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Short communication

MK-801 increases locomotor activity in a context-dependent manner in zebrafish

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HIGHLIGHTS

- MK-801 does not increase locomotor activity during exposure.
- MK-801 increases locomotor activity following pre-treatment.
- MK-801 pre-treatment does not increase whole-brain dopamine or DOPAC levels.

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ABSTRACT

Zebrafish have become a popular animal model for behavioral neuroscience with an increasing number of studies examining the effects of pharmacological compounds targeting the brain. Exposure to MK-801, a non-competitive *N*-methyl-D-aspartate receptor antagonist has been shown to increase locomotor activity in zebrafish. However, others have failed to replicate this finding as several contradicting studies report no changes in locomotor activity following exposure to similar doses. In the current study we reconcile these behavioral reports by demonstrating that zebrafish do not exhibit changes in locomotor activity during exposure to non-sedative doses of MK-801. Interestingly, zebrafish do exhibit significant increases in locomotion if pre-treated with MK-801 followed by subsequent testing in a novel environment, which suggests the effects of MK-801 are context-dependent. In addition, we examine the potential role of the dopaminergic system in mediating MK-801's locomotor stimulant effect by quantifying the levels of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the brains of zebrafish following a 30 min exposure to 10 μM of MK-801 (the dose found to induce the largest increase in locomotor activity). Our findings indicate that the MK-801-induced increase in locomotor activity is not accompanied by changes in whole-brain levels of dopamine or DOPAC. Overall, our results suggest that MK-801's context-dependent locomotor stimulant effect may be independent of whole-brain dopaminergic activation.

Locomotor activity is often quantified by an organism's total distance traveled, which is arguably one of the most common behavioral parameters measured by behavioral neuroscientists. A large number of complex neurochemical systems are thought to be involved in regulating this behavioral response (for a review see [1]), one of which is the glutamatergic neurotransmitter system. Glutamate is the central nervous system's primary excitatory neurotransmitter, and pharmacological manipulation of glutamate receptors has been shown to alter locomotor activity in rodents as well as in zebrafish [2–5]. A prime example in the rodent literature is the dose-dependent increase of locomotor activity following administration of [5R,10S-[+]-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801 or dizocilpine), a non-competitive antagonist of the *N*-methyl-D-aspartate receptor (NMDA-R) [3,4]. The NMDA receptor is a glutamate and glycine activated excitatory ionotropic receptor expressed in the vertebrate central nervous system (for a review see [6,7]).

MK-801 is one of the most commonly utilized NMDA-R antagonists in pre-clinical studies. It is often used to model symptoms

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or endophenotypes of schizophrenia in animal models—including decreased prepulse inhibition [8] as well as impairment of social behavior [9]. In addition, exposure to MK-801 has been found to have anxiolytic/antidepressant [10] as well as locomotor stimulant effects [3,4,11]. Recently, the effects of MK-801 have also started to be examined using zebrafish [9,12–14]. The high nucleotide sequence homology of the zebrafish genome relative to the human genome offers translational relevance of the former species. Additionally, the small size, high fecundity, and social nature of this simple vertebrate represents a number of cost-effective and practical advantages compared to rodent models in behavioral neuroscience [15,16]. However, unlike the rodent literature, the zebrafish literature illustrates inconsistent effects of MK-801 on locomotor activity.

For example, exposure to low doses of MK-801 (ranging from 2 to 20 μM) has been reported to increase total distance traveled in adult zebrafish [13,17] and also in zebrafish larvae [3,18]. Conversely, other studies administering similar doses, in addition to much higher doses (up to 200 μM), found no significant changes in the total distance traveled [19] or in locomotor activity quantified by number of crossings [12,14]. Inconsistencies found between these studies may be attributed to strain differences. For instance, different genetically well defined strains of zebrafish (AB vs. TU) have been reported to respond differently to MK-801 during development; the AB strain showing no increase in total distance traveled, while the TU strain showing a decrease in total distance traveled [19]. However, another possible explanation that has been overlooked concerns the differences in the experimental set-ups, procedures and designs of these studies. Several studies measured locomotor activity of zebrafish during exposure to the drug [12,14,19]. In other studies, zebrafish were pre-treated with MK-801 (often in a small volume exposure chamber), and the total distance traveled was quantified subsequently when the zebrafish was placed in a larger novel tank or testing apparatus without continual administration of the drug [9,13,17,18]. In the current study, we investigate whether the inconsistent reports on the effects of MK-801 on locomotor activity are potentially due to such context-dependent variables. Instead of systematically analyzing and parametrically varying all possible experimental variables, we decided to focus on a single question: whether locomotor activity is affected by MK-801 administration during the administration period and/or after it during a washout, i.e. during testing in a novel tank without the presence of the drug in the tank water.

In addition to the inconsistent reports regarding MK-801's locomotor activating effects, the mechanism of action of this drug in zebrafish is also unclear. The dopaminergic system is often investigated as a potential contributor to MK-801-induced locomotor activity in rodents [5,20–22]. However, dopaminergic neurotransmission (e.g. dopamine synthesis and metabolism) has not been examined in zebrafish [9,13,17]. To investigate potential changes in the functioning of the dopaminergic system, we quantified the levels of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) from whole-brain tissue of zebrafish following exposure to MK-801.

We obtained zebrafish of the AB strain from the University of Toronto Mississauga Vivarium (Mississauga, ON, CA). Zebrafish husbandry and housing have been described in detail elsewhere [23]. To examine whether MK-801's locomotor activating effects are context-dependent, we exposed adult 12-month-old zebrafish to different concentrations (0, 5, 10, 15, and 20 μM) of (+)-MK-801 (using hydrogen maleate powder of the drug dissolved in zebrafish system water) (Sigma Aldrich, Oakville, ON, CA). Fish were individually exposed to the corresponding concentrations in a 1.5 L Plexiglas tank for 30 min (n = 17–20 per group). Immediately following drug exposure, zebrafish were individually placed in a novel 37 L tank lined with white corrugated plastic on the bottom and sides for an additional 30 min without the drug. Behavioral responses during both exposures were recorded and the total distance traveled in each exposure was quantified using EthoVision XT 8.5 (Noldus Information Technology, Wageningen, The Netherlands). We conducted a temporal analysis (1 min intervals) and an endpoint analysis (last 10 min) for the total distance traveled during both exposures. The last 10 min of exposure was chosen since behavioral responses during this period were found most robust and stable following exposure to different pharmacological compounds [24,25].

Fig. 1A shows that zebrafish exposed to MK-801 exhibited no significant changes in locomotor activity in the last 10 min during exposure to the drug ($F(3, 72) = 0.409$, $p = 0.747$). Following the 30 min exposure to various concentrations of MK-801, zebrafish were then transferred to a 37 L novel tank without the drug and total distance traveled was quantified in the last 10 min (Fig. 1B). Notably, there was a significant main effect of MK-801 pre-treatment on locomotor activity ($F(3, 67) = 6.512$, $p = 0.001$). Tukey HSD post-hoc analysis confirmed that zebrafish pre-treated with 10 and 20 μM of MK-801 significantly increased their locomotor activity compared to zebrafish pre-treated with system water (exposed to 0 μM of MK-801) ($p \leq 0.015$).

To closely examine time-dependent changes in locomotor activity, we performed a temporal analysis of locomotor activity during drug exposure (Fig. 1C) and during the subsequent novel tank test (Fig. 1D). During drug exposure there was a time-dependent increase in the total distance traveled ($F(29, 2088) = 4.028$, $p < 0.001$) but there was no significant main effect of MK-801 ($F(3, 72) = 0.319$, $p < 0.812$) and there was no significant MK-801 × time interaction ($F(87, 2088) = 0.910$, $p < 0.709$). These results suggest a time dependent but drug exposure independent change of activity, which was likely due to a habituation induced increase of activity during the first five minutes of the session observed in fish of all treatment groups. During the novel tank exposure, we also found a time-dependent increase of total distance traveled ($F(29, 1943) = 3.252$, $p < 0.001$). Although the main effect of MK-801 did not reach significance ($F(3, 67) = 2.301$, $p = 0.085$), we found a significant MK-801 x time interaction ($F(87, 1943) = 2.709$, $p < 0.001$) with fish pre-treated with higher concentrations of MK-801 exhibiting a time-dependent increase in the total distance traveled while fish in the lowest MK-801 dose and control groups exhibiting no such increase.

Next, to quantify potential changes in the functioning of the dopaminergic system induced by MK-801, a separate group of zebrafish were pre-treated with either system water or 10 μM of MK-801 for 30 min (the dose previously found to induce the largest increase in locomotor activity) in 1.5 L Plexiglas tank and subsequently tested in a novel tank for 30 min. Following behavioral testing, fish were decapitated immediately and their brains were dissected. Whole-brain samples were used to quantify the levels of dopamine and its metabolite (DOPAC) using high precision liquid chromatography (HPLC) employing a previously established method [25].

Fig. 2A shows the effect of a 30 min MK-801 pre-treatment on whole-brain levels of dopamine in zebrafish that were subsequently tested in a novel environment. Independent samples t-test revealed no significant difference between the two groups ($t = 0.548$, $df = 30$, $p = 0.588$). Fig. 2B shows the effect of a 30 min MK-801 pre-treatment on whole-brain levels of dopamine's metabolite DOPAC in zebrafish that were subsequently tested in a novel environment. Similarly, independent samples t-tests revealed no significant difference between the two groups ($t = -0.281$, $df = 30$, $p = 0.780$).

In the current study we reconcile previous inconsistent findings regarding MK-801's locomotor stimulant effects in zebrafish. We found that zebrafish did not exhibit significant increases in