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Psychopharmacological effects of acute exposure to kynurenic acid (KYNA) in zebrafish

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ABSTRACT

A metabolite of the kynurenine pathway, kynurenic acid (KYNA) is an important endogenous neuromodulator and neuroprotector, that also exerts neurotropic effects following exogenous administration. In humans and animals, KYNA regulates affective and cognitive responses, acting mainly as an antagonist of glutamatergic receptors. However, the complete psychopharmacological profile of KYNA (which includes the activity of several neurotransmitter receptors) is poorly understood, and merit further studies. Aquatic models are rapidly emerging as useful tools in translational psychopharmacology research. Here, we exposed adult zebrafish (*Danio rerio*) to exogenous KYNA for 20 min, and assessed their behavior in the novel tank test. Exposure to KYNA (20 mg/L) in this paradigm evoked overt effects in fish, including decreased latency to enter the top half of the tank, increased number of top entries and longer top duration. In contrast, locomotor activity indices (swimming distance and velocity) were not affected by KYNA in this study. Overall, our results show KYNA has an anxiolytic-like pharmacological effect in zebrafish, and therefore strongly support the utility of zebrafish models in neurotropic drug screening, including drugs acting at central glutamatergic system. Robust phenotypic differences evoked by KYNA, revealed here using three-dimensional (3D) reconstructions of zebrafish locomotion in X, Y and time (Z) coordinates, confirm this notion, also demonstrating the value of 3D-based phenotyping approaches for high-throughput drug screening using zebrafish models.

1. Introduction

Kynurenic acid (KYNA) is the product of tryptophan degradation *via* the kynurenine pathway, which also leads to synthesis of neurotoxic quinolinic acid and as well as tryptamines, such as serotonin and melatonin (Lapin, 2000; Leklem, 1971; Schwarcz et al., 2012). KYNA acts as an antagonist of several brain receptors, including glutamatergic N-methyl-D-aspartate (NMDA) (Ganong and Cotman, 1986), kainate (Coleman et al., 1986), nicotinic (Hilmas et al., 2001; Wu et al., 2010) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Prescott et al., 2006; Schwarcz et al., 2012). In addition to its *exogenous* activity, KYNA is also an important, *endogenously* produced neuromodulator (Lapin, 2000; Vezzani et al., 1991; Wu et al., 1994) and neuroprotector (Amirkhani et al., 2002; Leib et al., 1996; Marosi et al., 2010; Urenjak and Obrenovitch, 2000).

In humans, endogenous levels of KYNA serve as a biomarker for various brain dysfunctions, including Alzheimer's (Hartai et al., 2007) and Parkinson's disorders (Hartai et al., 2005; Turski et al., 1991), as well as schizophrenia and depression (Kocki et al., 2012; Schwarcz et al., 2012). Paralleling clinical data, various pharmacological or experimental (e.g., chronic unpredictable stress) manipulations markedly alter endogenous levels of KYNA in rats (Wu and Schwarcz, 1996) and mice (Laugeray et al., 2011). Collectively, this strongly supports the role of KYNA as an important modulator of human and animal CNS functions (Lapin, 2000; Vezzani et al., 1991; Wu et al., 1994).

Exogenous administration of KYNA at various doses in rodent models also evokes biological responses, inducing ataxia, stereotyped behavior and learning/memory deficits (Klein et al., 2004; Maj et al., 1994; Vecsei and Beal, 1990, 1991). Suggesting a potential for anxiolytic-like action of this compound, central administration of KYNA in animals can also produce sedative and anti-stress responses (Yoshida et al., 2012; Dennison et al., 1992). Indeed, as an anti-excitatory modulator, KYNA evokes robust anticonvulsant and anxiolytic effects (Filippini et al., 1996; Foster et al., 1984; Lapin, 1998, also see Rasmussen et al., 1991), reducing the anxiogenic effects of caffeine, pentylenetetrazole, yohimbine and quinolinic acid in the mouse dark–light box, and showing an anxiolytic profile in the elevated plus-maze test (Lapin, 1998; Lapin et al., 1990; Schmitt et al., 1990).

Despite recent clinical and pre-clinical findings, the psychopharmacological profile of KYNA remains poorly understood. The development of novel high-throughput tests and expanding the range of model organisms are important strategic directions for screening small molecules and identifying potential drug candidates (Stewart et al., 2012a; Wong et al.,

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2010b). In addition, multiple KYNA analogs with varying pharmacological profiles (Fuvesi et al., 2004; Robinson et al., 1985; Wardley-Smith et al., 1989) merit further *in-vivo* screening for neurobehavioral effects.

Among several model species, zebrafish (*Danio rerio*) offer a low-cost, high-throughput and sensitive model that complements existing rodent animal models of brain disorders. Possessing high physiological similarity to humans (Alsop and Vijayan, 2009; Gonzalez-Nunez et al., 2006; Lillesaar, 2011; Panula et al., 2010; Panula et al., 2006; Sundvik and Panula, 2012; Tay et al., 2011), robust behavioral responses and a fully characterized genome (Beliaeva et al., 2010; Cheng et al., 2011), zebrafish are rapidly emerging as a useful organism for screening various neuroactive compounds (Cachat et al, 2011).

Previous studies in larval zebrafish have shown that KYNA modulates swimming (Buss and Drapeau, 2001) and suppresses glutamatergic activity (Patten and Ali, 2007; Zhu et al., 2009), suggesting the importance of KYNA in zebrafish brain mechanisms, including motor and behavioral control. Pilot studies using KYNA as an anticonvulsant agent have also demonstrated its ability to reduce epilepsy-like responses in zebrafish larvae (Baraban et al., 2007), confirming that zebrafish are indeed likely to be a sensitive *in-vivo* model for testing various central effects of KYNA and related compounds.

Emphasizing the role of specific receptor systems in the drug-induced phenotypes, recent studies have already reported the effects of lysergic acid diethylamide (LSD), mescaline, phencyclidine (PCP), dizocilpine (MK-801), ketamine, ibogaine, morphine and salvinorin A in adult zebrafish (Cachat et al., 2013; Ewald, 2009; Grossman et al., 2010a; Sison and Gerlai, 2011; Stewart et al., 2012b; Zakhary et al., 2011). Given a growing recognition of the importance of KYNA and kynurenic pathway as key modulators and potential drug targets in biological psychiatry (Lapin, 2000; Schwarcz et al., 2012; Stone et al., 2012), and capitalizing on fish's robust behavioral phenotypes highly sensitive to pharmacological manipulations, the present study examined the pharmacological effects of acute exposure to KYNA in adult zebrafish.

2. Methods

2.1. Animals and housing

A total of 80 adult (5–8 month old) "wild type" short-fin zebrafish (~50:50 male:female ratio) were obtained from a commercial distributor (50 Fathoms, Metairie, LA). All fish were given at least 14 days to acclimate to the laboratory environment and housed in groups of 20–30 fish per 40-L tank at the Animal Core of the ZENEREI Institute LLC. Tanks were filled with filtered system water and maintained at 25–27 °C. Illumination (1000–1100 lx) was provided by ceiling-mounted fluorescent lights on a 12-h cycle (on: 6.00 h, off: 18.00 h) according to the standards of zebrafish care (Westerfield, 2000). All animals used in this study were experimentally naïve and fed Tetramin Tropical Flakes (Tetra USA, Blacksburg, VA) twice a day. Following behavioral testing, the animals were euthanized in 500 mg/L Tricaine (Sigma-Aldrich, St. Louis, MO) and dissected on ice for further analysis. Animal experimentation in this study fully adhered to national and institutional guidelines and regulations, and was approved by the ZENEREI Institute.

2.2. Behavioral testing

Behavioral testing was performed between 11.00 and 15.00 h using tanks with water adjusted to the holding room temperature, assessing zebrafish behavior in the novel tank test. Prior to testing, fish were pre-exposed in a 1-L plastic beaker for 20 min to either drug-treated or drug-free vehicle, 0.1% solution of dimethyl sulfoxide (DMSO, Fisher Scientific, Waltham, MA, commonly used in zebrafish behavioral assays) (Goldsmith, 2004). Fish were then exposed to the novel tank test, used to assess zebrafish anxiety and locomotion (Levin et al., 2007; Stewart et al., 2011a; Stewart et al., 2011b). The apparatus used consisted of a 1.5-L trapezoidal tank (15 cm height × 28 cm top × 23 cm bottom × 7 cm width; Aquatic Habitats, Apopka, FL) maximally filled with water and divided into two equal virtual horizontal portions by a line marking the outside walls (Fig. 1).

During testing, zebrafish behavior was recorded by 2 trained observers blind to the treatments, who used the stopwatch and manually scored different behavioral endpoints (inter- and intra-rater reliability in all experiments > 0.85), which included the latency to reach the top half of the tank (s), time spent in top (s), number of transitions to top, as well as the number and duration (s) of freezing bouts. Freezing was defined as a total absence of movement, except for the gills and eyes, for > 2 s. Trials were also recorded to a computer using a USB webcam (2.0 megapixels, Gigaware, UK) and analyzed by Ethovision XT8.5 (Noldus IT, Wageningen, Netherlands), assessing swimming bouts, swimming duration (s), latency to top (s), top entries, time in top (s), distance traveled (m), and average velocity (m/s), as described elsewhere (Cachat et al., 2012; Grossman et al., 2010b). In order to assess intra-session (within-trial) habituation, reflecting spatial working memory of zebrafish, we examined their responses over a 6-min trial, analyzing the per-min distribution of behavioral endpoints mentioned above, and comparing the first *vs.* last 3 min (s) and the first *vs.* last (6th) minute values for each endpoint, as described previously (Wong et al., 2010a).

2.3. Pharmacological manipulations

The doses for KYNA (Sigma-Aldrich, St. Louis, MO) were chosen based on pilot studies (see below) as well as conversions from rodent literature. A standard 20-min pre-treatment time was chosen in our laboratory as the standard treatment test based on previous experiments with other psychotropic drugs (Cachat et al., 2013; Sison and Gerlai, 2011; Zakhary et al., 2011). In the pilot experiment, fish were individually pre-exposed to various doses of KYNA (5, 10, 20 and 40 mg/L) or drug-free vehicle (0.1% vol/vol DMSO) for 20 min, and tested in the standard 6-min novel tank test (n = 10–15 per group). Overall, the doses of 5 and 10 mg/kg did not significantly affect zebrafish behavior in any of the behavioral measures assessed, albeit producing a non-significant trend towards increased time spent in top and the number of top transitions ($P > 0.05$, *U*-test *vs.* control). In contrast, higher doses of KYNA (20 and 40 mg/L) both produced significant effects on these behaviors ($P < 0.05$, *U*-test), with the dose of 20 mg/L being the most effective. As this dose was deemed evoking the most overt behavioral effects, we used it for further detailed analyses in our study, utilizing a larger cohort of zebrafish (n = 20 per group).

2.4. Generation of spatiotemporal traces

During manual observation, videos were recorded in MPEG1 format with the maximum sample rate 30 fps for each trial by auto-focusing 2.0 MP USB webcams, placed 50 cm in front of or on top of the tanks, and attached to laptop computers. For each experiment, raw track data was exported into Excel spreadsheets, pre-processed and formatted to generate 3D swim path reconstructions, as described previously (Cachat et al., 2010; Cachat et al., 2011). Temporal 3D reconstructions were created in a Scatter 3D Color plot, in which X-center, time, and Y-center were attributed to the X, Y- and Z-axes, respectively. Dependent variables were actively cycled across the path using the color attribute, and tracks were explored using rotation and zooming features. For comparison, axis ranges were standardized, and reconstructions were saved as image files. Generated traces were independently rated, on a consensus basis from 1 to n, by three trained observers blinded to the treatments, as described elsewhere (Cachat et al., 2011; Grossman et al., 2010b; Kyzar et al., 2012). This visual assessment was based on general similarity of generated 3D traces (to each other) in terms of spatial distribution of activity (top/bottom), overall amount of locomotion (high/low), and pattern of observed activity (typical/aberrant)

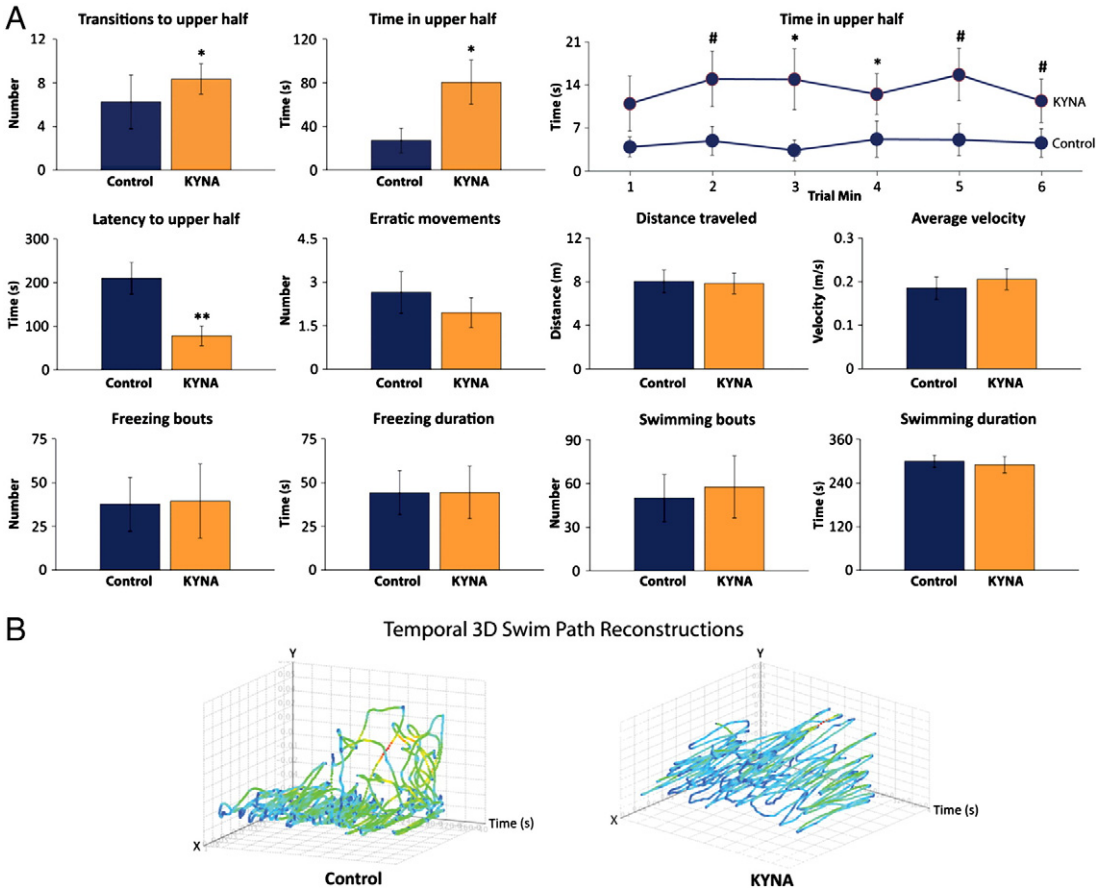

Fig. 1. Behavioral effects of acute 20-min kynurenic acid (KYNA) exposure in adult zebrafish tested in the novel tank. (A) Behavioral endpoints were obtained in the standard 6-min novel tank test for 20 mg/L KYNA (n = 20 per group). (B) Temporal 3D graphs plotted XY-coordinates (generated in Ethovision XT8.5) on respective XY-axes, with experimental time plotted across the Z-axis (Cachat et al., 2010; Cachat et al., 2011). Track color reflects changes in velocity (m/s; blue to green = lower velocity, yellow to red = higher velocity). $^{\#}P = 0.05–0.08$ (trend), $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control; *U*-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

within each group. Generally characterized by a tight clustering of the independent raters' scores, this approach enables a rapid selection of the *median* trace, to be used as representative of the group for the visual illustration (Fig. 1B) reflecting the global spatiotemporal pattern of zebrafish swimming (Cachat et al., 2011; Grossman et al., 2010b; Kyzar et al., 2012).

2.5. Statistical analyses

The behavioral data was analyzed using ANOVA (factors: time, dose) or Wilcoxon–Mann–Whitney *U*-test (with or without the Bonferroni correction, where appropriate). Inter- and intra-rater reliability for the observers was determined by Spearman correlation. Data were expressed as mean ± SEM, and significance was set at $P < 0.05$ in all experiments of this study. In habituation assays, data was analyzed using a two-sample paired *U*-test for significance between the initial (*i.e.*, min 1 or first 3 min) and the last observation time (min 6 or last 3 min, respectively), followed by the Bonferroni correction, where appropriate. Significance was set at $P < 0.05$ for *U*-test, but was adjusted accordingly for Bonferroni corrected post-hoc tests.

3. Results

In the novel tank test, acute (20-min) exposure to 20 mg/L KYNA induced a generally anxiolytic-like effect, significantly decreasing latency to the top half of the tank, and increasing the number of top entries and top duration (s) (Fig. 1A). Manual observations also paralleled the 3D traces generated through the video-tracking analysis, with KYNA-treated fish entering the top of the tank sooner and spending a greater duration of the trial there, while not altering general locomotion measures, such as velocity and distance traveled (Fig. 1A and B). KYNA also did not evoke overt circling behavior in this study, which was not observed in both control and experimental groups. Further analysis demonstrated no significant time or time × drug effects following exposure to 20 mg/L KYNA (ANOVA $P > 0.05$ for time and time × drug effects), suggesting that KYNA treatment does not uniquely affect the temporal patterning of zebrafish behavior. KYNA also did not significantly alter zebrafish habituation in the novel tank, since both first *vs.* last 3 min (s) and the first *vs.* 6th minute values for each endpoint were relatively similar between controls and KYNA-exposed fish ($P > 0.05$, *U*-test for all measures).

4. Discussion

This is the first study reporting the behavioral anxiolytic effects of KYNA in zebrafish (Fig. 1). While previous pilot investigation of circling and shoaling behavior following 1-h KYNA exposure (1.9, 19 and 190 mg/L in 1% DMSO) in adult zebrafish yielded no effects (Ewald, 2009), our results indicate that 20 mg/L KYNA produces an anxiolytic-like profile (increased top swimming) in zebrafish in the novel tank paradigm, without affecting general locomotor activity levels, as assessed by unaltered distance traveled and velocity (Fig. 1A). In general, this profile is consistent with the known anti-anxiety effects of KYNA in various other model organisms (Lapin, 1998; Lapin et al., 1990; Schmitt et al., 1990). The fact that KYNA exerts consistent behavioral effects in different species supports the anxiolytic profile of this compound, also emphasizing the utility of zebrafish high-throughput

in-vivo screens to study the psychopharmacology of KYNA and related compounds.

Recent evidence suggests that KYNA may act *via* multiple brain receptors, including the alpha7 nicotinic receptors (Stone, 2007), (but see conflicting data in (Dobelis et al., 2012)) which may contribute to some neurophysiological effects of the drug. Nevertheless, as already mentioned, the key, well-established mechanism of KYNA action is the antagonism of glutamatergic receptors (Ganong and Cotman, 1986; Schwarcz et al., 2012; Wu et al., 1994). It was therefore interesting to compare the effects of KYNA with the activity of other glutamatergic antagonists in zebrafish models. Several agents sharing this mode of action have recently been tested in zebrafish. For example, ketamine (Riehl et al., 2011), PCP (Kyzar et al., 2012), MK-801 (Sison and Gerlai, 2011) and ibogaine (Cachat et al., 2013) all increase top-swimming and exert anxiolytic-like action in various zebrafish models. Given elevated top swimming produced by KYNA here (Fig. 1), this collectively implies a *shared* anxiolytic profile of various NMDA antagonists in zebrafish, further supporting the utility of aquatic models for *glutamatergic* drug discovery and small molecule screening.

Importantly, clinical and rodent literature generally shows anxiolytic effects associated with NMDA antagonism (Bubser et al., 1992; Corbett et al., 1995; Engin et al., 2009; Garcia et al., 2009; Inta et al., 2012; Irwin and Iglewicz, 2010; Kehne et al., 1991; Laugeray et al., 2011; Liu et al., 2009; Loss et al., 2012; Louzada-Junior et al., 1992; Plaznik et al., 1994; Riaza Bermudo-Soriano et al., 2012; Turgeon et al., 2011). Taken together, this raises the possibility that zebrafish may represent efficient and sensitive screens for anxiolytic responses mediated through the central glutamatergic system. On the other hand, the above-mentioned similarity of drug-induced profiles across different species strongly supports the translational value of zebrafish models for targeting evolutionarily conserved molecular pathways, including mimicking human phenotypes associated with modulation of anxiety by glutamatergic compounds.

Since KYNA and several other glutamatergic antagonists can also impair reference and working memory in rats (Klein et al., 2004), we assessed the effects of KYNA on zebrafish spatial working memory in the habituation task. While KYNA exposure did not significantly affect habituation in this study, this profile was similar to the effects of other anxiolytics (*e.g.*, fluoxetine and ethanol; Wong et al., 2010a) on zebrafish habituation, albeit deviating from some rodent findings (File and Mabbutt, 1990; Kaneko et al., 2007). Inter-species differences in habituation to novelty may explain these observations, since rodents generally reduce locomotion with increasing familiarity to a novel environment (Leussis and Bolivar, 2006; Mar et al., 2000), while zebrafish do the opposite (Best et al., 2008; Wong et al., 2010a). Clearly, more specific memory/learning tasks may be necessary to more fully investigate the effects of KYNA on zebrafish cognitive responses (also see recent innovative 'integrative', more global approaches to zebrafish cognitive phenotyping in (Stewart et al., 2012b)).

There were several other limitations of this study. For example, since we only focused on anxiety-, habituation- and motor-related phenotypes, further investigation may assess other neurobehavioral domains in zebrafish. As KYNA and other glutamatergic antagonists may modulate learning/memory and social behavior in rodents (Hlinak and Krejci, 1995), future analysis of KYNA modulation of those behaviors in zebrafish is warranted. Another aspect to consider is the potential role of sex- and strain-differences in zebrafish behaviors. This study used wild type short-fin zebrafish with an approximate 50:50 male/female ratio, similar to multiple published studies from other groups (Khor et al., 2011; Pather and Gerlai, 2009; Rosemberg et al., 2011). Given known sex/strain differences in behavioral responses of zebrafish to various drugs (Dlugos et al., 2011; Vital and Martins, 2011), the analyses of these factors in KYNA effects require further investigation. Since the role of KYNA in fish biology remains poorly understood, it will also be important to examine the role of endogenous *vs.* exogenous KYNA levels in the observed responses, as well as to assess their sustainability and the potential long-term (*e.g.*, delayed) effects of KYNA administration.

Furthermore, we focused on the acute effects of KYNA here, and therefore potential differences in acute and chronic KYNA effects in zebrafish merit further scrutiny, especially given its well-known effects on neuroprotection (Andine et al., 1988), brain plasticity (Schwarcz et al., 2012) and long-term responses in rodents (Dennison et al., 1992; Maj et al., 1994). The potential of drug–drug interaction, especially targeting possible additive/synergistic interactions between KYNA and other anti-glutamatergic agents, may be another application for zebrafish-based screens developed here. Likewise, while KYNA and other kynurenines form an evolutionarily conserved molecular pathway (Schwarcz et al., 2012), they play a key role in both the regulation of brain processes and peripheral (*e.g.*, immune or metabolic) mechanisms. Therefore, their central and peripheral modulation in zebrafish merits further studies. Also relevant here is the ability of KYNA to cross the blood–brain barrier (BBB). For example, KYNA does not readily cross the BBB in rodents (Fukui et al., 1991), necessitating the use of its analogs that cross this barrier more easily (Fuvesi et al., 2004), or applying KYNA centrally (Ericson et al., 1990; Fuvesi et al., 2004; Schmitt et al., 1990; Yoshida et al., 2012), in order to exogenously modulate brain phenotypes. At the same time, the fact that various rodent studies (Filippini et al., 1996; Lapin, 1998; Lapin et al., 1990) successfully used i.p., i.v. or s.c. administration of KYNA, indicates that psychopharmacological effects of this drug can be evoked exogenously and following systemic treatment. In zebrafish, systemic administration of various pharmacological compounds by immersion was efficient for their crossing the BBB (Watanabe et al., 2012), and this aspect may underlie the behavioral effects evoked here by KYNA (Fig. 1). Thus, the possibility of using zebrafish for *in-vivo* small molecule testing becomes particularly promising, given the ease and throughput of systemic drug administration *via* immersion in this model, such as used here.

Moreover, our analyses reveal interesting aspects of the efficacy of KYNA (relative to other glutamatergic antagonists) across several different species (Table 1). For example, in the present study, behavioral effects were observed acutely following a 20-mg/L treatment with KYNA (Fig. 1). Based on published data (Table 1), this exogenous dose was equally potent to ketamine and ibogaine, but ~10 times less potent than PCP and ~100 times less potent than MK-801. In rodents, exogenous KYNA appears to be slightly less potent than ketamine and ibogaine, but again was markedly less potent than PCP (30–40 times) and MK-801 (200 times). Taken together, this indicates that the effects of KYNA and other glutamatergic antagonists in fish generally parallel those observed in mammals, with the ranking of relative efficacy (exogenous MK-801 > PCP > ketamine, ibogaine > KYNA) similar across different species (Table 1). The lack of drug-evoked circling behavior here and in earlier observations (Ewald, 2009) was somewhat surprising, but is generally in line with a lower glutamatergic antagonism produced by KYNA relative to other NMDA agents, such as ketamine, MK-801 and PCP, which all induce overt circling in both zebrafish and rodent models (see Ewald, 2009; Kyzar et al., 2012; Riehl et al., 2011 for details).

Notably, the ability of KYNA to evoke physiological (anti-glutamatergic) effects in larval models (Buss and Drapeau, 2001; Patten and Ali, 2007) further supports the utility of zebrafish to study KYNA-induced phenotypes. The robust anxiolytic phenotypes identified for KYNA in this study in adult zebrafish (Fig. 1) are similar to profiles evoked by other glutamatergic antagonists, emphasizing the translational value of zebrafish models for psychopharmacology research. Given the growing importance of glutamatergic compounds in biological psychiatry (Javitt, 2004; Riaza Bermudo-Soriano et al., 2012; Yasuhara and Chaki, 2010), the discovery of glutamatergic drug targets becomes a critical task. As our present study suggests (also see fish data in Table 1), zebrafish can offer a promising and sensitive novel model for achieving this goal. However, given the possibility of multiple other receptor

Table 1
Comparison of effective acute behavioral doses of *exogenously* administered kynurenic acid (KYNA) and other glutamatergic antagonists in various experimental animal models.

Species	Dizocilpine (MK-801)	Phencyclidine (PCP)	Ketamine	Ibogaine	KYNA
Zebrafish	0.1 mg/kg (Sison and Gerlai, 2011) 0.02, 0.2 mg/kg (Swain et al., 2004)	0.5, 1, 3 mg/L (Cachat et al., 2013)	20, 40 mg/L (Riehl et al., 2011)	10, 20 mg/L (Cachat et al., 2013)	20 mg/L (Fig. 1)
Mice	0.0025, 0.05, 0.1, 0.2 mg/kg (Jessa et al., 1996) (Brosnan-Watters et al., 1996) (Hlinak and Krejci, 2006)	5 mg/kg (Bird et al., 2001)	20, 30, 50, 100, 150 mg/kg (Irifune et al., 1991)	10, 40 mg/kg (Popik and Wrobel, 2001)	200 mg/kg (Lapin, 1998) 40, 50 mg/kg (Filippini et al., 1996)
Rats	0.2 mg/kg (Andine et al., 1999) 0.2, 0.5 mg/kg (al-Amin and Schwarzkopf, 1996)	3 mg/kg (Wessinger et al., 1985)	30 mg/kg (Becker and Grecksch, 2004)	30 mg/kg (Helsley et al., 1997)	100 mg/kg (Chess et al., 2007) 150 mg/kg (Bespalov et al., 1994) 50, 200 mg/kg (Maj et al., 1994) 30 mg/kg (Hlinak and Krejci, 1995)
Non-human primates	0.003–0.075 mg/kg (Buffalo et al., 1994)	0.13–0.18 mg/kg (Frederick et al., 1995)	1, 2.5, 5 mg/kg (Shiigi and Casey, 1999)	5–25 mg/kg (Kubiliene et al., 2008)	N/A[a]

[a] N/A: no available published literature.

targets for KYNA (*e.g.*, Hilmas et al., 2001; Wu et al., 2010), zebrafish models may be useful for investigating these additional neuroactive pathways as well.

Overall, our results show high sensitivity of zebrafish to exogenously administered KYNA, revealing an anxiolytic pharmacological profile of this compound, consistent with its known action in clinical and rodent studies. Finally, robust phenotypic differences revealed here using 3D reconstructions and visualization of zebrafish locomotion (Fig. 1B), further confirm this notion, also demonstrating the utility of 3D-based phenotyping approaches for high-throughput drug screening in adult zebrafish (Cachat et al., 2010; Cachat et al., 2011). Collectively, these findings strongly support the developing utility of zebrafish models in *in-vivo* neurotropic drug screening and drug discovery.

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