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Research report

Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (*Danio rerio*)

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ABSTRACT

Glutamate N-methyl-D-aspartate (NMDA) receptor antagonists, such as dizocilpine (MK-801), elicit schizophrenia-like symptoms in humans and a behavioral syndrome in rodents, characterized by hyperlocomotion and stereotyped actions, which is antagonized by antipsychotic drugs. Animal models of schizophrenia have been established and used for the development of new antipsychotic drugs. In this work we characterized the behavioral effects of MK-801 and investigated the effect of typical and atypical antipsychotic treatments on locomotor activity as well on the hyperlocomotion induced by MK-801 in zebrafish. MK-801 (20 μM) increased the locomotor behavior as measured by the number of line crossings, distance traveled, and the mean speed in the tank test after 15, 30, and 60 min of exposure. All tested antipsychotics counteracted MK-801-induced hyperactivity on all parameters analyzed and at doses that, given alone, had no effect on spontaneous locomotor activity. The results suggest a similar profile between typical and atypical antipsychotics in the reversal of locomotor disorders induced by MK-801. Moreover, an anxiolytic effect was verified at 30 and 60 min of MK-801 exposure, which was not reversed by antipsychotics tested in this work. In addition, olanzapine, which alone caused an anxiolytic response, when given with MK-801 potentiated the latter's effect on anxiety. In this work we demonstrated the value of the zebrafish, a simple to use animal model, in developing some behavioral features observed in schizophrenia, which may indicate a new approach for drug screening.

1. Introduction

Locomotor behavior in vertebrates, such as walking or swimming, relies upon neural networks in the brain and spinal cord [47]. *Danio rerio*, the so-called zebrafish, exhibits several features that have made it an increasingly popular research system to examine the development and functioning of these networks [10,18]. Consequently, this animal has become consolidated as a model system in neurochemical, toxicological, and behavioral studies [21,61,63]. Compared to rodent models, zebrafish have many practical advantages, including a high fertility level (up to 200 eggs in one mating), small size, rapid generation time (days as opposed to weeks), and optical transparency during early embryogenesis [28,67]. There is a paucity of studies on complex behavior in zebrafish, even though it is recognized as having great potential as a model for understanding the genetic basis of human behavioral disorders [26,56], for the investigation of neuropharmacological mechanisms in mammals, and for applications in drug discovery [33,55].

Schizophrenia is a complex psychiatric disorder which is characterized by three main types of symptoms: positive (e.g. hallucinations, delusions), negative (e.g. social withdrawal, anhedonia), and cognitive deficits (e.g. impaired working memory and attention) [5]. Motor symptoms are frequent in schizophrenia and about 50% of psychotic patients display at least one motor symptom [57]. Increasing evidence supports the view that the development of motor systems in schizophrenic patients is impaired at very early life stages [41]. For example, motor development during infancy and early childhood was shown to be delayed or deviant in subjects later to suffer from schizophrenia [59]. In addition, several studies reported motor disturbances in both treated and untreated schizophrenia [19,46,72]. Changes in several neurotransmitter systems as well as neuroanatomical changes have been reported in the

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brains of schizophrenic patients [73]. Nevertheless, the pathophysiology of schizophrenia remains unclear and this lack of information might be attributed in part to the difficulties in modeling this disorder [62].

At least two classes of psychotomimetics have been used as a model of schizophrenia in animals: dopamine agonists, such as amphetamine, which produce and exacerbate positive symptoms [36,65], and NMDA (N-methyl-D-aspartate) antagonists, such as ketamine, phencyclidine (PCP) or MK-801 (dizocilpine maleate) that can cause a profile of behavioral changes that are similar to the positive, negative, and cognitive symptoms of schizophrenia [23,49,60]. It is well known that systemic administration of the non-competitive NMDA receptor antagonist MK-801 causes an increase in rodent locomotion and, at higher doses, stereotypic behaviors including head weaving and uncoordinated, ataxic gaits [14,16].

Behavioral abnormalities induced in rodents by NMDA, such as hyperactivity, are prevented by antipsychotic drugs, more potently by the atypical than the typical type [12,23,29]. The principal mechanism of action of typical antipsychotics is the blockade of central DA receptors [58]. By contrast, atypical antipsychotics, while less potent than their typical counterparts in blocking central D2 receptors, have affinity for a wide range of other receptors including D1, D4, 5-HT2A, 5-HT6, α1, H1, and M1 [30]. Typical antipsychotic medications, although effective in treating psychotic symptoms, are limited by their propensity to cause higher rates of motor side effects [2], leading to non-adherence to treatment and increasing the risk of relapse [31]. Meanwhile, the main disadvantages of using atypical antipsychotic medications include higher costs and adverse effects on metabolism [39,69], although they present fewer extrapyramidal side effects and do alleviate negative symptoms while improving cognitive deficits [70].

In the present study we characterized the behavioral syndrome produced by MK-801 exposure in the zebrafish. Since various antipsychotics have been reported to modulate MK-801-induced hyperlocomotion, we also examined the effects of typical and atypical antipsychotics and their interaction with the MK-801-induced behavioral changes in zebrafish through the analysis of swimming activity and anxiety responses using the novel tank diving test.

2. Materials and methods

2.1. Animals

Adult wild type zebrafish strains (3–5 cm) of both sexes were obtained from a specialized commercial supplier (Redfish, RS, Brazil) and were of genetically heterogeneous (randomly bred) stock. The fish were acclimatized to the laboratory environment for at least 14 days and housed in a 50-l thermostated aquarium filled with continuously unchlorinated water at a targeted temperature of 28 ± 2 °C, with constant filtration and aeration (7.20 mg O_2/l) and a density of up to five animals per liter [71]. Animals were kept on a day:night cycle of 14:10 h and fed twice a day with flaked fish food that was supplemented with live brine shrimp.

Fish were manipulated healthy and free of any signs of disease, according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996). The Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (PUCRS) approved the protocol under license number CEUA 09/00135.

2.2. Pharmacological treatments

A group of animals was individually exposed in a 300-ml beaker to 20 μM MK-801 hydrogen maleate (Sigma–Aldrich, Brazil), dissolved in tank water, for 15, 30 or 60 min before analysis in the tank diving behavioral test. Control animals were maintained individually in a 300-ml beaker with tank water for the same time as the MK-801 treatment.

The effects of typical and atypical antipsychotics were investigated in the same way as MK-801 in the apparatus test. Groups of animals were individually treated for 15 or 30 min in a 300-ml beaker with 9 μM haloperidol, 100 μM olanzapine or 250 μM sulpiride (all from Sigma–Aldrich, Brazil). Tank water was used as the vehicle for haloperidol and olanzapine and tank water with 5% DMSO was used as the vehicle to dissolve sulpiride.

To assess the effects of antipsychotics on MK-801-induced behavioral changes in the zebrafish, the following treatments were performed: (i) a control group was exposed to tank water in a beaker for 30 min; (ii) a MK-801 group was exposed to 20 μM MK-801 for 30 min; and (iii) a MK-801 plus antipsychotic group was pre-treated in a beaker with 20 μM MK-801 for the first 15 min and subsequently the same animals were transferred to another beaker containing 20 μM MK-801 plus 9 μM haloperidol or 100 μM olanzapine or 250 μM sulpiride, and remained there for an additional 15 min.

The MK-801 and haloperidol doses were chosen based on previous studies with zebrafish [68,25]. The doses of other antipsychotic agents used in this study were chosen based on drug potencies observed in human [45,34] and rat [27,54] studies.

2.3. Behavioral assessment

Behavioral testing of drug effects took place during the light phase between 10:00 a.m. and 5:00 p.m. Animals were individually placed in the experimental tank (30 cm × 15 cm × 10 cm, length × height × width) immediately after the pharmacological manipulation and were first habituated to the tank for 30 s, as previously described [22]. There was no drug exposure during behavioral experiments. The animals' locomotor activity was recorded on video for 5 min after the habituation period and simultaneously analyzed using the ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA). The tank was divided into equal sections with four vertical lines and one horizontal line, and the following behavior patterns were measured: number of line crossings (vertical and horizontal lines), distance traveled and mean speed. The time spent in each tank position (bottom vs. upper levels) was considered as the index of anxiety. This task exploits the natural tendency for zebrafish to spend most of the time at the bottom when introduced into a novel environment and then gradually to extend the swimming range, over a period of minutes, to include the upper portions of the test tank [37]. A longer time spent in the bottom and less time spent in the top part of the tank indicates heightened anxiety [37]. Visual observations throughout the experimental periods allow the documentation of erratic movements, defined as sharp changes in direction or velocity and repeated rapid darting behaviors [37]. In addition, these movements may be manifested by bouts of vertical swimming or sideways swimming, suggesting a problem with coordination [25].

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. The behavioral effects of MK-801, antipsychotic drugs, and MK-801 plus antipsychotics were examined by one-way ANOVA, followed by Newman–Keuls *post hoc* test. A significant difference was attributed to p values less than 0.05.

3. Results

3.1. MK-801 induces changes in locomotor activity

Distinct parameters of zebrafish swimming activity were examined in the tank diving behavioral test. As indicated by the number of line crossings in the apparatus there was increased locomotor activity of animals treated with MK-801 in both the 15 (76.6%) and 30 min (100.6%) groups when compared with the control group (170.5 ± 20 line crossings) (Fig. 1A). MK-801 treatment (15, 30, and 60 min) increased the distance traveled (89.4%, 81.9%, and 85.1%, respectively) and the mean speed (89.8%, 99.8%, and 85.1%, respectively) in relation to control animals (11.4 ± 1.1 m; 0.04 ± 0.004 m/s, respectively) (Fig. 1B and C). Fig. 1D shows that when compared with the control group there was a significant, gradual increase in the time spent in the upper portion of the test tank in animals exposed to MK-801 for 30 (373.9%) or 60 min (464.7%), which may be interpreted as an indicator of anxiolytic behavior. As can be seen in Fig. 1E, there was no change in the behavioral parameters with MK-801 treatment when the data were subjected to minute-by-minute analysis. A representative trace of control and MK-801 treatment time-effects is shown in Fig. 1F. After assessing the effects of MK-801 on the zebrafish behavior we chose 30 min of MK-801 exposure as an adequate time to investigate the interaction with antipsychotics.

3.2. Effects of antipsychotic drug administration on the locomotor activity

Haloperidol, sulpiride, and olanzapine administered alone for 15 or 30 min before test sessions did not affect the number of line crossings, distance traveled and mean speed (Fig. 2A–C). However,

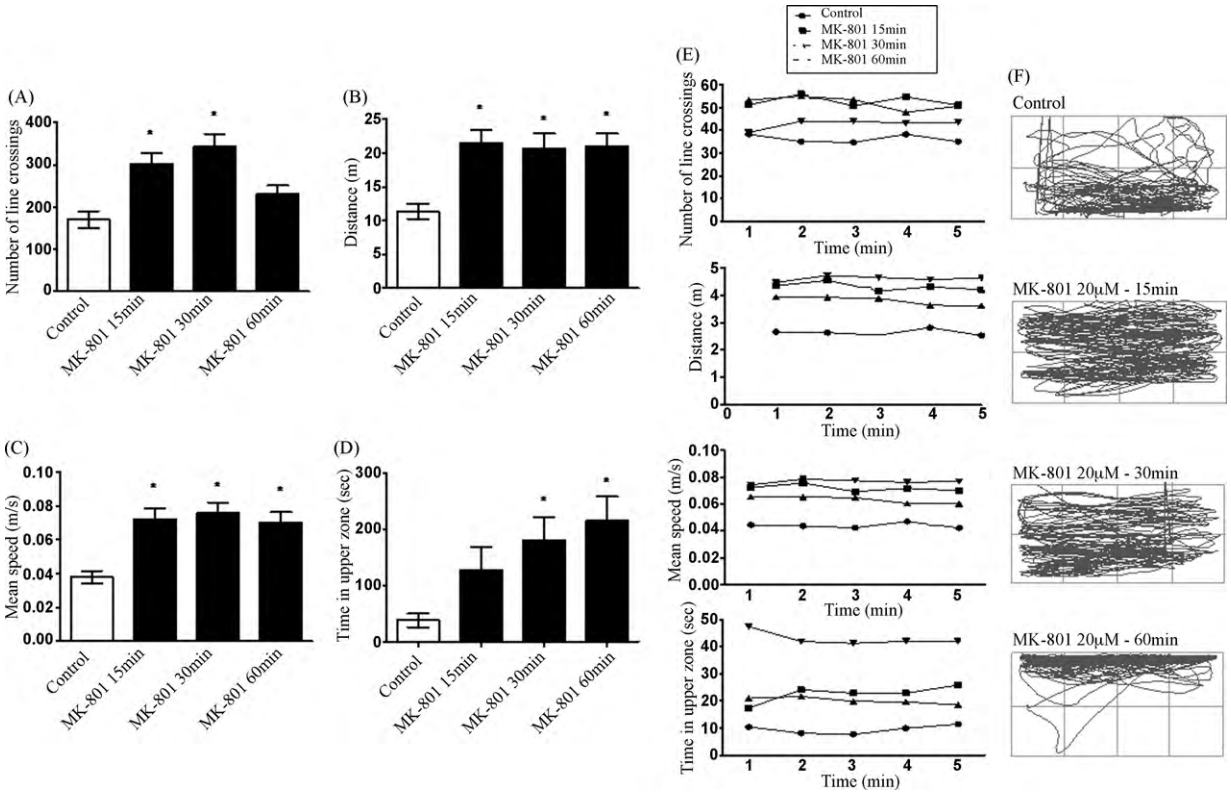

Fig. 1. Effect of exposure to 20 μM MK-801 for different times (15, 30 or 60 min) on the number of line crossings (A), distance traveled (B), mean speed (C), and time spent in the upper zone (D) determined during 5 min of videorecording in the tank diving behavioral test. (E) Minute-by-minute analysis; (F) representative traces. Data were expressed as mean ± S.E.M. of 10 animals for each group and were analyzed by one-way ANOVA followed by Newman–Keuls post hoc test. $^{*}p < 0.05$ denotes a significant difference from the control group.

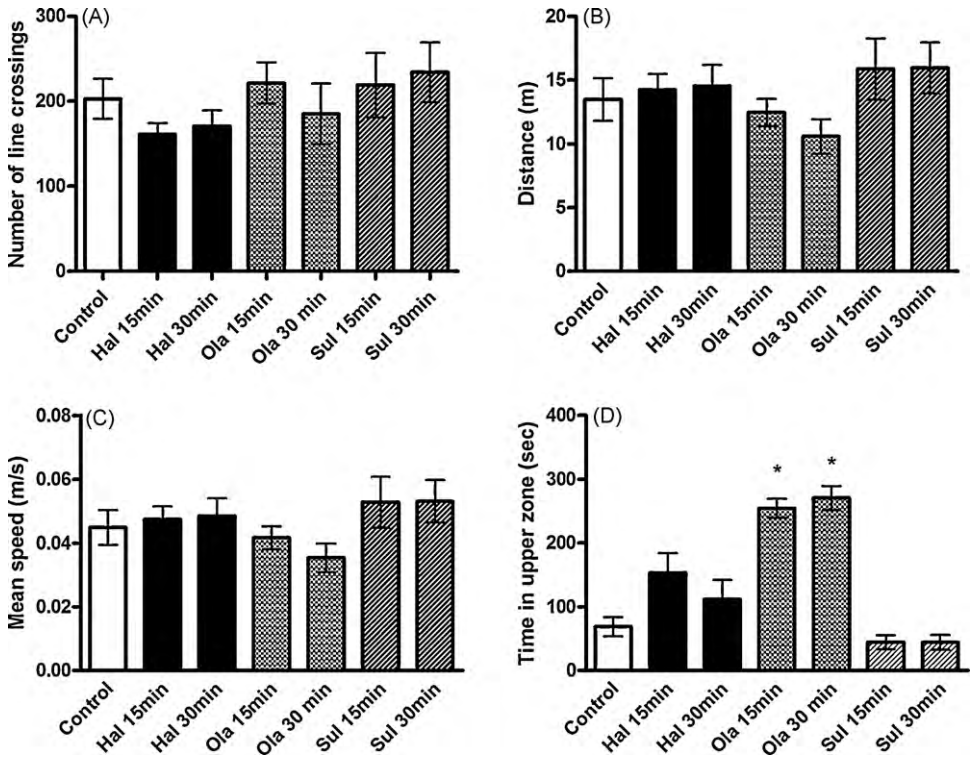

Fig. 2. Effect of 15 and 30 min treatment with haloperidol (Hal; 9 μM), olanzapine (Ola; 100 μM) and sulpiride (Sul; 250 μM) on the number of line crossings (A), distance traveled (B), mean speed (C), and time spent in the upper zone (D) determined during 5 min of videorecording in the tank diving behavioral test. Data were expressed as mean ± S.E.M. of 10 animals for each group and were analyzed by one-way ANOVA followed by Newman–Keuls post hoc test. $^{*}p < 0.05$ denotes a significant difference from the control group.

in relation to the anxiolytic profile, only olanzapine at 15 (269.7%) and 30 min (293.2%) induced a significant anxiolytic response (control group, 68.8 ± 15.02 s) (Fig. 2D).

3.3. Behavioral changes induced by MK-801 pretreatment are reversed by co-administration with different antipsychotic drugs

Based on pronounced MK-801-induced hyperlocomotor behavior, we examined the effects on behavioral activity of co-administration of MK-801 with typical or atypical antipsychotic drugs. When co-administered with MK-801 both classes of antipsychotic drugs were able to reverse the increase in the number of line crossings, distance traveled, and mean speed promoted by 30 min of MK-801 exposure (Fig. 3A–C, respectively).

However, all antipsychotic drugs tested in the co-treatment with MK-801 failed to reverse the anxiolytic effect of MK-801 determined by the time spent in the upper zone of the tank (Fig. 3D). Moreover, it is important to note that olanzapine, when co-administered with MK-801, actually potentiated the anxiolytic effect of the NMDA antagonist (Fig. 3D).

4. Discussion

The use of preclinical tests and animal models is essential in understanding and developing the pharmacological profile of novel antipsychotics. It is now recognized that zebrafish possess a great deal of similarity to mammals and are an extremely useful model for screening compounds at several stages of the drug discovery process [7]. Therefore, the present study was designed to determine if MK-801, previously described as a psychotomimetic drug of schizophrenia, causes behavioral changes in zebrafish.

Schizophrenia is a psychotic disorder marked by severely impaired thinking, emotions, and behaviors that affects about 1% of the general population worldwide. Although the etiology remains unknown, studies with animal models have begun to reveal possible mechanisms. It is difficult to produce animal models of hallucinations and delusions [24]; however, impairments in cognition, memory, emotion and social interaction, impaired sensorimotor gating, and hyperactivity in response to different drugs may be investigated [42,50,64]. Kilts [35] have described drug-induced hyperactivity as corresponding to psychomotor agitation, which is also a characteristic of schizophrenia, and studies have shown that MK-801 and PCP increase locomotor activity and induce stereotypic effects, which can be reversed with both typical and atypical antipsychotics [15,20,50].

Since the effects of NMDA antagonists on locomotor activity are well known and the latter is altered in schizophrenia, this behavioral measure was used in the present study. Our data show that locomotor activity in zebrafish was increased after MK-801 administration, in agreement with previous results obtained in mammals [13,11]. The assessment of locomotor activity may be a valuable method to identify the effect of antipsychotics on behavioral changes induced by psychotomimetics, and it has previously been demonstrated that antipsychotic agents, including haloperidol, clozapine, and olanzapine antagonize MK-801-induced hyperlocomotion. However, haloperidol exerted this antagonistic effect only at a dose that also decreased spontaneous activity, whereas clozapine and olanzapine reduced MK-801-induced hyperactivity at doses that had no effect on spontaneous activity [51,53]. Moreover, although haloperidol tended to decrease the locomotor activity, the three antipsychotics tested in this study caused no significant changes in this parameter when tested alone. A possible reason for this effect could be the unusual body position exhibited in swimming during the behavioral test after exposure to a typical antipsychotic. While control animals maintain a position parallel to the water surface during swimming, we observed that animals treated with haloperidol were unable to maintain such a position, indicating a lack of postural balance, as described in previous studies [25]. In addition, we found that haloperidol-treated zebrafish exhibited erratic swimming patterns, manifested by bouts of vertical swimming or sideways swimming, suggesting a problem with coordination.

The mechanisms by which antagonists of serotonin 5-HT2A receptors, such as sulpiride and olanzapine, inhibit MK-801-induced hyperlocomotion have not been fully elucidated. Some neurochemical data suggest that NMDA antagonists increase serotonin release, which in turn activates serotonin 5-HT2A receptors on glutamatergic neurons in the cortex to release glutamate [1,3]. The increased glutamate release may act on post-synaptic AMPA/kainate receptors causing changes in behavioral responsiveness and inducing the neuropathological changes observed with NMDA antagonist exposure [48,52]. Su et al. [66] showed that the atypical antipsychotic risperidone inhibits NMDA antagonist-induced glutamate release in the medial prefrontal cortex by blocking serotonin 5-HT2A receptors on glutamatergic terminals, leading to attenuation of the activity of cortico-subcortical glutamatergic neurons. This attenuation, in turn, decreases MK-801-induced hyperlocomotion [66]. Therefore, it is possible that sulpiride and olanzapine inhibit the MK-801-induced hyperlocomotion through a similar mechanism.

Clearly, other mechanisms may also be involved in the inhibitory effect of antipsychotic drugs on MK-801-induced hyperlocomotion. As we know, atypical antipsychotics block not only serotonin 5-HT2A receptors but also dopamine D2, α1-adrenoreceptors, and histamine H1 receptors [38], and therefore these agents may attenuate MK-801-induced hyperlocomotion via such blockade. Many reports have shown that atypical antipsychotics are more potent than typical antipsychotic drugs in inhibiting MK-801-, phencyclidine- or ketamine-induced locomotor activity [12,29,53]. Our findings demonstrate that haloperidol, sulpiride, and olanzapine acutely inhibited MK-801-induced hyperlocomotion, differing only in their influence upon the anxiolytic effect of MK-801.

Additionally, our results showed an MK-801-induced anxiolytic profile in zebrafish after 30 and 60 min of exposure. This is in agreement with previous studies conducted in mammals, in which rats treated with MK-801 and submitted to the elevated plus-maze test presented an increase in time spent in the open arms, indicative of an anxiolytic-like effect [6,17,32]. A further notable finding in our study was the anxiolytic effect presented after 15 and 30 min of exposure to olanzapine, although this drug did not have any effect on locomotor activity. Atypical antipsychotic drugs (risperidone, olanzapine, and quetiapine) have been increasingly used to treat anxiety-related disorders in addition to their use in the treatment of psychosis. Indirect evidence suggests that the effects of clozapine on allopregnanolone, a progesterone metabolite, may be responsible for its anxiolytic effect. It has been found that clozapine and olanzapine increase allopregnanolone in rat cerebral cortex and hippocampus in a dose-dependent manner [43,44], and since allopregnanolone acts as a positive modulator of the $GABA_A$ receptor [40] and shows a strong anxiolytic effect in the elevated plus-maze task and the Geller–Seifter conflict test [4,8,9], it is possible to suggest that clozapine or olanzapine-induced elevations in allopregnanolone contribute to their anxiolytic-like effect. Finally, our results show that haloperidol and sulpiride did not cause significant changes in the behavioral parameters observed.

In conclusion, both typical and atypical antipsychotics attenuated MK-801-induced hyperlocomotion in zebrafish at doses that did not reduce spontaneous locomotor activity. Testing the efficacy of drugs in alleviating MK-801-induced behavioral changes is one of the experimental approaches for screening a new therapeutically

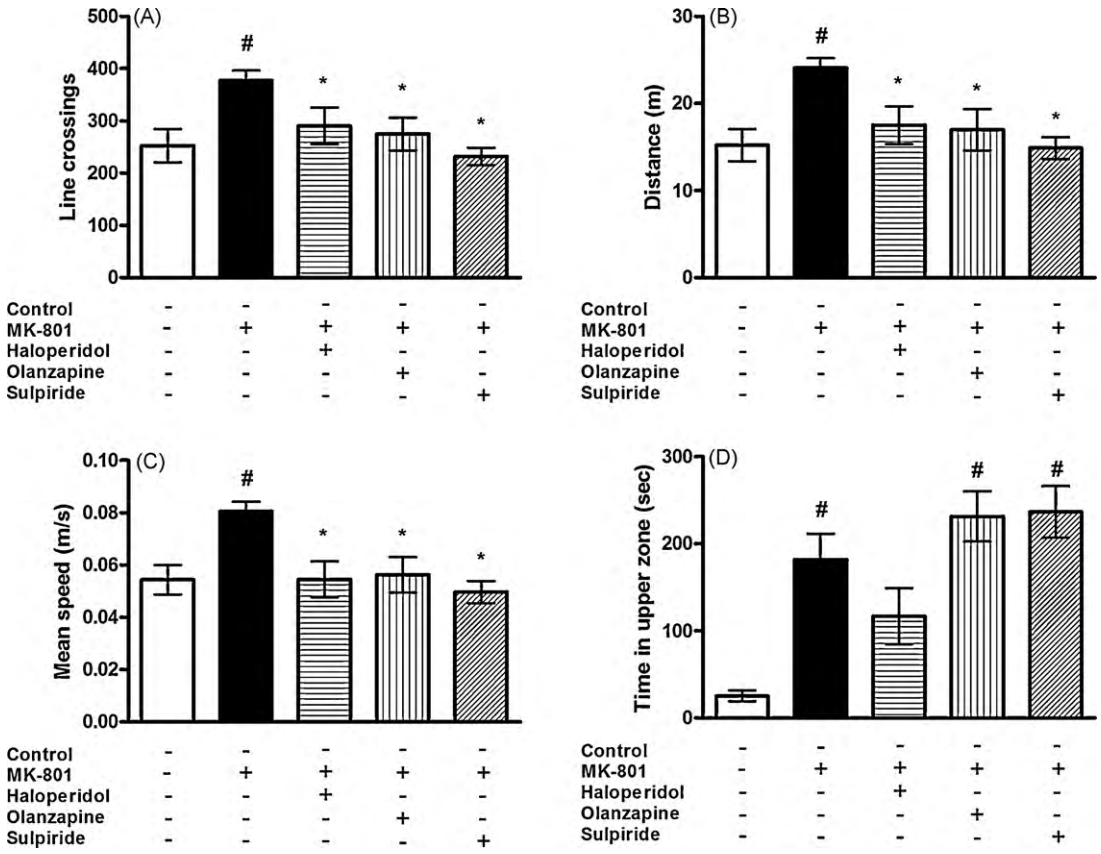

Fig. 3. Effect of 15 min pretreatment with 20 μM MK-801 and later 15 min co-treatment with 20 μM MK-801 and haloperidol (Hal; 9 μM), olanzapine (Ola; 100 μM), or sulpiride (Sul; 250 μM) on the number of line crossings (A), distance traveled (B), mean speed (C), and time spent in the upper zone (D) determined during 5 min of videorecording in the tank diving behavioral test. Data were expressed as mean ± S.E.M. of 10 animals for each group and were analyzed by one-way ANOVA followed by Newman–Keuls post hoc test. $^{\#}p < 0.05$ denotes a significant difference from the control group; $^{*}p < 0.05$ denotes a significant difference from the MK-801 treatment.

relevant compound, and merits further investigation in this animal model.

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References

[1] Adams BW, Moghaddam B. Effect of clozapine, haloperidol, or M100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. Biol Psychiatry 2001;50:750–7.
[2] Adams CE, Coutinho ES, Davis J, Duggan L, Leucht S, Li C, et al. Cochrane schizophrenia group. Schizophr Bull 2008;34:259–65.
[3] Aghajanian GK, Marek GJ. Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. Brain Res 1999;825:161–71.
[4] Akwa Y, Purdy RH, Koob GF, Britton KT. The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. Behav Brain Res 1999;106:119–25.
[5] Austin J. Schizophrenia: an update and review. J Genet Couns 2005;14:329–40.
[6] Bertoglio LJ, Carobrez AP. Anxiolytic-like effects of NMDA/glycine-B receptor ligands are abolished during the elevated plus-maze trial 2 in rats. Psychopharmacology (Berl) 2003;170:335–42.
[7] Best JD, Alderton WK. Zebrafish: an in vivo model for the study of neurological diseases. Neuropsychiatr Dis Treat 2008;4:567–76.
[8] Bitran D, Klibansky DA, Martin GA. The neurosteroid pregnanolone prevents the anxiogenic-like effect of inescapable shock in the rat. Psychopharmacology (Berl) 2000;151:31–7.
[9] Brot MD, Akwa Y, Purdy RH, Koob GF, Britton KT. The anxiolytic-like effects of the neurosteroid allopregnanolone: interactions with GABA(A) receptors. Eur J Pharmacol 1997;325:1–7.
[10] Brustein E, Saint-Amant L, Buss RR, Chong M, McDearmid JR, Drapeau P. Steps during the development of the zebrafish locomotor network. J Physiol (Paris) 2003;97:77–86.
[11] Bubenikova-Valesova V, Svoboda J, Horacek J, Vales K. The effect of a full agonist/antagonist of the D1 receptor on locomotor activity, sensorimotor gating and cognitive function in dizocilpine-treated rats. Int J Neuropsychopharmacol 2009:1–11.
[12] Carlsson M, Carlsson A. Interactions between glutamatergic and monoaminergic systems within the basal ganglia—implications for schizophrenia and Parkinson's disease. Trends Neurosci 1990;13:272–6.
[13] Chartoff EH, Heusner CL, Palmiter RD. Dopamine is not required for the hyperlocomotor response to NMDA receptor antagonists. Neuropsychopharmacology 2005;30(1):324–33.
[14] Clineschmidt BV, Martin GE, Bunting PR, Papp NL. Central sympathomimetic activity of (+)-5-methyl-10,11-dihydro-5*H*-dibenzo [d]cyclohepten-5,10-immine(MK-801), a substance with potent anticonvulsant, central sympathomimetic, and apparent anxiolytic properties. Drug Dev Res 1982;2:135–45.
[15] Corbett R, Camacho F, Woods AT, Kerman LL, Fishkin RJ, Brooks K, et al. Antipsychotic agents antagonize non-competitive N-methyl-D-aspartate antagonist-induced behaviors. Psychopharmacology (Berl) 1995;120:67–74.
[16] Deutsch SI, Rosse RB, Mastropaolo J. Behavioral approaches to the functional assessment of NMDA-mediated neural transmission in intact mice. Clin Neuropharmacol 1997;20:375–84.
[17] Dunn RW, Corbett R, Fielding S. Effects of 5-HT1A receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. Eur J Pharmacol 1989;169:1–10.
[18] Fetcho JR, Bhatt DH. Genes and photons: new avenues into the neuronal basis of behavior. Curr Opin Neurobiol 2004;14:707–14.
[19] Flyckt L, Sydow O, Bjerkenstedt L, Edman G, Rydin E, Wiesel FA. Neurological signs and psychomotor performance in patients with schizophrenia, their relatives and healthy controls. Psychiatry Res 1999;86:113–29.
[20] Gattaz WF, Schummer B, Behrens S. Effects of zotepine, haloperidol and clozapine on MK-801-induced stereotypy and locomotion in rats. J Neural Transm Gen Sect 1994;96:227–32.

[21] Gerlai R, Fernandes Y, Pereira T. Zebrafish (*Danio rerio*) responds to the animated image of a predator: towards the development of an automated aversive task. Behav Brain Res 2009;201:318–24.
[22] Gerlai R, Lahav S, Guo S, Rosenthal A. Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. Pharmacol Biochem Behav 2000;67:773–82.
[23] Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 2001;156:117–54.
[24] Geyer MA, Moghaddam B. Animal models relevant to schizophrenia disorders. In: Davis KL, Charney D, Coyle JT, Nemeroff C, editors. Neuropsychopharmacology: the fifth generation of progress. Washington, DC: Lippincott Williams & Wilkins; 2002. p. 689–701.
[25] Giacomini NJ, Rose B, Kobayashi K, Guo S. Antipsychotics produce locomotor impairment in larval zebrafish. Neurotoxicol Teratol 2006;28:245–50.
[26] Guo S. Linking genes to brain, behavior and neurological diseases: what can we learn from zebrafish? Genes Brain Behav 2004;3:63–74.
[27] Ichikawa J, Kuroki T, Dai J, Meltzer HY. Effect of antipsychotic drugs on extracellular serotonin levels in rat medial prefrontal cortex and nucleus accumbens. Eur J Pharmacol 1998;351:163–71.
[28] Irons TD, Macphail RC, Hunter DL, Padilla S. Acute neuroactive drug exposures alter locomotor activity in larval zebrafish. Neurotoxicol Teratol 2010;32:84–90.
[29] Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1999;20:201–25.
[30] Jones DNC, Gartlon JE, Minassian A, Perry W, Geyer M. In: McArthur RA, Borsini F, editors. Developing new drugs for schizophrenia: from animals to the clinic. Animal and translational models for CNS drug discovery volume 1: psychiatric disorders. New York: Elsevier; 2008. p. 199–261.
[31] Kane JM. Schizophrenia. N Engl J Med 1996;334:34–41.
[32] Karcz-Kubicha M, Jessa M, Nazar M, Plaznik A, Hartmann S, Parsons CG, et al. Anxiolytic activity of glycine-B antagonists and partial agonists—no relation to intrinsic activity in the patch clamp. Neuropharmacology 1997;36: 1355–67.
[33] Kari G, Rodeck U, Dicker AP. Zebrafish: an emerging model system for human disease and drug discovery. Clin Pharmacol Ther 2007;82:70–80.
[34] Kiang M, Daskalakis ZJ, Christensen BK, Remington G, Kapur S. Actigraphic measurement of the effects of single-dose haloperidol and olanzapine on spontaneous motor activity in normal subjects. J Psychiatry Neurosci 2003;28:293–9.
[35] Kilts CD. The changing roles and targets for animal models of schizophrenia. Biol Psychiatry 2001;50:845–55.
[36] Laruelle M, Abi-Dargham A. Dopamine as the wind of the psychotic fire: new evidence from brain imaging studies. J Psychopharmacol 1999;13:358–71.
[37] Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. Physiol Behav 2007;90:54–8.
[38] Leysen JE, Janssen PM, Megens AA, Schotte A. Risperidone: a novel antipsychotic with balanced serotonin-dopamine antagonism, receptor occupancy profile, and pharmacologic activity. J Clin Psychiatry 1994;55:5–12.
[39] Lindenmayer JP, Nathan AM, Smith RC. Hyperglycemia associated with the use of atypical antipsychotics. J Clin Psychiatry 2001;62:30–8.
[40] Majewska MD. Steroid regulation of the GABAA receptor: ligand binding, chloride transport and behaviour. Ciba Found Symp 1990;153:83–97.
[41] Manschreck TC, Maker BA, Candela SF. Earlier age of first diagnosis in schizophrenia is related to impaired motor control. Schizophr Bull 2004;30:351–60.
[42] Marquis JP, Audet MC, Doré FY, Goulet S. Delayed alternation performance following subchronic phencyclidine administration in rats depends on task parameters. Prog Neuropsychopharmacol 2007;31:1108–12.
[43] Marx CE, VanDoren MJ, Duncan GE, Lieberman JA, Morrow AL. Olanzapine and clozapine increase the GABAergic neuroactive steroid allopregnanolone in rodents. Neuropsychopharmacology 2003;28:1–13.
[44] Marx CE, Shampine LJ, Duncan GE, VanDoren MJ, Grobin AC, Massing MW, et al. Clozapine markedly elevates pregnenolone in rat hippocampus, cerebral cortex, and serum: candidate mechanism for superior efficacy? Pharmacol Biochem Behav 2006;84:598–608.
[45] McClelland GR, Cooper SM, Pilgrim AJ. A comparison of the central nervous system effects of haloperidol, chlorpromazine and sulpiride in normal volunteers. Br J Clin Pharmacol 1990;30:795–803.
[46] McCreadie RG, Srinivasan TN, Padmavati R, Thara R. Extrapyramidal symptoms in unmedicated schizophrenia. J Psychiatr Res 2005;39:261–6.
[47] McKeown KA, Downes GB, Hutson LD. Modular laboratory exercises to analyze the development of zebrafish motor behavior. Zebrafish 2009;6:179–85.
[48] Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci 1997;17:2921–7.
[49] Murray J. Phencyclidine (PCP): a dangerous drug, but useful in schizophrenia research. J Psychol 2002;136:319–27.
[50] Newcomer JW, Farber NB, Jevtovic-Todorovic V, Selke G, Melson AK, Hershey T, et al. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. Neuropsychopharmacology 1999;20:106–18.
[51] Ninan I, Kulkarni SK. Preferential inhibition of dizocilpine-induced hyperlocomotion by olanzapine. Eur J Pharmacol 1999;368:1–7.
[52] Olney JW, Newcomer JW, Farber NB. NMDA receptor hypofunction model of schizophrenia. J Psychiatr Res 1999;33:523–33.
[53] O'Neill MF, Shaw G. Comparison of dopamine receptor antagonists on hyperlocomotion induced by cocaine, amphetamine, MK-801 and the dopamine D1 agonist C-APB in mice. Psychopharmacology (Berl) 1999;145:237–50.
[54] Parikh V, Terry AV, Khan MM, Mahadik SP. Modulation of nerve growth factor and choline acetyltransferase expression in rat hippocampus after chronic exposure to haloperidol, risperidone, and olanzapine. Psychopharmacology 2004;172:365–74.
[55] Parng C, Seng WL, Semino C, McGrath P. Zebrafish: a preclinical model for drug screening. Assay Drug Dev Technol 2002;1:41–8.
[56] Patton EE, Zon LI. The art and design of genetic screens: zebrafish. Nat Rev Genet 2001;2:956–66.
[57] Peralta V, Cuesta MJ. Motor features in psychotic disorders. II. Development of diagnostic criteria for catatonia. Schizophr Res 2001;47:117–26.
[58] Porsolt RD, Moser PC, Castagne V. Behavioral indices in antipsychotic drug discovery. J Pharmacol Exp Ther 2010;333:632–8.
[59] Ridler K, Veijola JM, Tanskanen P, Miettunen J, Chitnis X, Suckling J, et al. Fronto-cerebellar systems are associated with infant motor and adult executive functions in healthy adults but not in schizophrenia. Proc Natl Acad Sci USA 2006;103:15651–6.
[60] Rujescu D, Bender A, Keck M, Hartmann AM, Ohl F, Raeder H, et al. A pharmacological model for psychosis based on N-methyl-d-aspartate receptor hypofunction: molecular, cellular, functional and behavioral abnormalities. Biol Psychiatry 2006;59:721–9.
[61] Seibt KJ, Oliveira RL, Rico EP, Dias RD, Bogo MR, Bonan CD. Typical and atypical antipsychotics alter acetylcholinesterase activity and *ache* expression in zebrafish (*Danio rerio*) brain. Comp Biochem Physiol C 2009;150: 10–5.
[62] Seillier A, Giuffrida A. Evaluation of NMDA receptor models of schizophrenia: divergences in the behavioral effects of sub-chronic PCP and MK-801. Behav Brain Res 2009;204:410–5.
[63] Senger MR, Rosemberg DB, Rico EP, Arizi MB, Dias RD, Bogo MR, et al. In vitro effect of zinc and cadmium on acetylcholinesterase and ectonucleotidase activities in zebrafish (*Danio rerio*) brain. Toxicol In Vitro 2006;20:954–8.
[64] Simpson SM, Menard JL, Reynolds JN, Beninger RJ. Post-weaning social isolation increases activity in a novel environment but decreases defensive burying and subchronic MK-801 enhances the activity but not the burying effect in rats. Pharmacol Biochem Behav 2010;95:72–9.
[65] Snyder SH. Amphetamine psychosis: a 'model' schizophrenia mediated by catecholamines. Am J Psychiatry 1973;130:61–7.
[66] Su YA, Si TM, Zhou DF, Guo CM, Wang XD, Yang Y, et al. Risperidone attenuates MK-801-induced hyperlocomotion in mice via the blockade of serotonin 5-HT2A/2C receptors. Eur J Pharmacol 2007;564:123–30.
[67] Sumanasa S, Lin S. Zebrafish as a model system for drug target screening and validation. Drug Discov Today Targets 2004;3:89–96.
[68] Swain HA, Sigstad C, Scalzo FM. Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (*Danio rerio*). Neurotoxicol Teratol 2004;26:725–9.
[69] Taylor DM, McAskill R. Atypical antipsychotics and weight gain—a systemic review. Acta Psychiatr Scand 2000;101:416–32.
[70] Voruganti L, Cortese L, Ouyewumi L, Cernovsky Z, Zirul S, Awad A. Comparative evaluation of conventional and new antipsychotic drugs with reference to their subjective tolerability, side effect profile and impact on quality of life. Schizophr Res 2000;43:135–45.
[71] Westerfield M. The zebrafish book. 5th ed. Eugene: University of Oregon Press; 2007.
[72] Whitty P, Clarke M, Browne S, McTigue O, Kamali M, Feeney L, et al. Prospective evaluation of neurological soft signs in first-episode schizophrenia in relation to psychopathology: state versus trait phenomena. Psychol Med 2003;33:1479–84.
[73] Wong AH, Van Tol HH. Schizophrenia: from phenomenology to neurobiology. Neurosci Biobehav Rev 2003;27:269–306.