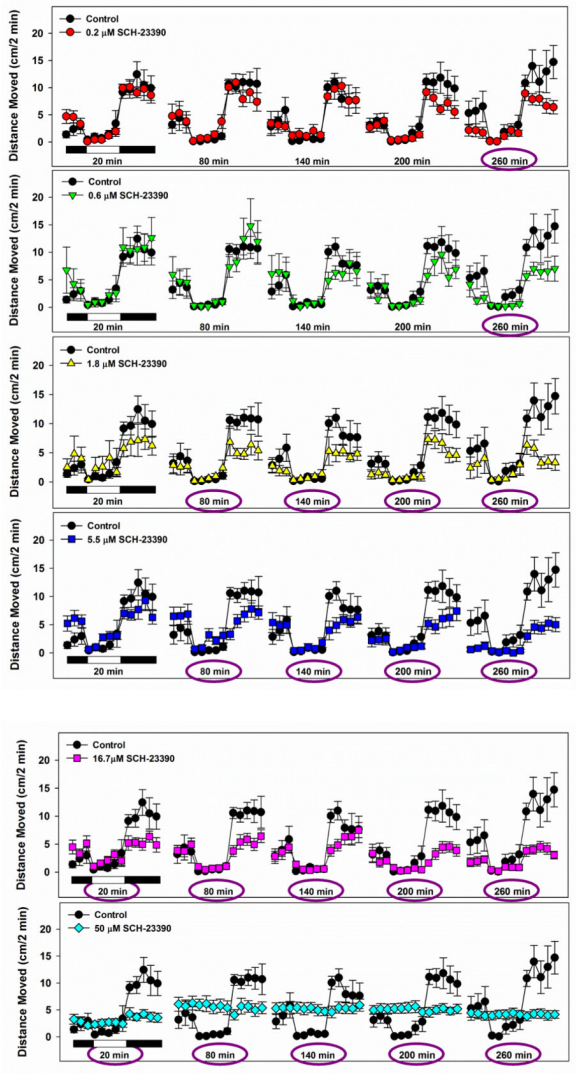

Figure B.5.

SCH-23390 time of peak effect. The time of peak effect for SCH-23390 was determined by identifying the time point at which the most doses produced maximal effects. Data are presented as mean ± SEM. The controls are the same in each panel, and all dose groups were present on the same experimental plate (n = 12/dose). The black and white bar indicates periods of dark and light, respectively and applies to each recording time point (shown at 20 min for example) in each panel. Time points circled in purple denote maximal effects considered in the determination. Because the greatest number of maximum effects was reached at 260 min and above, 260 min was determined to be the time of peak effect for SCH-23390.

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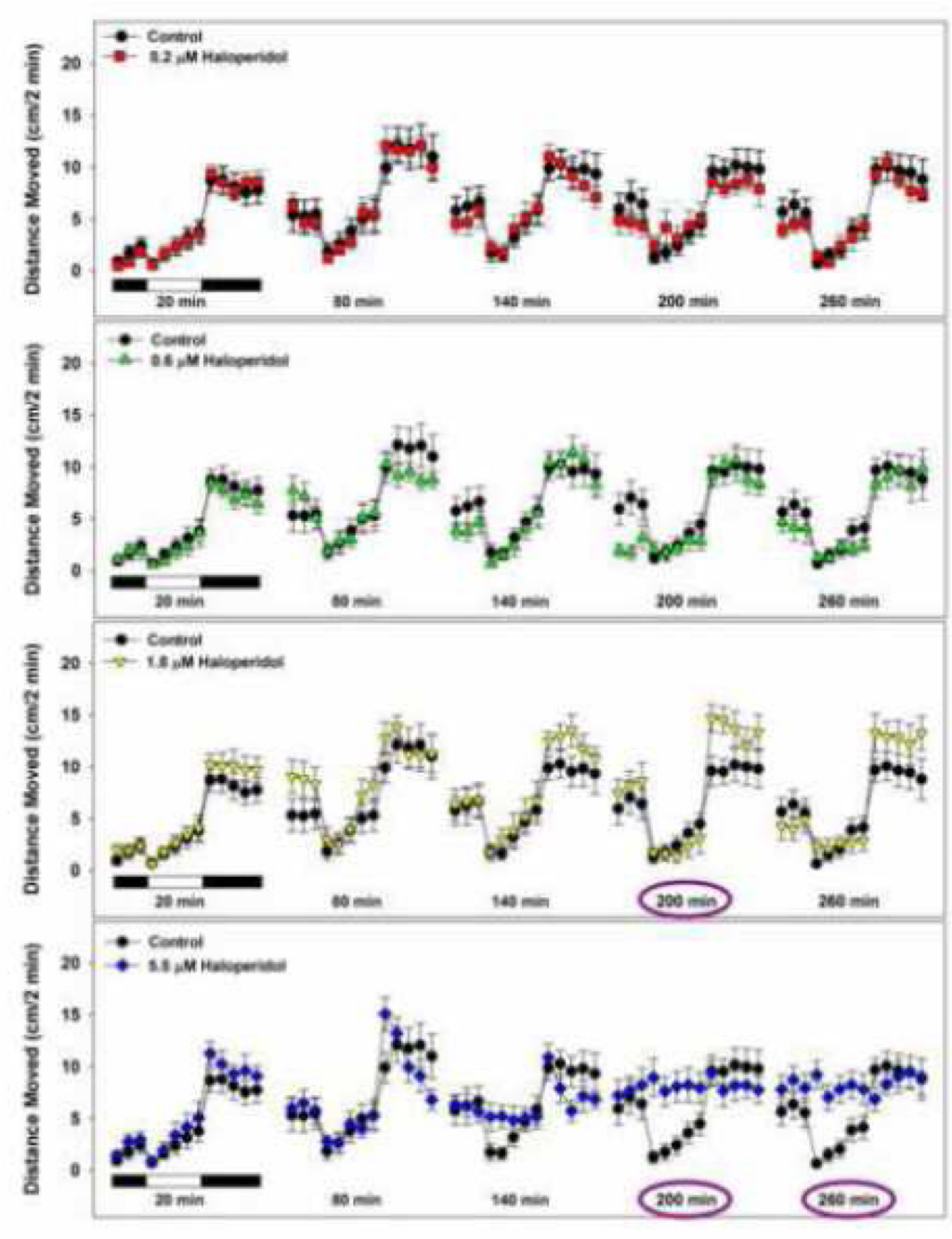
Highlights

- Larval locomotion was altered by dopamine receptor agonists and antagonists.
- Receptor agonists and antagonists generally had opposite effects.
- Larval zebrafish are promising models for dopamine behavioral pharmacology.



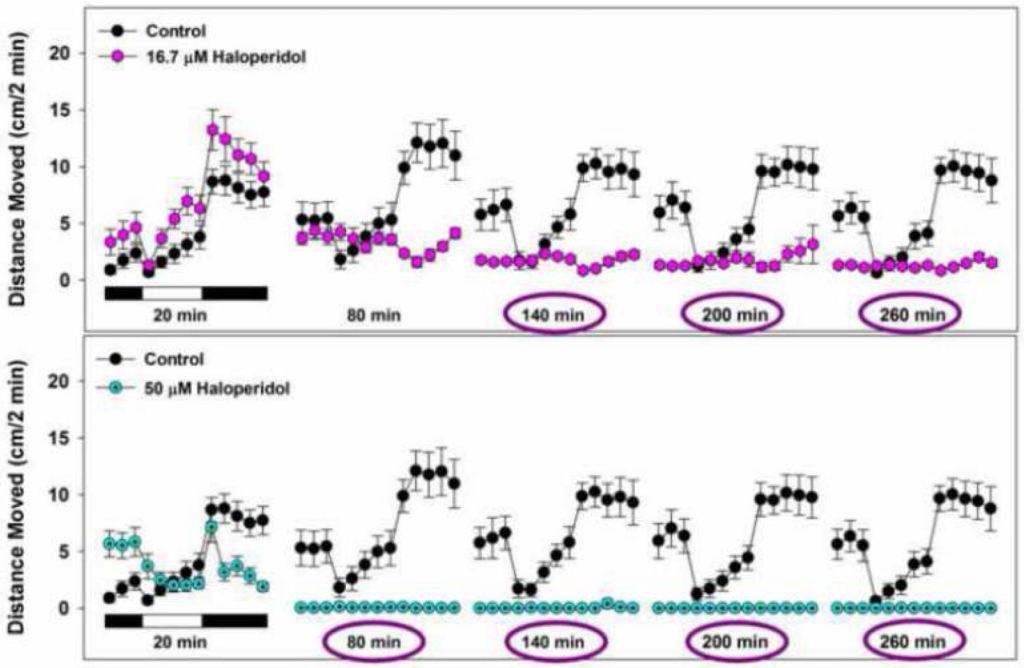


Figure 1.

Representative time of peak effect determination (Haloperidol). The time of peak effect for haloperidol was determined by identifying the time point at which the most doses produced maximal effects. Data are presented as mean ± SEM. The controls are the same in each panel, and all dose groups were present on the same experimental plate (n = 12/dose). The black and white bars indicate periods of dark and light, respectively, and apply to each recording time point (shown at 20 min for example) in each panel. Time points circled in purple denote maximal effects considered in the determination. The greatest number of maximum effects was reached at 200 min, which was determined to be the time of peak effect for haloperidol.

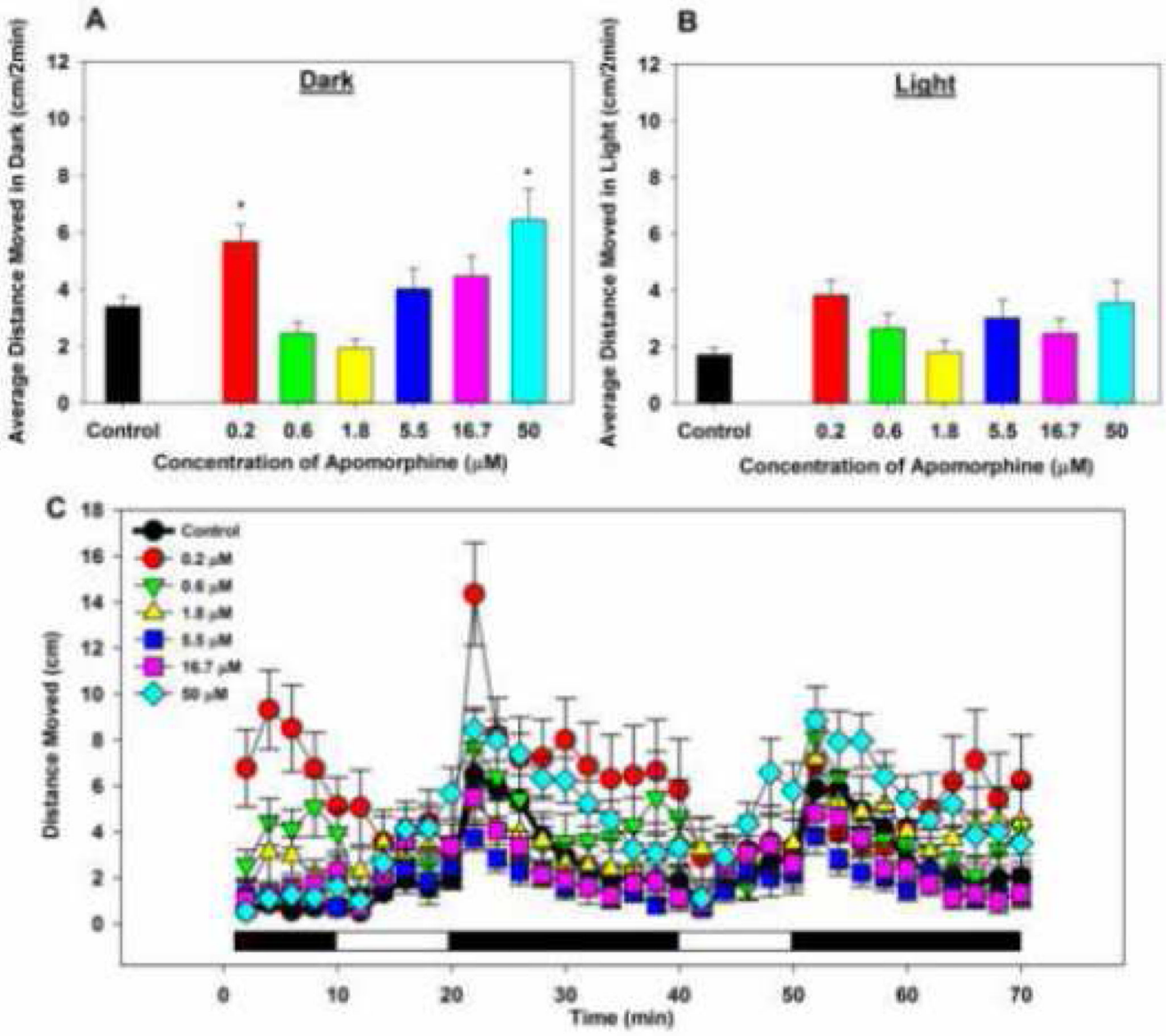


Figure 2.

Behavioral effects of apomorphine. Apomorphine administration had biphasic effects on locomotion in dark, but not in light. (A) In the dark, apomorphine increased activity at the lowest and highest doses (0.2 and 50 μM). (B) In the light, locomotor activity was not significantly altered by apomorphine administration. Data in (A) and (B) are presented as mean activity in 2 min ± SEM (*$p \leq 0.05$). (C) The changes in activity over time are illustrated. Data in (C) are expressed as mean ± SEM (n = 24/dose). The black and white bars denote periods of dark and light, respectively. Statistical Summary: An initial two-way, repeated-measures ANOVA yielded a significant effect of apomorphine ($F_{1,178} = 3.05$, $p = 0.007$) and lighting condition ($F_{1,178} = 26.97$, $p < 0.0001$) and a significant apomorphine×lighting condition interaction ($F_{6,178} = 2.43$, $p = 0.028$). Step-down one-way ANOVAs revealed a significant effect in dark ($F_{6,178} = 3.50$, $p = 0.003$) but no significant

effect of apomorphine in light ($F_{6,178} = 1.90$, $p = 0.083$). Post-hoc tests indicated that dark activity was increased at 0.2 μM ($p = 0.005$) and 50.0 μM ($p = 0.036$).

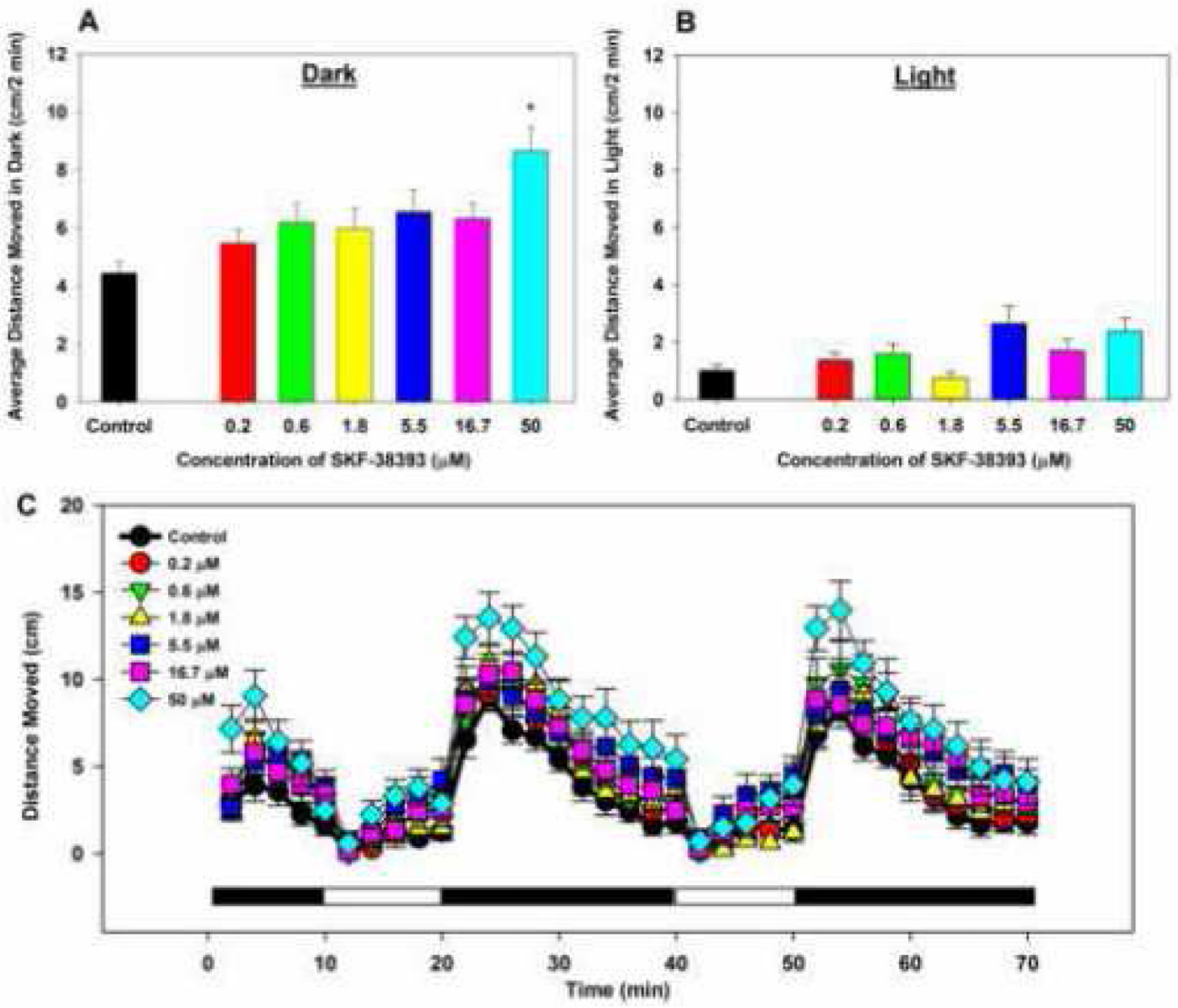


Figure 3.

Behavioral effects of SKF-38393. SKF-38393 administration increased larval locomotion in dark only. (A) In the dark, SKF-38393 increased activity only at 50 μM. (B) In the light, no effects were noted. Data in (A) and (B) are presented as mean activity in 2 min ± SEM (*$p \leq 0.05$). (C) The changes in activity over time are illustrated. Data in (C) are expressed as mean ± SEM (n = 24/dose). The black and white bars denote periods of dark and light, respectively. Statistical Summary: An initial two-way, repeated-measures ANOVA yielded a significant effect of SKF-38393 ($F_{6,125} = 2.43$, $p = 0.030$) and lighting condition ($F_{1,125} = 201.91$, $p < 0.0001$), but no SKF-38393×lighting condition interaction occurred ($F_{6,125} = 1.23$, $p = 0.30$). Step-down one-way ANOVAs revealed a significant effect in dark ($F_{6,125} = 2.22$, $p = 0.044$), but no significant effect of SKF-38393 in light ($F_{6,125} = 1.52$, $p = 0.177$). Post-hoc tests indicated that dark activity was increased at 50 μM only ($p = 0.0006$).

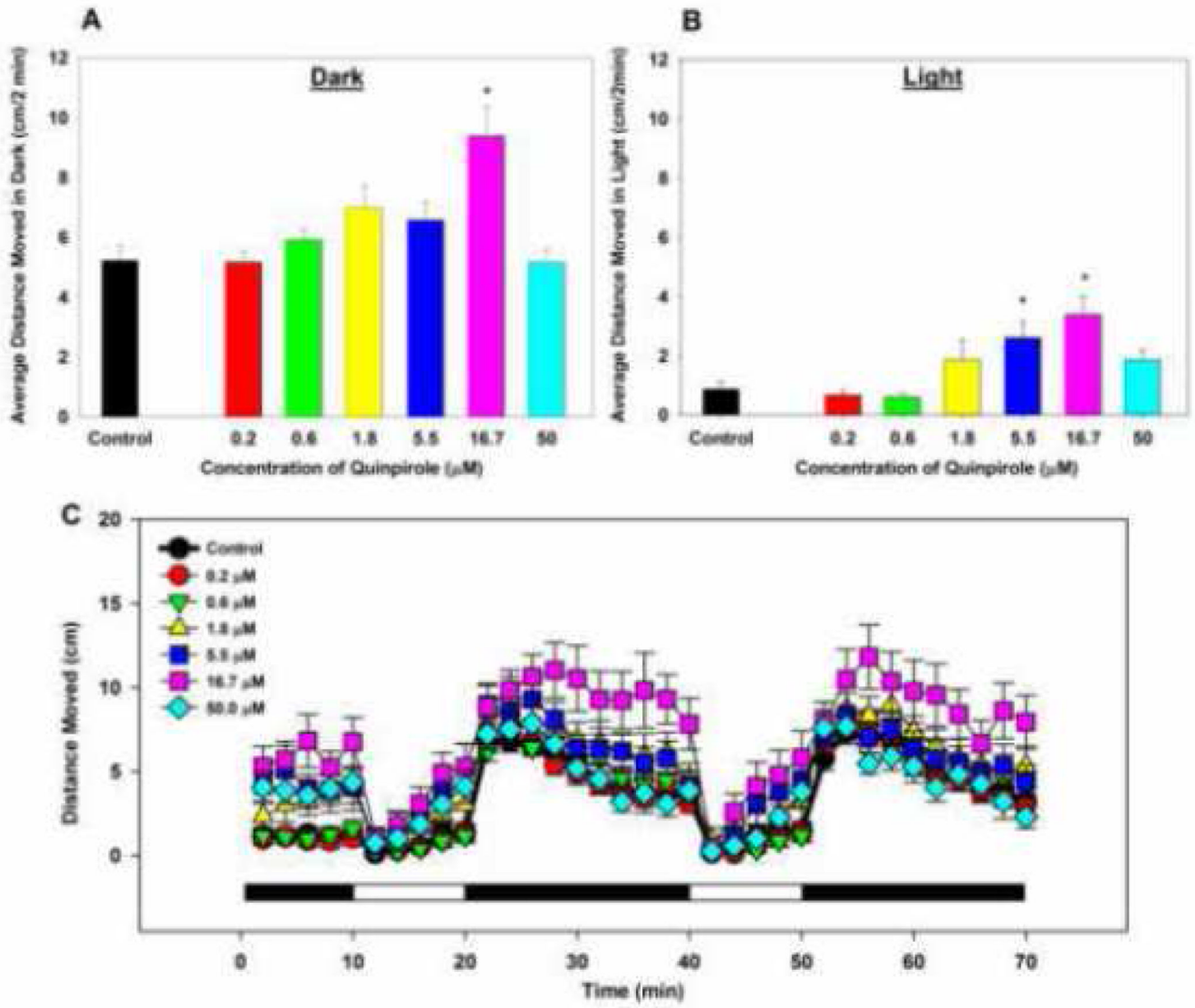


Figure 4.

Behavioral effects of quinpirole. Quinpirole administration increased larval locomotion in light and dark. (A) In the dark, quinpirole increased activity at 16.7 μM. (B) In the light, increases were seen at 5.5 and 16.7 μM. Under both conditions, activity was not significantly different from control at the highest dose (50 μM). Data in (A) and (B) are presented as mean activity in 2 min ± SEM (*$p \leq 0.05$). (C) The changes in activity over time are illustrated. Data in (C) are expressed as mean ± SEM (n = 24/dose). The black and white bars denote periods of dark and light, respectively. Statistical Summary: An initial two-way, repeated-measures ANOVA yielded a significant effect of quinpirole ($F_{6,166}$ = 3.40, p = 0.003) and lighting condition ($F_{1,166}$ = 338.55, $p < 0.0001$), but no quinpirole×lighting condition interaction occurred ($F_{6,166}$ = 1.717, p = 0.120). Step-down one-way ANOVAs revealed significant effects of quinpirole in both dark ($F_{6,166}$ = 3.09, p = 0.007) and light ($F_{6,166}$ = 3.08, p = 0.007). Post-hoc tests indicated that dark activity was increased at 16.7 μM (p = 0.0002) and light activity was increased at both 5.5 (p = 0.029) and 16.7 μM (p = 0.001).

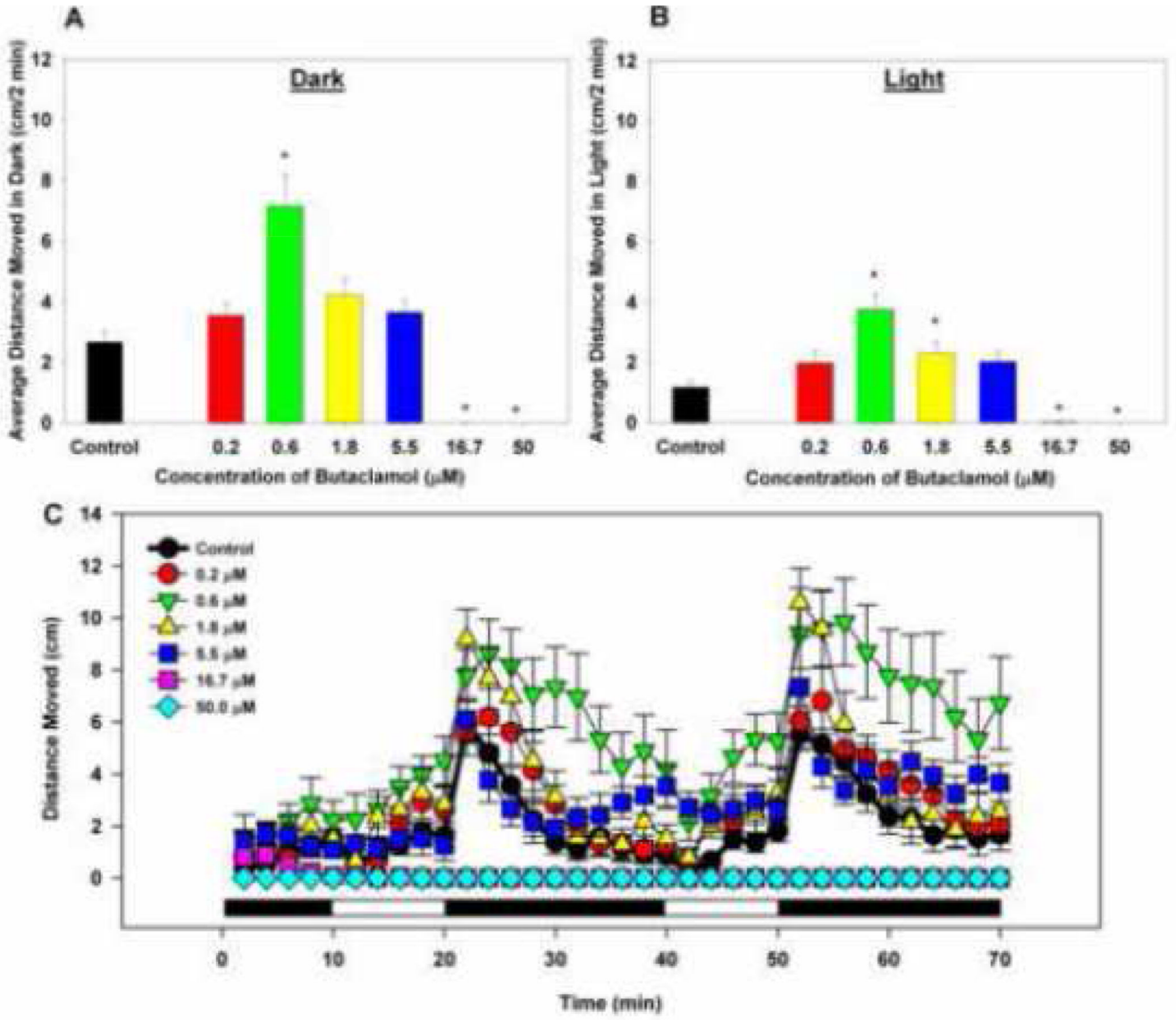


Figure 5.

Behavioral effects of butaclamol. Butaclamol administration had biphasic effects on locomotion in both light and dark. (A) In the dark, butaclamol increased activity at an intermediate dose 0.6 μM, while abolishing activity at the highest doses (16.7 and 50 μM). (B) In the light, activity was increased at both 0.6 and 1.8 μM, and abolished at the highest doses (16.7 and 50 μM). Data in (A) and (B) are presented as mean activity in 2 min ± SEM (*$p \leq 0.05$). (C) The changes in activity over time are illustrated. Data in (C) are expressed as mean ± SEM (n = 24/dose). The black and white bars denote periods of dark and light, respectively. Statistical Summary: An initial two-way, repeatedmeasures ANOVA yielded a significant effect of butaclamol ($F_{6,159} = 16.803$, $p < 0.0001$) and lighting condition ($F_{1,159} = 36.46$, $p < 0.0001$) and a significant butaclamol×lighting condition interaction ($F_{6,159} = 3.55$, $p = 0.0025$). Step-down one-way ANOVAs revealed significant effects of butaclamol in both dark ($F_{6,159} = 12.979$, $p < 0.0001$) and light ($F_{6,159} = 11.84$, $p < 0.0001$). Post-hoc tests indicated that activity in the dark was increased at 0.6 μM ($p < 0.0001$) and decreased

at 16.7 ($p = 0.008$) and 50 μM ($p = 0.007$), and light activity was increased at both 0.6 ($p < 0.0001$) and 1.8 μM ($p = 0.041$) and decreased at 16.7 ($p = 0.039$) and 50 μM ($p = 0.033$).

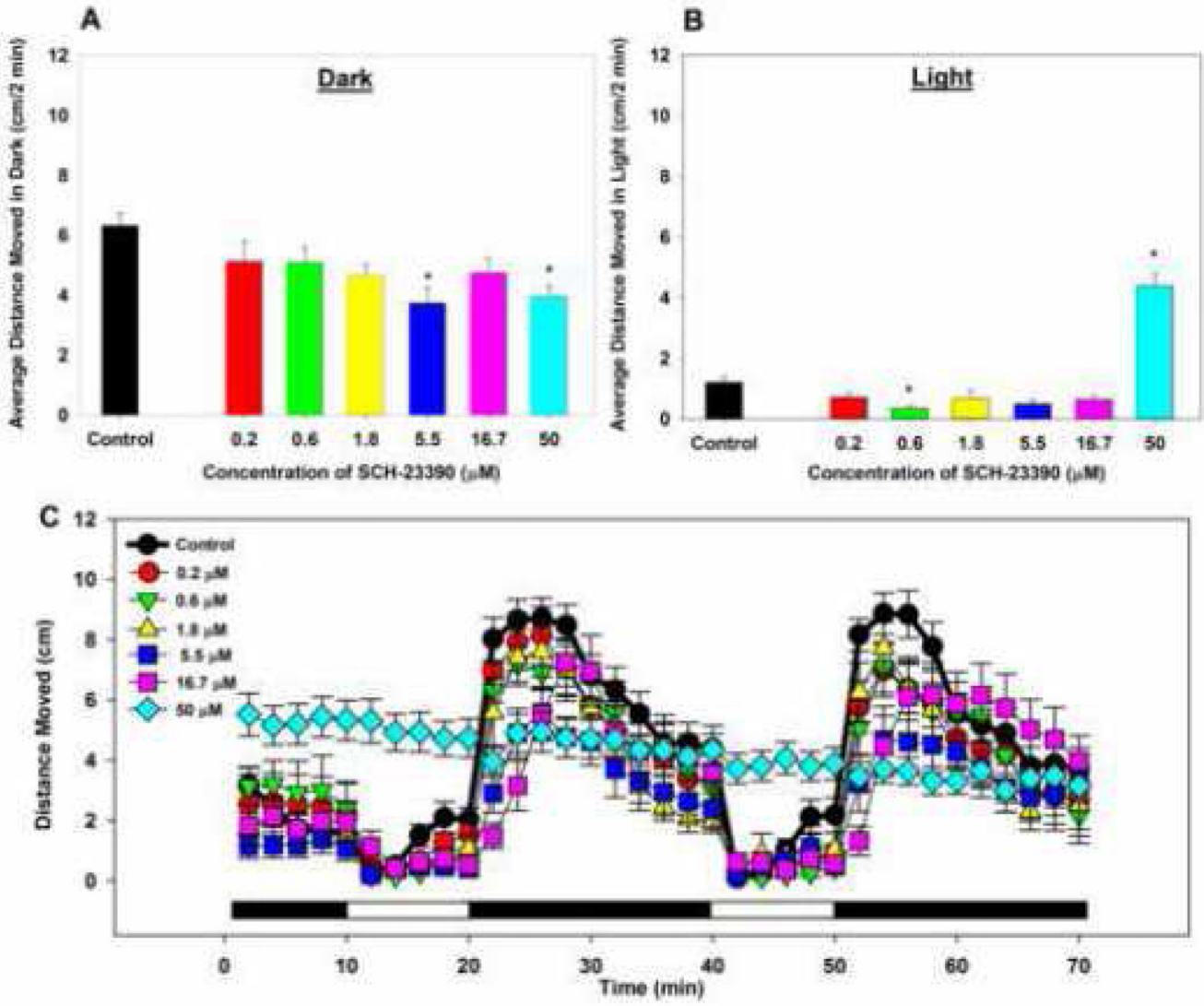


Figure 6.

Behavioral effects of SCH-23390. SCH-23390 administration decreased larval locomotion in the dark and had a biphasic effect on locomotion in the light. (A) In the dark, SCH-23390 decreased activity at 5.5 and 50 μM. (B) In the light, activity decreased at 0.6 μM and increased at 50 μM. Data in (A) and (B) are presented as mean activity in 2 min ± SEM (*$p \leq 0.05$). (C) The changes in activity over time are illustrated. At 50 μM, it appears that larvae no longer respond to changes in lighting conditions, as the activity levels are similar under both conditions. Data in (C) are expressed as mean ± SEM (n = 24/dose). The black and white bars denote periods of dark and light, respectively. Statistical Summary: An initial two-way, repeated-measures ANOVA yielded a significant effect of SCH-23390 ($F_{6,162}$ = 3.328, p = 0.004) and lighting condition ($F_{1,162}$ = 228.97, $p < 0.0001$) and a significant SCH-23390×lighting condition interaction ($F_{6,162}$ = 9.49, $p < 0.0001$). Step-down one-way ANOVAs revealed significant effects of SCH-23390 in both in dark ($F_{6,162}$ = 2.29, p = 0.038) and light ($F_{6,162}$ = 22.489, $p < 0.0001$). Post-hoc tests indicated that in the dark, activity was decreased at both 5.5 μM (p = 0.003) and 50 μM (p = 0.005), and in the light, activity was decreased at 0.6 μM ($p < 0.0001$) and increased at 50 μM ($p < 0.0001$).

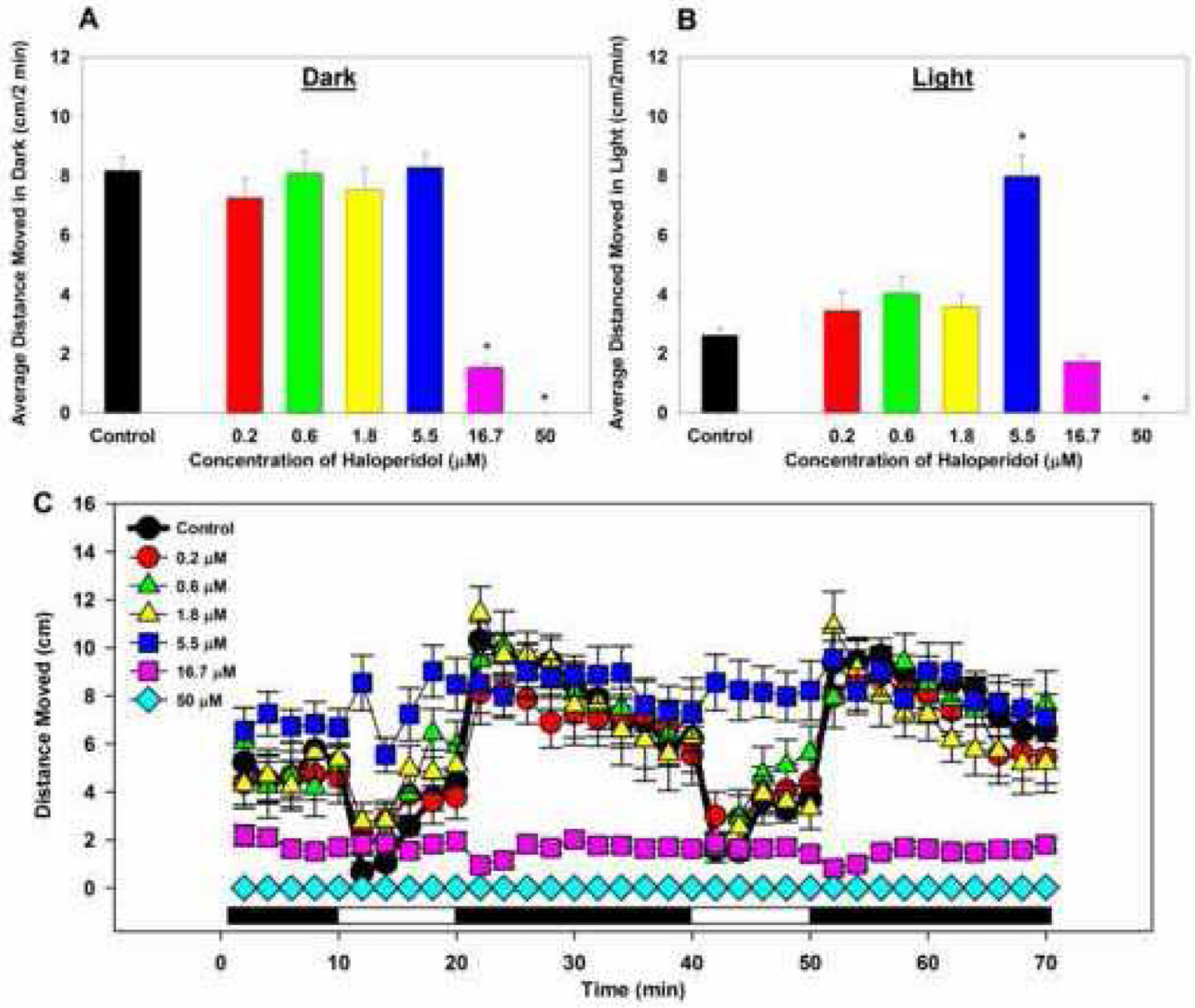


Figure 7.

Behavioral effects of haloperidol. Haloperidol administration affected larval locomotion differently depending on lighting condition. (A) In the dark, haloperidol decreased activity at 16.7 and 50 μM. (B) In the light, however, decreased activity was only seen at 50 μM, while activity was increased at 5.5 μM. Data (A) and (B) are presented as mean activity in 2 min ± SEM (*$p \leq 0.05$). (C) The changes in activity over time are illustrated. As dose increases, the response of larvae to changes in lighting conditions is blunted, accounting for the appearance of hyperactivity in light at 5.5 μM. Data (C) are expressed as mean ± SEM (n = 24/dose). The black and white bars denote periods of dark and light, respectively. Statistical Summary: An initial two-way, repeatedmeasures ANOVA yielded a significant effect of haloperidol ($F_{6,155} = 21.48$, $p < 0.0001$) and lighting condition ($F_{1,155} = 84.35$, $p < 0.0001$) and a significant haloperidol×lighting condition interaction ($F_{6,155} = 12.67$, $p < 0.0001$). Step-down one-way ANOVAs revealed significant effects of haloperidol in both dark ($F_{6,155} = 20.609$, $p < 0.0001$) and light ($F_{6,155} = 16.39$, $p < 0.0001$). Post-hoc tests indicated that activity in the dark was decreased at both 16.7 μM ($p < 0.0001$) and 50 μM (p

< 0.00013), and light activity was increased at 5.5 μM ($p < 0.0001$) and decreased at 50 μM ($p = 0.0006$).

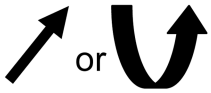
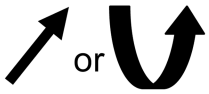
Table 1

Summary of time of peak effect determinations. Data show the number of doses that produced a maximal effect at each time point (total of 6 doses used).

Drug	20 min	80 min	140 min	200 min	260 min	Peak Effect
Apomorphine	4	3	0	1	2	20 min
SKF-38393	3	4	5	5	5	140 min
Quinpirole	2	4	4	3	3	80 min
Butaclamol	5	3	5	4	2	20 min
SCH-23390	2	4	4	4	6	260 min
Haloperidol	0	1	2	4	3	200 min

Table 2

Summary of dose-response profiles. Arrows with positive slopes denote dose-response profiles of increasing activity, while arrows with negative slopes denote dose-response profiles of decreasing activity. U-shaped arrows () represent biphasic dose-responses, where activity either increases at low doses, then again at high doses, or activity decreases at low doses, then increases at high doses (i.e. with apomorphine/quinpirole in mammals). Inverted U-shaped arrows () represent patterns in which activity increases at low/intermediate doses, then decreases at high doses. Angular shapes () represent cases in which activity increased at intermediate doses and then returned to control levels, but did not decrease further.

Drug	Type of Drug	Mammalian Activity	Zebrafish - Activity in Dark	Zebrafish - Activity in Light
Apomorphine	Non-selective Agonist			-
SKF-38393	D1-like Agonist			-
Quinpirole	D2-like Agonist			
Butaclamol	Non-selective Antagonist			
SCH-23390	D1-like Antagonist			
Haloperidol	D2-like Antagonist