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Research report

Behavioral performance altering effects of MK-801 in zebrafish (*Danio rerio*)

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ABSTRACT

MK-801, a non-competitive NMDA-R antagonist, has been utilized in the analysis of mammalian learning and memory. The zebrafish is a novel vertebrate study species that has been proposed for the analysis of the mechanisms of learning and memory. Although learning paradigms have been developed for this species, psychopharmacological characterization of its behavioral responses is rudimentary. Before one attempts the analysis of the effects of MK-801 on learning and memory in zebrafish, one needs to know whether this drug affects motor function, perception and/or motivation, factors that may influence performance in learning tasks. Here we conduct dose response analyses investigating the effects of 0, 2, 20 and 100 μM MK-801 administered 24 h or 30 min before the behavioral test, or during the test. We analyze responses in the open tank to measure motor and posture patterns, in the light dark paradigm to evaluate visual perception, and in a group preference task to attempt to quantify motivation. Our results show a significant performance alteration only in the highest (100 μM) dose groups. These fish spent more time on the bottom of their tank, showed elevated Erratic movement, increased their clockwise and counterclockwise turning frequency, and reduced the time spent near a shoal stimulus, behavioral alterations that also depended upon the timing of drug administration. Thus, using the current delivery procedures and outbred zebrafish population, the highest dose that may not lead to significant performance deficits is 20 μM, a concentration we propose to use in a future learning study in zebrafish.

1. Introduction

The zebrafish is a small (4 cm long) freshwater teleost that inhabits slowly moving streams and small lakes of the Indian subcontinent [8]. It has been exported and bred for the pet trade for a long time and has also been well studied in the laboratory. For example, for the past three decades the zebrafish has been in the forefront of developmental biology and genetics [17]. As a result of the accumulated genetic information and the development of a sophisticated genetics tool set, numerous scientific fields have taken notice of this fish. Among these is behavioral brain research [10,28]. Briefly, several authors have argued that the zebrafish represents an excellent compromise between system complexity (it is a vertebrate) and practical simplicity (it is small, easy to keep and breed in large numbers) [11,18]. There are some drawbacks, however, when one wants to study mechanisms of brain function and behavior using this species. The number of behavior and/or brain related studies of the zebrafish is orders of magnitude less than those devoted to rodents, for example [11,28]. Most recently, however, several papers have appeared that started to ameliorate this problem. For example, behavioral characteristics of this species have been increasingly explored [5] and analysis of the psychopharmacological properties of zebrafish has also been started [19].

Learning and memory have been well studied and numerous molecular players and neurobiological mechanisms involved in these processes have already been revealed [32]. However, despite the large number of studies devoted to the understanding of the biological mechanisms of these brain functions, a lot remains to be discovered. The zebrafish has been proposed as a potentially useful laboratory tool for the analysis of these mechanisms [29]. In addition to, or in combination with, molecular genetic approaches, psychopharmacological tools have been particularly useful in this research [9]. However, little is known about the psychopharmacological characteristics of zebrafish. For example, only very few studies have investigated the function of N-Methyl-D-Aspartate Receptor (NMDA-R) in zebrafish.

NMDA-R is a ligand (glutamate) and voltage gated calcium/sodium ion channel that is believed to play fundamental roles in learning and memory [32]. As a coincidence detector of presynaptic activation (glutamate release) and postsynaptic activation (depolarization), it is argued to be crucial for processes including long-term potentiation (LTP) and long-term depression (LTD), synapse level phenomena proposed to underlie associative learning and memory [32]. Importantly, NMDA-R and several genes corre-

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sponding to its subunits have been identified in zebrafish [7,24] and the nucleotide sequence of its genes has been found highly similar (approaching 90% identity in some cases) to those of mammalian genes [7]. It is thus expected that the zebrafish NMDA-R functions similarly to the mammalian receptor [22,5] and that psychopharmacological tools developed for the mammalian receptor work with zebrafish NMDA-R [27].

MK-801 (dizocilpine, or (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) is a non-competitive NMDA-R antagonist that has been utilized in learning and memory research, mostly using rodents and other mammalian species [33,35]. In addition to its learning and memory altering properties [35], it is also known that MK-801 has many "side effects", i.e. it can potentially alter behavioral performance other than learning and memory itself [34]. Performance alterations in a learning paradigm may be mistaken to learning or memory deficits, thus it is important that one tries to dissociate these effects [13].

The psychopharmacology of MK-801 has started to be explored with zebrafish. For example, hyperactivity induced by this drug was recently shown to be reversible by administration of antipsychotic drugs [27]. The effects of MK-801 on circling and place preference have also been explored [31]. The effect of MK-801 on learning performance of zebrafish has also started to be explored. One study demonstrated that when administered after training, MK-801 inhibited learning performance (consolidation of memory) in a one-trial avoidance task [3] while another study showed that it did not have such an inhibitory effect in an active avoidance task [36]. Given the potentially complex psychopharmacological profile of MK-801 and the above controversies, we decided to expand on these findings and attempted to identify potential performance altering properties of MK-801 that may impede behavior of zebrafish in learning paradigms. Briefly, the goal of the current study is to help us select a dose that elicits no such performance impairing effects, a concentration that may be appropriate for the analysis of learning and memory in zebrafish.

Performance factors that can alter behavior in learning tasks may be classified into three distinct categories: motor function, perception and motivation (e.g. Gerlai, 2001 [13]). We have developed a learning task for zebrafish in which the test fish are expected to actively swim in a maze to a target [29]. The target is a group of conspecifics placed in a separate tank [1]. This tank is visible from inside the test maze of the experimental fish. We have shown previously that zebrafish are highly social [26,20,21] and are motivated to join a shoal and that this motivation is sufficiently strong to support good learning performance [1]. In the current paper we utilize behavioral tests that can potentially tap into performance characteristics required for the above learning task. We investigate whether MK-801 can disrupt motor function (important for navigating through the maze), visual perception (i.e. whether the drug can disrupt the ability to see the target stimulus), and motivation (i.e. whether the drug can reduce/alter shoaling tendencies) in zebrafish. Last, we also study the effect of the timing of drug delivery and study how MK-801 may influence behavioral performance when administered 24 h or 30 min before the behavioral test, or during the test. Analysis of the effects of MK-801 administered at these time points we hope will help us in future learning studies aimed at dissociating temporally distinct mechanisms associated with acquisition, consolidation and recall of memory in zebrafish.

2. Methods

2.1. Animals and housing

The experimental and stimulus subjects were all short-fin wild type (SF) zebrafish (*Danio rerio*), which were of the second filial generation that originated from breeders purchased in a local pet store (Big Al's Aquarium Services, Mississauga, Ontario, Canada). SF zebrafish are a genetically uncharacterized heterogeneous stock whose advantage over genetically well defined standard zebrafish strains is the expected lack of strain specific idiosyncratic features, i.e. absence of inbreeding induced genetic drift (random fixation of alleles). Briefly, in addition to being robust and good breeders these fish are expected to possess behavioral characteristics typical of wild type zebrafish. Young sexually mature adults (6–8 months of age, approximately 50–50% males and females) were tested in the behavioral paradigms. Importantly, each fish was tested in only one behavioral paradigm and only once, a between subject experimental design. Fish were bred, raised and maintained in house (University of Toronto Mississauga Vivarium) in 2.8 L acrylic aquaria (15 fish per tank) on a high density rack system (Aquaneering Inc., San Diego, CA, USA), which provided multistage filtration (mechanical filter, a fluidized glass bed biological filter, activated carbon chemical filter, and a fluorescent UV light sterilizing unit). 10% of the water was replaced daily with deionized water supplemented with 60 mg/L Instant Ocean Sea Salt (the system water). The water temperature was maintained at $26 \pm 2\,^{\circ}C$ and the aquaria were illuminated by fluorescent light tubes from the ceiling of the holding room (lights turned on at 08:00 h and off at 20:00 h). Fish were fed a mixture of ground flake food (4 parts, Tetramin Tropical Flakes, Tetra, USA) and powdered spirulina (1 part, Jehmco Inc., Lambertville, NJ, USA).

2.2. MK-801 dosing procedures

We employed four concentrations of MK-801 hydrogen maleate (M107, Sigma–Aldrich) dissolved in system water: 0 μM, 2 μM (674 ng/mL), 20 μM and 100 μM. We administered the drug at three different time points: (a) fish received the drug during the 30 min long behavioral session, (b) fish received the drug for a 30 min period immediately before the behavioral session, and (c) fish received the drug for 30 min 24 h before the behavioral session. A particular fish received only one dose and only once, i.e. the experimental design was a 4 (concentration) × 3 (timing) between subject design. The chosen concentrations were based upon a previous study on zebrafish [31], the mammalian literature as well as on our own pilot experiments. The rationale for the different time points of administration was as follows. Detailed ADME (absorption, distribution, metabolism and excretion) information has not been obtained for MK-801 in zebrafish. The pharmacokinetic and pharmacodynamic properties of this drug are unknown for this species. From a previous study with zebrafish (e.g. Swain et al., 2004, [31]) and from the mammalian literature, however, it appears reasonable to assume that a 30 min drug exposure should allow the drug to reach the brain and occupy a significant amount of NMDA-R, thus we employed the 30 min prior to test dosing. MK-801 may reach the brain sooner and/or may have direct immediate peripheral effects. For example, perhaps unlikely, but the drug may irritate the gills or the skin and thus induce behavioral responses without a central (brain) action (fish are swum in MK-801 solution and passively uptake this water soluble drug). Therefore, we explored if MK-801 affects behavior if it is present in the water only during behavioral testing. Last, learning trials often need to be performed repeatedly. For example, in the plus maze, we trained fish across multiple days [29] and thus it is important to know whether a drug administered 24 h before could have a residual effect on performance tested at a subsequent trial the following day, hence the 24 h prior to test drug administration. In all cases, MK-801 was administered using a 2 L rectangular exposure tank in which the single fish received the corresponding drug solution for 30 min and absorbed the drug (via the skin, gills and orally) while swimming in the solution.

Three separate behavioral tests were performed, each on a set of naïve fish. In each of the three behavioral experiments 12 groups of fish (3 drug exposure time points × 4 concentrations) were tested, and each of these groups had a sample size (n) equaling 8, i.e. a total of 386 fish were analyzed. This test battery consisted of the following behavioral paradigms: open tank, light–dark preference, and group preference.

2.3. Recording of behavior: general procedures

Two 13 W fluorescent light bulbs illuminated the experimental tanks from above. In each test a single fish was monitored at a time. Once the fish completed the tests, they were netted from the experimental apparatus and returned to a collecting tank. All experiments were conducted with the experimenter absent from the testing room, and occurred during the light phase of the light/dark cycle, i.e. in between 1000 and 1600 h. All of the behavioral experiments were videotaped from the side using a digital hard drive video camera (JVC Everio GZ-MG500, Yokohama, Japan). The digital video-files were later transferred to a computer, replayed and scored using the event recorder application Observer Color Pro 5.0 (Noldus, Wageningen, The Netherlands).

2.4. The open tank test

Motor responses were assessed in a novel empty, i.e. open, 2 L (21 cm × 10 cm × 10 cm) tank made of Plexiglas. The behavioral recording session was 30 min long. Exposure to a novel environment as well as handling by the experimenter is an inherent part of any experiment and is expected to induce passive and active avoidance reactions [e.g. 11,14]. In a novel open tank thus zebrafish may perform numerous motor and posture patterns [12,4] including Freezing (complete immobility, only the eyes or the pectoral fins may move, fish