A microfluidic device to study electrotaxis and dopaminergic system of zebrafish larvae

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The zebrafish is a lower vertebrate model organism offering multiple applications for both fundamental and biomedical research into the nervous system from genes to behaviour. Investigation of zebrafish larvae's movement in response to various stimuli, which involves the dopaminergic system, is of interest in the field of sensory-motor integration. Nevertheless, the conventional methods of movement screening in Petri dishes and multi-well plates are mostly qualitative, uncontrollable, and inaccurate in terms of stimulus delivery and response analysis. We recently presented a microfluidic device built as a versatile platform for fluid flow stimulation and high speed time-lapse imaging of rheotaxis behaviour of zebrafish larvae. Here, we describe for the first time that this microfluidic device can also be used to test zebrafish larvae's sense of the electric field and electrotaxis in a systemic manner. We further show that electrotaxis is correlated with the dopamine signalling pathway in a time of day dependent manner and by selectively involving the D2-like dopamine receptors. The primary outcomes of this research opens avenues to study the molecular and physiological basis of electrotaxis, the effects of known agonist and antagonist compounds on the dopaminergic system, and the screen of novel pharmacological tools in the context of neurodegenerative disorders. We propose that this microfluidic device has broad application potential, including the investigation of complex stimuli, biological pathways, behaviors, and brain disorders. Published by AIP Publishing. <https://doi.org/10.1063/1.5016381>

I. INTRODUCTION

Danio rerio or zebrafish is the simplest vertebrate model organism with high genomic homology to humans and wide usage in drug discovery,¹ brain mapping,² disease modeling,^{3,4} and behavioral screening^{5–7} applications. At larval stages, zebrafish offer several unique advantages including small size, ease of manipulation and handling,⁸ optical transparency,^{2,9–11} and rapid and low-cost development in a laboratory setting,^{1,4,12,13} which make it an appealing model for a variety of high throughput biological assays.^{5,14–17} Moreover, by the age of 5 day-post-fertilization (dpf), zebrafish larvae fully develop numerous sensory-motor functions, making them ideal organisms for systemic behavioral and neural investigations.^{2,3,9,12,18,19} However, in neurobehavioral studies, conducting quantitative analysis of zebrafish larvae's behavior^{20–24} has proven to be a critical and challenging task.

Some of the most widely studied behaviors of zebrafish larvae are prey capturing,²⁵ avoidance,¹⁶ escape,¹² phototaxis,²⁶ visuomotor,¹⁹ and orientation.^{22,23,27,28} These studies have two major components in common, i.e., application of an external stimulus and consequent investigation of larvae's body movements. One of the most popular conventional techniques to study movement phenotypes is the use of agarose gel to partially immobilize the larvae for exposure to different stimuli such as sound^{29,30} and optical^{25,31} signals, followed by quantification of

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mouth, eyes, and tail movements. A few disadvantages associated with conventional techniques are time-consuming agar preparation and thermal processes, expertise needed for larvae manipulation, irreversibility, and possible oxygen deprivation and morphological damages to the zebrafish.^{32,33} Another conventional technique adopted is the behavioral investigation of freely swimming zebrafish larvae inside Petri dishes and multi-well plates. Despite its advantages in terms of revealing several complex motor behaviors such as re-orientation, O-bends,³⁴ and avoidance responses,¹⁶ this technique is limited in controlling the stimuli and quantification of movement phenotypes inside the complex 3D environment of the plate.

Microfluidic technology has recently emerged as a powerful tool for neural and behavioral investigation of small model organisms such as *C. elegans*,^{35–42} *D. melanogaster*,^{43–46} and *D. rerio*.^{47–53} Most recently, various microfluidic techniques have been introduced for precise manipulation, stimulation, and monitoring of zebrafish larvae's behavior.^{51–53} For instance, neural and motor responses of zebrafish larvae have been investigated inside a microfluidic chip that used agarose to partially immobilize a larva while monitoring its tail movements in exposure to a spatiotemporally controlled chemical stimulus.⁵² Another microfluidic device has been used to study the neural and motor activities of zebrafish larvae under exposure to ethanol by employing a zebrafish-body-mimicking channel to thoroughly immobilize and a funnel-like channel to trap the larvae for neural imaging and locomotor recording, respectively.⁵³ We have also utilized a microfluidic device to study zebrafish larvae's rheotaxis in an enclosed environment.⁴⁷

The introduced microfluidic technologies have provided us with powerful systems to study immobilized and partially immobilized zebrafish larvae and hold a great potential for behavioral investigation of freely swimming larvae, which is yet to be demonstrated. Moreover, to the best of our knowledge, the inherent sensing and movement response of zebrafish larvae under electric signal stimulation (termed electrotaxis) and its underlying mechanisms in Petri dish or microfluidic environments have not been investigated. In this paper, we introduce a versatile microfluidic device customized for on-demand Direct Current (DC) electrical stimulation of swimming zebrafish larvae and the quantitative analysis of their electrotaxis movement towards the anode inside a controllable environment. We observed that the electrotaxis response diminishes significantly at nighttime, while exposure of the larvae to dopamine agonists could enhance night electrotaxis significantly. Our findings in this paper shed light on the electrotactic behavior of zebrafish larvae and its potential regulation by the dopaminergic system, while demonstrating the usefulness of this screening method in studying many sensory-motor systems of the zebrafish larva that may be involved in electrotaxis.

II. MATERIALS AND METHODS

A. Zebrafish larvae growth and maintenance

Zebrafish (*Danio rerio*) of Tüpfel long fin strain (TL) were used for all experiments in the present study. Adult zebrafish were maintained at 28 °C with a 14:10 h light:dark photoperiod in a recirculation system (Aquaneering, CA, USA). Fish were fed *ad libitum* twice a day with brine shrimps (Brine Shrimp Direct, Ogden, Utah, USA). Eggs were collected from natural spawning typically 30 min after the light was switched on. Embryos were raised in egg water, i.e., 60 mg l⁻¹ of instant ocean sea salt (Instant Ocean, Blacksburg, VA, USA), containing 0.1% methylene blue (M291-100 Fisher Scientific, CA) in an incubator (28 °C). All experiments and procedures were performed according to the CACC guidelines of the Canadian Council for Animal Care (CCAC) after approval of the ACC protocol [GZ 2014-19 (R3)]. The number of experiments including zebrafish larvae was kept to the necessary minimum following the York University-approved biosafety permit 02-19 and the ACC protocol.

B. Chemical preparation and larvae exposure

Apomorphine hydro-chloride hemihydrate, SKF-38393 hydrochloride, and Quinpirole hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). A standard protocol

by Irons *et al.*⁷ was followed to prepare various concentrations of these drugs for our studies. The drugs were first dissolved in deionized (DI) water to make 0.2 ml stock solutions of 0.5, 10, and 1 mM concentrations for the apomorphine, SKF 38-393, and Quinpirole, respectively. Then, the stock solutions were serially diluted in DI water to obtain three final apomorphine concentrations of 0.2, 1.8, and 50 μ M, as well as 50 μ M SKF-38393 and 16.7 μ M Quinpirole hydrochloride (all used in studies by Irons *et al.*⁷). These concentrations were used for zebrafish larvae exposure and electrotaxis screening in our microfluidic device.

For exposure, individual 5–7 dpf zebrafish larvae were loaded into the wells of a 24-well plate. Animals were exposed to the specified apomorphine concentrations for a duration of 20 min. The exposure duration for SKF-38393 and Quinpirole hydrochloride was 140 min and 80 min, respectively. Exposures were stacked 5 min apart to provide enough time for testing each larva in the device. Before each experiment, the selected larva was washed in E3 water thoroughly in order to avoid any over-exposure effect of the drug on electrotactic behavior.

C. Design and fabrication of the microfluidic device

The microfluidic device [Fig. 1(a)] used for electrotaxis screening of zebrafish larvae consisted of a 45°-tilted inlet tube, a 0.9 mm-wide 43.2 mm-long larva loading channel, a U-shape channel gradually expanding in width from 0.9 mm to 1.6 mm, a 1.6 mm-wide 63.3 mm-long main screening channel, and a vertical outlet tube. All channels were 0.55 mm deep. The inlet and outlet reservoirs were extended by two side channels (0.1 mm wide, 0.2 mm deep, and 26 mm long) to two electrode reservoirs at their anterior side. Introducing a simultaneous flow inside the side and tilted inlet channels during the animal loading precluded the larva from colliding with the base and the walls of the channel and resulted in smooth delivery of the animals into the loading channel (see Fig. S1 in the [supplementary material](#)). The loading channel was implemented to visually confirm a larva entering the device, and the U-shape channel was used (i) to reduce the footprint of the device and (ii) to act as an electro-fluidic valve that prevented the larva from escaping the device during the electrotactic movement towards the narrowing bend.³⁸ All movement screening experiments were conducted in the main channel, using protocols discussed in section II D. Electric signals were applied to the channel via wire electrodes that were inserted into the side channel tubes.

The microfluidic device shown in Fig. 1(a) was fabricated by replica molding of polydimethylsiloxane (PDMS) against a plastic master mold. The master mold was 3D designed using SolidWorks (SolidWorks Corp. MA, USA) and printed with an Objet260 Connex3 printer (Stratasys Ltd., MN, USA). To fabricate the device, PDMS base and curing agent (Sylgard 184 kit from Dow Corning, MI, USA) were mixed in a 10:1 ratio and de-bubbled for up to 30 minutes at room temperature. The de-bubbled PDMS pre-polymer was then casted on the master mold, while the inlet and outlet tubes (Cole-Parmer Canada, QC, Canada) with an inner diameter of 1.6 mm were already PDMS glued to their respective reservoirs. Afterwards, the casted PDMS was cured at 60 °C on a hot plate for 6 h. Subsequently, the cured PDMS was peeled off the master mold, oxygen-plasma treated at 1 Torr pressure and 50 W power for 90 s (PDC-001-HP Harrick Plasma, USA), and bonded to a flat glass slide [Fig. 1(a)]. The electrical resistance of the channel, filled with water in all tests, was ~ 30 M Ω . Using this method, the device could be fabricated with high reproducibility, so our studies did not involve any device replicates and trials.

D. Experimental setup and procedure

The experimental setup shown in Fig. 1(b) consisted of the microfluidic device that was positioned on an inverted microscope (BIM-500FLD, Bioimager Inc., Canada) equipped with a camera (GS3-U3-23S6M-C, Point Grey Research Inc., Canada) for recording the animals' orientation responses in the main channel of the device. Meanwhile, for further analysis of the immediate speed and motion pattern of the larvae (explained in section II E), a Samsung Galaxy S6 camera (Samsung Company, Suwon, South Korea) mounted vertically on top of the device was used. Two syringe pumps connected to the tilted inlet and side channel tubes were employed to load the larva into the device. A sourcemeter (Model 2410, Keithley, OH, USA)

FIG. 1. Images of (a) the microfluidic device and (b) the experimental setup used for electrotactic movement screening of 5–7 dpf zebrafish larvae. (a) Top (i) and side (ii) views of the microfluidic device consisting of inlet and outlet tubes, a U-shaped channel, a main channel, and two side channels with tubes at the ends instrumented with two electrode wires. The length, width, and height of the main channel were 63.3, 1.6, and 0.55 mm, respectively. (b) The experimental setup consisted of the microfluidic device, two syringe pumps, direct current sourcemeter, microscope, and two cameras, one on the microscope for close-up imaging and another on a stand for movement recording of the zebrafish larvae in the main channel of the device.

was utilized to apply the desirable electric currents ($1\text{--}25\ \mu\text{A}$ in a continuous mode) across the channel via two wire electrodes that were connected from the sourcemeter outlets to the device electrode reservoirs.

The day and night electrotaxis experiments were conducted at 11 am–4 pm and 8 pm–11 pm, respectively. To conduct an experiment, a single 5–7 dpf larva was pipetted into the device inlet tube. Flow rates of 10 ml min^{-1} and 3 ml min^{-1} in the inlet and side channel tubes were used to load the larva into the main channel (Fig. S1 in the [supplementary material](#)). Both syringe pumps were turned off once the loaded larva reached the main channel. The larva was given $\sim 60\text{ s}$ to recover from any loading stress condition. The longitudinal orientation of the larva with respect to the two electrodes was first determined under the microscope (e.g., head towards the outlet or the inlet). Afterwards, a continuous-mode electric current with a constant magnitude in the range of $1\text{--}25\ \mu\text{A}$ was applied to the larva in the direction of tail-to-head (e.g., anode at the tail) in order to explore zebrafish's reaction to the electric stimulus. The larva's electrotaxis was video-recorded at a speed of 60 frames per second (fps) for approximately 20 s of stimulation using the Samsung Galaxy S6 camera. Screening lasted until the larva stopped moving, caused either by the electric current or the channel length limits. Subsequently, the tested larva was ejected

through the outlet and the device was prepared for the next experiment with a fresh larva. Zebrafish larvae exposed to various concentrations of apomorphine, SKF-38393, and Quinpirole hydrochloride (see Sec. II B for details), and unexposed larvae (control group) were tested in our studies.

E. Data analysis and statistics

In our microfluidic device, zebrafish larvae's movement response to the electric current stimulation were video-recorded by the microscope and cellphone cameras, as discussed in section II D. ImageJ software⁵⁴ was used to convert the videos to a series of images and to analyze the images for quantifying the horizontal (X) and vertical (Y) positions of the larva along the channel axis and width, respectively. For this purpose, the *Binary Process* function in ImageJ was used to convert each frame to a black and white image and to obtain XY coordinates of the center of mass of the fish in the channel. These coordinates were also used to calculate the speed of the larvae as they moved along the channel in response to the electrical current stimulus.

All statistical analyses were performed by using Microsoft Excel (Microsoft Corp., WA, USA). The results were reported as the average $\pm$ SD (standard deviation). Two-tailed Student t-test with assumption of unequal variances was selected as the statistical method to identify any significant differences between two groups of data. Power analysis was performed to estimate the required sample sizes by maintaining the upper threshold of the significance level at 0.05 and the power at 80%. The effect size, which is defined as the significance of the experimental effects when comparing two sets of data, was assumed to be 0.5, 1.0, and 1.5 as small, medium, and large effects, respectively.

F. Viability test

To examine whether electric stimulation in our microfluidic device had harmed zebrafish, we assessed the morphological abnormalities and the survival of the larvae following procedures reported by Pardo-Martin *et al.*¹⁷ The stimulated larvae were recovered from the device through the tilted inlet tube by using the withdrawal function of the syringe pump. This approach reduced the chances of damaging the larvae as they were being removed from the device for the subsequent viability and morphological tests. The retracted larvae along with a control group of zebrafish, which were not exposed to any electrical current in the microfluidic device, and a reference control group, which were not exposed to neither electric current nor the device, were then delivered to a fish tank and placed inside a 28 °C incubator. The larvae were raised for four weeks while being fed twice a day with paramecia. For survival rate assessment, we counted the number of days that the larvae remained alive under the controlled conditions. We used visual confirmation of the mortality rate in addition to the larvae's reaction to a gentle nudge. To evaluate morphological abnormalities, we visually monitored the fish inside the tank on a daily-basis during the 4-week period and counted any spinal bends (e.g., lordosis, kyphosis, and scoliosis) as an abnormality.

III. RESULTS AND DISCUSSIONS

We first report an efficient technique to induce and quantitatively study zebrafish larvae's electrotaxis for the first time. As a significant advantage of this behavioural screening technique, the electric current stimulus used in our device can be easily and rapidly applied and manipulated inside the microfluidic environment.^{35–40} After assessing zebrafish larvae's electrotaxis at various times in a day, we also show the application of this technique in investigating the involvement of D1- or D2-like dopamine receptors in modulating this sensory-motor response by treating the larvae with various selective and non-selective dopamine agonists.

A. Electrotaxis of zebrafish larva in a channel

Model organisms such as *C. elegans* have been shown to respond robustly to electric stimulus in a microchannel.³⁶ The electrotaxis behavior has been used for investigating the effect of

various chemicals, such as neurotoxins, on *C. elegans*.³⁹ In this study, we questioned whether 5–7 dpf zebrafish larvae also respond to the electrical signal inside a channel. Accordingly, the microfluidic device shown in Fig. 1(a) was fabricated to study zebrafish larvae's electrotaxis. Individual animals were loaded into the channel, monitored under the microscope for being healthy and morphologically intact, and then exposed to various electric currents along the channel axis in a manner that the anode electrode was positioned at the larva's tail (determined after some preliminary experiments). The fish's immediate response and their pre- and post-experimental morphologies were captured under the microscope (Movie S1 in the [supplementary material](#)). To assess the movement pattern and speed parameters, the larvae's response was video-recorded by the cellphone camera (Movie S2 in the [supplementary material](#)) and quantitatively analyzed with ImageJ software. Figure 2(a) shows the sequential images of the electrotactic response [Figs. 2(a-i) and 2(a-ii)] and movement [Figs. 2(a-iii) and 2(a-iv)] of a 7 dpf zebrafish larva in the channel, under exposure to an electric current of 3 μA . It was observed that the zebrafish larvae had a tendency to move towards the anode electrode in a channel, which is opposite to electrotaxis in the hermaphrodite *C. elegans*.

We investigated a wide range of electric currents between 1 μA and 25 μA with 0.5 μA intervals to determine the current window within which electrotactic response could be reliably obtained. This range was selected in a set of preliminary experiments because the zebrafish did not respond to electric currents under 1 μA , while severe paralysis and movement inability were observed at currents beyond 25 μA . Within the experimental current range of 1–25 μA , we were interested in minimizing the electric current magnitude to a level that electrotactic response could be evoked robustly. Moreover, we sought to determine the maximum magnitude of electric current to which zebrafish could respond without any significant paralysis and fatality. Our preliminary experiments revealed that the fish respond inconsistently to the electric currents in 1–2.5 μA range (Fig. S2 in the [supplementary material](#)). Starting from 3 μA , a robust electrotactic orientation response (>80%) towards the anode was observed. After the orientation, the fish continued swimming towards the anode in the channel. A visual assessment revealed that the overall morphology of the fish in terms of the eye position and body shivering were in a normal health state. We continued to increase the electric current from 3 to 15 μA and observed a similar directed response towards the anode. However, an immediate partial paralysis was observed in the fish exposed to the electric currents beyond 10 μA although most of the paralyzed fish could recover upon removing the electric stimulus. By increasing the electric current from 15 μA to 25 μA , although responding, the larvae were found completely paralyzed and

FIG. 2. Electrotaxis of 5–7 dpf zebrafish larvae in a microfluidic channel. (a) Electrotactic response of a 7 dpf zebrafish larva to a 3 μA electric current stimulus [(i) and (ii)] and its subsequent movement in the channel towards the anode [(iii) and (iv)] with a speed of 37.5 mm s^{-1} . (b) Electrotactic orientation of 5–7 dpf zebrafish larvae ($N = 30$ in three independent trials) stimulated with the electric currents of 3 μA and 15 μA . The results are compared to a control group of 30 animals, not exposed to any electric current, which shows statistically significant differences for both groups (two-tailed t-test, **: $p\text{-value} < 0.01$). There was no significant difference in the response between the two electrically stimulated groups (two-tailed t-test, $p\text{-value} > 0.05$). (c) Electrotactic speed of 5–7 dpf zebrafish larvae ($N = 15$ in three independent trials) in response to electric currents in the channel. An increase in the electric current resulted in a significant (two-tailed t-test, **: $p\text{-value} < 0.01$) decrease in the electrotactic speed.

sometimes dead right after the exposure due to the severe effect of electric stimulation. The majority of the fish lost their normal morphology, which was determined as a change in their eye position and a shivering effect during the exposure. The alive fish were also unable to swim for several minutes after the exposure (Movie S3 in the [supplementary material](#)). In a separate pilot study, no effect of age on electrotaxis response of 5–7 dpf zebrafish larvae was observed, which agreed with the general locomotion of larvae reported by Colwill and Creton.²⁰

Based on the initial experiments, electric currents of $3\ \mu\text{A}$ and $15\ \mu\text{A}$ were selected for investigation of the electrotaxis response and speed of 5–7 dpf zebrafish larvae. Of a total number of 30 zebrafish tested per exposure condition, $86.7 \pm 11.5\%$ and 100.0% responded immediately to $3\ \mu\text{A}$ and $15\ \mu\text{A}$ electric currents, respectively, by orienting towards the anode electrode [Fig. 2(b)]. In comparison with the control group, which were loaded into the channel but not exposed to any electric current, the response to both $3\ \mu\text{A}$ and $15\ \mu\text{A}$ signals was statistically different (two-tailed t-test, $p\text{-value} < 0.01$). Within the $3\text{--}15\ \mu\text{A}$ window, the larvae exhibited sensitivity to the magnitude of electric current, as evident by a more robust response to the higher electric currents in Fig. 2(b) (in-between data not shown because of their similarity). However, the overall response of the fish to $15\ \mu\text{A}$ was not statistically different from their response to $3\ \mu\text{A}$ current (two-tailed t-test, $p\text{-value} > 0.05$).

We also monitored and quantified larvae's electrotactic movement speed ($N=15$) towards the anode in the channel right after their responses to $3\ \mu\text{A}$ and $15\ \mu\text{A}$ electric currents [Fig. 2(c)]. The calculated electrotactic speeds for $3\ \mu\text{A}$ and $15\ \mu\text{A}$ currents were $58.5 \pm 19.0\ \text{mm s}^{-1}$ and $41.5 \pm 13.1\ \text{mm s}^{-1}$, respectively. The measured speeds were significantly different from each other (two-tailed t-test, $p\text{-value} < 0.01$). The reduction of electrotactic speed at higher current values can be explained as an effect of electric current on the body of larvae and their partial paralysis during the movement towards the anode. The results above imply that although the larvae were more sensitive to the stronger electric currents, they were adversely influenced by these currents resulting in their slower moving speed in the channel.

We then investigated the reproducibility of the electrotactic response in zebrafish larvae. For this aim, we exposed $N=30$ fish to electric currents of $3\ \mu\text{A}$ and $15\ \mu\text{A}$ and allowed the larvae to recover from the electric stimulation for 5–10 min in the channel. Subsequently, we exposed the larvae to a reverse electric current of the same magnitude and observed that they displayed electrotactic behavior for the second time. This conveys that the 5–7 dpf zebrafish larvae's response to electric current is reproducible in a channel. We observed a more than three times higher electrotaxis of the larvae in response to electric currents of $10\ \mu\text{A}$ and higher.

We also performed electrotaxis experiments at $3\ \mu\text{A}$ current on 30 animals (three trials) in which the anode was positioned at the head side of the larvae. In all cases, we observed a forward movement towards the anode with no rotation involved. This confirmed that the observed behaviour was indeed electrotaxis and not a triggered startle response. To rule out the possible effect of electrokinetic flow in orienting the zebrafish, we exposed the dead larvae, which were morphologically intact, to the abovementioned electric currents in the channel. No movements were observed which revealed that there was no significant electrokinetic effect on the larvae during the electrotaxis experiments. Our calculations also showed that temperature elevation due to electric current is not significant in the channel. Therefore, we ruled out the possibility of any thermotaxis response. Based on the experiments above, we conclude that zebrafish larvae can sense the electric current magnitude and direction, and it is possible to speculate that they might share similar mechanisms like *C. elegans*. Our device provides a platform in which the mechanism of electrotaxis can be studied in the future.

B. Viability and morphological state of zebrafish larvae after electrotaxis in the device

We were intrigued to find out if loading the larvae into the device and exposing them to various electric currents have any significant impact on their post-exposure health status. The morphology and survival were selected as the criteria to monitor the short- and long-term effects of the aforementioned conditions on the zebrafish, respectively.¹⁷ Four groups of larvae

were tested, including a *Reference Control* group that was not exposed to the device or any electric current, a *Device Control* group that was loaded into the device but not exposed to any electric current, and two groups of larvae which were exposed to the electric currents of $3\ \mu\text{A}$ and $15\ \mu\text{A}$ inside the device. For survival assessment, the fish were removed from the device and their viability was monitored daily over a period of 30 days [Fig. 3(a)]. For morphological assessment, the larvae were inspected for physiological damages to their bodies each day [Fig. 3(b)].

As shown in Fig. 3, during the 30 days of post-experimental monitoring, the survival of *Reference Control* larvae was 90%. The zebrafish processed in the device and the ones exposed to $3\ \mu\text{A}$ electric current revealed almost similar survivals during the assay, with 70% and 80% of them being alive at the end of the 30-day period, respectively. The larvae exposed to $15\ \mu\text{A}$ current had a 30% survival rate after 30 days, which was different from the two control groups and the larvae exposed to $3\ \mu\text{A}$ current. For statistical comparison, we monitored 20 animals in three independent trials during the first 4-days of post-exposure.⁵⁵ It was observed that the larvae exposed to $15\ \mu\text{A}$ were more likely to die (Fig. S3 in the [supplementary material](#)). Their survival was significantly different from the *Reference Control* larvae (two-tailed t-test, p -value < 0.05). The survival rate of the *Device Control*, $3\ \mu\text{A}$ exposure, and the *Reference Control* group was indistinguishable (two-tailed t-test, p -value > 0.05 ; Fig. S3 in the [supplementary material](#)).

We also assessed the morphology of larvae during a 4-week period [Fig. 3(b)]. Similar to the survival criterion, more than 70% of the fish passed through the device or exposed to $3\ \mu\text{A}$ current showed a normal morphology when compared to the *Reference Control* group (two-tailed t-test, p -value < 0.05) [Fig. 3(c-i)]. In contrast, 70% of the larvae exposed to a $15\ \mu\text{A}$ current experienced spinal bending abnormalities [Fig. 3(c-ii)], which was statistically different from the *Reference Control* group (two-tailed t-test, p -value < 0.01).

In summary, the electrotaxis experiments and survival and morphological abnormality assays of 5–7 dpf zebrafish larvae let us conclude that electrical currents, ideally $< 10\ \mu\text{A}$, should be used for future studies.

FIG. 3. Survival and morphological abnormality of 5–7 dpf zebrafish larvae tested in the microfluidic electrotaxis screening device and then monitored off-chip for 30 days ($N = 10$ per condition). (a) Off-chip survival of zebrafish larvae, after exposure to $3\ \mu\text{A}$ and $15\ \mu\text{A}$ electric currents (ECs) in the device, compared to that of the fish not exposed to any EC in the device as the *Device Control* group and the fish that were not exposed to the device and EC as the *Reference Control* group. (b) Morphological abnormality of the above-mentioned zebrafish larvae during 4 weeks of observation after experiments (two-tailed t-test, **: p -value < 0.01). (c) Microscope images of (c-i) an intact *Reference Group* larva and (c-ii) a morphologically damaged zebrafish after exposure to $15\ \mu\text{A}$ EC.

C. Time of day dependency of electrotactic behavior in zebrafish larvae

According to the literature, the circadian rhythms (i.e., readily day-night cycles) affect the biological and behavioral activities of zebrafish.⁵⁶ For instance, it has been shown that the locomotion activities of 5–7 dpf zebrafish larvae reduce significantly at nighttime.⁵⁷ Thus, we asked if zebrafish larvae would show behavioral changes in response to the electric current exposure at different times of the day. Electrical stimulation with 3 μ A electric current was conducted on 5–7 dpf zebrafish larvae at night, i.e., 8–11 pm, and compared to the daytime responses at 11 am–4 pm and control groups of larvae not exposed to the electric current (Fig. 4). The electrotaxis response at early hours of each cycle was similar to that of the middle-hours (data not shown).

As shown in Fig. 4, the control groups showed an arbitrary movement with less than 10% reorientation or no movement at all at day and night, respectively. Zebrafish larvae's electrotaxis stimulated by 3 μ A electric current increased significantly at different times of the day. Interestingly at night, the response level was $26.8 \pm 5.8\%$, as compared to $86.7 \pm 11.5\%$ for the larvae tested during daytime. This drop-off in the night electrotactic response was significantly different from the day response level (two-tailed t-test, p-value < 0.01). Our results correlated with the reduced level of general locomotion activities observed in zebrafish larvae at nighttime.⁵⁷ Apparently, this behavioral abnormality was provoked due to the circadian rhythm in zebrafish larvae that could be studied quantitatively with our novel electrotaxis screening assay. We became interested in further understanding the potential causes of such an anomalous response, as discussed in section IIID.

D. Microfluidic electrotaxis as a tool for screening dopaminergic compounds

The observed attenuation of electrotactic response at nighttime opened new lines of inquiry. Specifically, we wanted to evaluate the possibility to screen biological pathways involved in sensory-motor processes using the microfluidic electrotaxis assay. It is well understood that the motor behavior of the zebrafish larva is mediated by the dopaminergic system.⁷ For instance, it has been demonstrated that the activities of dopamine receptors alter in zebrafish during the light-dark cycle.⁵⁸ It is also shown that mutation in the circadian gene *period1b* (*per1b*)⁵⁷ or exposure of zebrafish larvae to the dopamine agonist apomorphine⁷ can alter the dopamine level or receptor activities and result in significant changes in zebrafish's general locomotion behavior. For instance, exposing zebrafish to 0.2 μ M and 50 μ M apomorphine in darkness resulted in a strong increase in larvae's average travel distance, and an intermediate exposure to 1.8 μ M apomorphine significantly lowered that activity.⁷

The above knowledge about involvement of the dopaminergic system in general locomotion and the results shown in Fig. 4 led us investigate whether the activities of the dopaminergic

FIG. 4. Zebrafish larvae's electrotaxis (N = 20 fish in three independent trials) stimulated by an electric current of 3 μ A during day (11 am–4 pm) and night (8–11 pm) along with the control group of zebrafish not exposed to the current (two-tailed t-test, **: p-value < 0.01).

system regulate the electrotaxis behavior. Specifically, we asked if non-selective stimulation of the dopaminergic receptors could affect the electrotaxis response of zebrafish larvae at night. Accordingly, we exposed 5–7 dpf zebrafish larvae to dopamine agonist apomorphine and investigated the electrotactic response at night in the microfluidic device. Dose dependency was tested with three different apomorphine concentrations of 0.2 μM , 1.8 μM , and 50 μM , at three electric currents of 3, 6, and 9 μA , and including untreated controls (Fig. 5).

As shown in Fig. 5, only $26.8 \pm 5.8\%$ of the control larvae responded to a 3 μA electric current at night, while the larvae exposed to 0.2 μM apomorphine (low-dose: LD) exhibited an enhanced response of $53.9 \pm 4.2\%$. There was a significant difference (two-tailed t-test, p-value < 0.01) between the two groups which confirmed the positive effect of LD apomorphine on electrotaxis at night. This is consistent with the previous studies showing an enhanced level of general activities in zebrafish larvae in a similar condition.⁷ At the 3 μA current level, exposing the larvae to medium-dose (MD) apomorphine resulted in an electrotaxis response of $28.5 \pm 4.3\%$ which was significantly lower than the LD response (two-tailed t-test, p-value < 0.01) but not the control group (two-tailed t-test, p-value > 0.05). This was also consistent with the results of Irons *et al.*⁷ At high dose (HD) apomorphine, electrotaxis response stayed at a low level of $19.3 \pm 12.6\%$ and did not increase as opposed to the high-level general activities of zebrafish in a similar condition.⁷ This may suggest that the higher doses of apomorphine fail to evoke the larvae's response to low electric current (3 μA) due to the possible saturation of the dopamine receptors after treatment.^{59,60} At the HD apomorphine, we also observed abnormal movement behaviors such as dizziness, drowsiness, and confusion which might be due to any possible side effects of the higher dosage treatment.

At 6 μA and 9 μA electric currents, the electrotaxis response saturated due to the over-strength of the stimuli. This effect was also observed in the larvae that were exposed to the same-concentration apomorphine and tested at different current levels (i.e., 3–9 μA). Their electrotactic response increased with the increase in electric current magnitude. At the 6 μA current level, we noticed a gradual reduction in electrotaxis response as the apomorphine concentration was increased. However, only the MD response was significantly lower than the control group (two-tailed t-test, p-value < 0.05). The HD response was only marginally different from the control group with a p-value of 0.07. At 9 μA electric current, the larvae stayed dominantly sensitive to electrical stimulus and the alteration of apomorphine dosage up to MD did not lead to any changes in electrotaxis. However, the HD apomorphine was again influential in disrupting electrotaxis, causing abnormal behavior, and plummeting the mean response rate in the larvae.

Overall, we showed that the dopaminergic system of zebrafish is potentially involved in electrotaxis, and a low electric current electrotaxis assay can be used to interrogate the effect of dopaminergic compounds on zebrafish larvae movement.

FIG. 5. Effect of apomorphine at different concentrations on electrotaxis of 5–7 dpf zebrafish larvae at night. The larvae were treated with three apomorphine concentrations of 0.2, 1.8, and 50 μM and then tested in the microfluidic device at three electric current levels in three independent trials. Each drug concentration involved $N = 30$ fish for 3 μA and 6 μA and $N = 15$ fish for 9 μA electric currents (two-tailed t-test, *: p-value < 0.05 , **: p-value < 0.01).

E. Electrotactic speed, place preference, and swimming pattern of zebrafish larvae

It has recently been reported that apomorphine at different concentrations can influence the maximum speed and place preference of the zebrafish larvae.⁶¹ Upon observing that exposure to 0.2 μ M apomorphine improves electrotaxis of zebrafish at night at low current levels, we became interested to see whether the movement of larvae in this condition is different from their untreated counterparts tested for electrotaxis during the daytime. To study this in detail, we quantified the movement speed and the absolute lateral position of zebrafish larvae as they electrotactically moved along the main channel of the device. Two groups of larvae were tested at 3 μ A electric current, one group not exposed to any drug at daytime and one group exposed to 0.2 μ M of apomorphine at night. We observed no significant differences in the movement speed of the larvae that responded to the electric current in these two conditions (data not shown). However, assessment of the lateral positions, defined as larvae's center of masses in the channel, demonstrated differences as shown in Fig. 6.

As shown in Fig. 6, the lateral distances of the two groups from the centerline of the channel were significantly different from each other (two-tailed t-test, p-value < 0.01). The treated animals by apomorphine tested at night tended to stay closer to the wall of the channel, while the movement of untreated zebrafish at daytime was smoother at the centerline of the channel. The assessment of the movement patterns conveyed more struggling swimming for the treated larvae compared to untreated zebrafish that moved dominantly with forward swimming in the channel, as defined by Fajardo *et al.*²¹ The dominance of struggling swimming in the treated zebrafish resulted in more bouncing of the larvae in the channel and their overall positioning towards the wall of the device. Determining whether the body bend pattern differences were attributed to the time of the assay or the drug exposure requires further investigations in the future.

F. Role of D1- and D2-dopamine receptors in zebrafish larvae's electrotaxis

After showing the possible involvement of the dopaminergic system in electrotaxis of the zebrafish larvae, we examined potential roles of specific dopamine receptors in regulating the electrotaxis, as a first line to elucidate the underlying molecular and physiological mechanisms. Two widely used selective dopamine agonists, SKF-38393 (for D1-like receptors) and Quinpirole (for D2-like receptors), were selected because of their prominent role in the dopamine signalling

FIG. 6. Absolute lateral distance of larvae's center of mass from the centerline of the channel (dashed-line in the inset graph) during electrotaxis of 5–7 dpf zebrafish larvae. Using a current of 3 μ A, N = 18 zebrafish were tested at daytime, while N = 21 larvae were exposed to a 0.2 μ M dose of apomorphine and tested at night (two-tailed t-test, **: p-value < 0.01).

FIG. 7. Electrotaxis of zebrafish larvae treated with 50 μ M SKF-38393 (D1-like receptors) and 16.7 μ M Quinpirole (D2-like receptors) at night. $N = 15$ larvae in three independent trials for each condition (two-tailed t-test, *: p -value < 0.05).

pathway. Treating 5–7 dpf zebrafish larvae with 50 μ M of SKF-38393 and 16.7 μ M of quinpirole has been shown to significantly affect the zebrafish movement activity at night.⁷ Therefore, larvae were tested at 3 μ A electric current after exposing them to the drugs above at night (See Sec. II for exposure times). As shown in Fig. 7, for $N = 15$ samples per condition, the electrotaxis responses were $13.3 \pm 11.5\%$, $13.3 \pm 11.5\%$, and $53.3 \pm 11.5\%$ for control (not exposed), SKF-38393, and quinpirole treatments, respectively.

SKF-38393 appeared to be ineffective on electrotaxis (two-tailed t-test, p -value > 0.05), while quinpirole significantly affected larvae's electrotaxis compared to the control group (two-tailed t-test, p -value < 0.05). This finding suggests a significant involvement of the D2-dopamine receptors in modulating zebrafish larvae's electrotaxis. Further investigations are required to prove the exact roles of dopamine receptors in electrotaxis. We propose that the microfluidic device introduced in this research will be instrumental to explore the molecular and physiological basis of electrotaxis and the role of the dopaminergic pathway, including exploring a wider range of selective agonist drugs and concentrations or using genetic approaches targeting the dopamine signalling pathway.

IV. CONCLUSIONS

We presented zebrafish larvae's behavioral response to the electric current (i.e., electrotaxis) in a channel for the first time and demonstrated the application of our technique in screening the role of dopamine receptors in electrotaxis. The device and presented technique are simple to use for on-demand stimulation of movement and screening the effect of chemicals on the zebrafish larva as a model. The tested zebrafish in our daytime assays responded robustly to a minimum electric current threshold of 3 μ A by swimming towards the anode electrode. The response increased with the magnitude of the electric current up to 15 μ A. However, the larvae exhibited slower electrotactic swimming speed, mortality, and morphological abnormality at 15 μ A. At currents in the range of 15–25 μ A, we observed severe paralysis in the channel and elevated mortality after the assays.

Similar experiments with 3 μ A electric current at nighttime revealed a significant drop in electrotactic response. We benefited from the device and showed a possible link between zebrafish's electrotaxis and the dopamine receptors activities. Treating the larvae with a non-selective dopamine agonist, apomorphine, resulted in alteration of electrotactic response at night, proving the considerable involvement of the dopamine receptors in regulating this behavior. The effect of apomorphine on electrotaxis was more apparent at low-level electric currents. For instance, at 3 μ A, low-dose apomorphine exposure significantly increased the response due to the enhancement of dopamine receptors activities, while higher doses failed to boost the

behavior in the larvae potentially due to oversaturation effects.^{59,60} Moreover, higher electric currents (6 and 9 μ A) resulted in more robust electrotaxis responses at night, with and without treatment of the larvae by different apomorphine dosages. This demonstrated the dominant role of electric current magnitude in zebrafish's electrotaxis and the importance of controlling this variable to achieve sensitive assays. Interestingly, for apomorphine-exposed zebrafish that demonstrated enhanced electrotaxis at night, their movement pattern within the channel was dominated by struggling swimming and a center of mass trajectory that was closer to the wall of the channel, whereas the larvae tested during the day with no drug exposure exhibited more forward swimming while moving closer to the centerline of the channel. Furthermore, performing assays with selective dopamine agonists SKF-38393 and Quinpirole revealed a dominant involvement of D2-dopamine receptors in electrotaxis.

All in all, our device provides an accurate, sensitive, and versatile platform to evoke zebrafish's electrotactic movement behavior on-demand and investigates the effect of various drugs on it quantitatively. In addition, the biological basis of electrotaxis, which can be studied with this device, may lead to a better understanding of the pathways involved in sensory-motor processes of zebrafish larvae.

SUPPLEMENTARY MATERIAL

See [supplementary materials](#) for videos of zebrafish larva electrotaxis and supporting data for larva loading into the device, electrotaxis response at low currents, and survival during the first four days after experimentation.

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