Introduction

Startle reflex is a universal sensorimotor response that is rapidly produced after the initiation of a sudden or strong stimulus (Abel et al. 1998; Geyer et al. 1990). This primitive reflex displays several forms of plasticity such as prepulse inhibition (PPI) and habituation which are important that they allow the nervous system to filter out irrelevant stimuli (Basnet et al. 2019). Since the past few decades, plasticity of startle reflex has been the focus of interest in a multitude of psychiatric and neurological diseases where the functions of sensorimotor pathways are thought to be impaired, such as schizophrenia (Bolino et al. 1994, 1992; Geyer & Braff 1982; Parwani et al. 2000). The sensitivity of PPI and habituation to pharmacological manipulations...
habituation response in the current work, we evaluate whether tap stimuli can induce becomes sealed at 5 dpf (O’Brown et al. 2019). In the only by 10 dpf (Fleming et al. 2013), their midbrain barrier blood-brain barrier in zebrafish larvae is fully developed habituation at 6 dpf (Basnet et al. 2019). Although the neurobehavioral effects due to environmental toxicants associated to human diseases, but more so for the studies zebrafish to study impairment in habituation response However, this startle modality is less popularly in use in (Eddins et al. 2010; Glazer et al. 2018; Rice et al. 2011). Consistency and reproducibility of the tap stimuli based on solenoid was later developed to improve the evo...stimulus, which is acoustic startle. These studies require dedicated startle apparatus for generating acoustic stimuli of varying intensities and frequencies, as well as for controlling and measuring the direction and the motion of acoustic particles (Bhandiwad et al. 2013; Burgess & Granato 2007). Importantly, the experiments need to be performed in sound-attenuated chambers (Bhandiwad et al. 2018, 2013; Burgess & Granato 2007) to prevent interference of external noise and vibrations. While these studies often provided us with reliable and crucial insights on the sensorimotor function deficits endowed by the disease, the startle apparatus is rather costly and complicated, and considerable knowledge is required for setting up and performing the experiments.

Mechanical or tap stimulus presents as a simpler and more economical modality for studying startle response in zebrafish. In earlier studies, mechanical startle was generated by using a rubber hammer or by releasing a masking tape to a flat surface (Chanin et al. 2012). These methods were shown to produce adequate stimulus to evoke startle response in zebrafish. Automated method based on solenoid was later developed to improve the consistency and reproducibility of the tap stimuli (Eddins et al. 2010; Glazer et al. 2018; Rice et al. 2011). However, this startle modality is less popularly in use in zebrafish to study impairment in habituation response associated to human diseases, but more so for the studies of neurobehavioral effects due to environmental toxicants (Eddins et al. 2010; Glazer et al. 2018; Rice et al. 2011).

Zebrafish has been demonstrated to produce startle response as early as 5 days post fertilization (dpf), and habituation at 6 dpf (Basnet et al. 2019). Although the blood-brain barrier in zebrafish larvae is fully developed only by 10 dpf (Fleming et al. 2013), their midbrain barrier becomes sealed at 5 dpf (O’Brown et al. 2019). In the current work, we evaluate whether tap stimuli can induce habituation response in the MK801-induced schizophrenia model of zebrafish at 6 dpf using our custom-built startle apparatus.

**MATERIALS AND METHODS**

**ANIMALS**

Embryos were obtained from natural spawning of AB wild-type zebrafish (Danio rerio). They were reared in 96-well microtiter plates in embryo (E3) medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl, and 0.33 mM MgSO, pH 7.4) upon collection. Unfertilized and dead embryos were removed on the next day. The remaining embryos were maintained at 28 °C on a 14:10 light: dark cycle. To prevent the medium in the wells from drying out, the E3 medium was replenished daily until the days of experiments. All procedures performed in this study were approved by UTAR Scientific and Ethical Review Committee.

**DRUG TREATMENT**

Dizocilpine hydrogen maleate (MK801), a noncompetitive NMDA receptor antagonist was purchased from Sigma-Aldrich (St. Louis, USA). MK801 was dissolved in DMSO and diluted in E3 medium to a final concentration of 0.1% DMSO. On 6 dpf, a group of larval zebrafish (n = 18) was treated with 20 µM of MK801 for 3 h to model the symptoms of schizophrenia (Chen et al. 2010). Larvae in the control group (n = 18) was applied with 0.1% DMSO for 3 h.

**STARTLE APPARATUS**

The startle apparatus consists of a single swim arena that is 30 mm in diameter and 10 mm in height. A 9 V push and pull solenoid is attached to the side of the arena to provide sudden taps. The two are mounted on a solid platform that holds them in place. The solenoid is toggled on and off by an Arduino Uno microcontroller that is programmed to control the timing of the taps. A recording camera at 30 frames per second was centrally positioned above the arena, 8 cm above the water level. A backlight illuminator is attached underneath the stage to provide additional lighting along with the fluorescent ceiling lights for video recording. The setup and the circuit diagram for the startle apparatus are illustrated in Figures 1 and 2, respectively.

**STARTLE HABITUATION ASSAY**

Larvae were transferred to the swim arena of the startle apparatus that was filled with 3 mL of E3 medium following drug treatment. They were allowed to acclimate in the arena for at least 30 min prior to the test. Each
larva was subjected to 36 consecutive taps at the preset intervals. Videos were captured and processed offline using ToxTrac (Rodriguez et al. 2018) for fish tracking and activity analysis. All studies were performed between 11 am and 4 pm as the activity of zebrafish larvae was found to be most stable within these hours (MacPhail et al. 2009).

STATISTICAL ANALYSIS
The statistical analysis was performed using Prism (Graphpad software Inc., San diego, CA). Welch’s t-test was used to compare the difference in the area under the curve (AUC) between the control and the MK801-treated larvae. Statistical power analysis was performed
using G*Power (version 3.1.9.4; Dusseldorf University, Dusseldorf, Germany) to evaluate the performance of the current experiment.

RESULTS

Tap stimuli induced startle response in zebrafish, measured as the increase in distance moved across the swim arena compared to baseline, in both control and MK801-treated groups (Figure 3(A)). However, unlike the control, the response of the MK801-treated larvae did not habituate over startle trials. To quantify the overall startle response, the AUC was calculated (Best et al. 2008). The AUC of the MK801-treated larvae was significantly higher compared to the control (Figure 3(B), Welch’s t-test, $p<0.0001$), confirming an impairment in the habituation response. Statistical power analysis showed that the study has a power of 99.92%, indicating that our sample size assured an adequate power to detect at least 24% difference in the startle response of the two groups (assuming a type I error of 0.05).

Apart from the difference in the travelling distance, larvae from the two groups also demonstrated different swimming trajectory. Larvae from the control group exhibited a relatively ordered swimming pattern (Figure 3(C)) while larvae from the MK801-treated group swam chaotically through the arena without any deterministic

![Figure 3](image-url)

**FIGURE 3.** MK801 impaired habituation response in larval zebrafish. (A) Distance moved against startle trials for larvae from control and MK801-treated groups. For visual clarity purpose only the first 16 trials were shown. (B) Comparison of AUC of the two treatment groups. Data shown in (A) and (B) are mean ± SEM ($n=18$/data point) (C and D) Representative activity trajectory and occupancy plots for control (C) and MK801-treated (D) larvae, respectively. Color bar indicates how frequent a larva occupied a particular zone in the arena.
patterns (Figure 3(D)). Note that larvae from the control group spent a longer time at the center of arena, while those from the MK801-treated group tend to linger more frequently near the periphery of the arena.

**DISCUSSION**

This study provides a brief and simple technique for assessing the startle and habituation responses in larval zebrafish. As of acoustic startle, the tap stimuli produced using our custom apparatus adequately evoked startle response in larval zebrafish that habituated after the first few taps. Previous studies based on acoustic startle have shown that the rate of habituation varied with inter-trial intervals (ITIs), with a more rapid habituation response seen at a shorter ITI (Best et al. 2008; Thompson & Spencer 1966). We similarly observed the same with mechanical startle and found that larval zebrafish habituated the greatest and fastest when subjected to tap stimuli at 1 Hz (data not shown).

There is strong evidence for deficient habituation among a substantial portion of schizophrenia patients (Bolino et al. 1994, 1992; Parwani et al. 2000). A significant impairment of habituation response was likewise observed in our pharmacological model of schizophrenia induced using MK801. Additional analysis of the swimming trajectories showed that the larvae treated with MK801 displayed a rather chaotic swimming pattern compared to the control in response to tap stimuli, mimicking the disordered or ataxia movements seen in schizophrenia rat models (Marcotte et al. 2001) and patients (Picard et al. 2008). Despite of the randomized swimming pattern, larvae administered with MK801 was found to spend a longer time at the periphery of the swim arena, reflecting a state of anxiety. Remarkably, the startle and habituation responses exhibited by MK801-treated larvae were also comparable to that elicited by acoustic startle in the zebrafish model of schizophrenia induced using donepezil (Best et al. 2008), suggesting tap stimuli to be an effective modality for assessing information processing deficits in schizophrenia.

Like most of the automated commercial and custom mechanical startle apparatus (Eddins et al. 2010; Glazer et al. 2018), DC solenoid is used as the actuator for producing sudden taps in our study. However, instead of controlling the timing of the solenoid using software running on the computer, the timing of the stimuli was pre-programmed in the microcontroller to increase the portability. For high throughput analysis, the system can be modified by increasing the number of solenoids and by adding relays at the output pins of the microcontroller to control the individual solenoids. Importantly, since the design uses only off-the-shelf components, it can be readily and be inexpensively reproduced in other laboratories.

**CONCLUSION**

Overall, our results suggest that mechanical startle is an appropriate and reliable alternative for studying habituation response in the schizophrenia model of zebrafish. Although the present technique was tested only in the schizophrenia model induced using MK801, it may be extended for the studies of other pharmacological models in zebrafish that associated with impaired habituation response.

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