

## SEROTONIN MODULATES ELECTROSENSORY PROCESSING AND BEHAVIOR VIA 5-HT<sub>2</sub>-LIKE RECEPTORS

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**Abstract**—Efficient sensory processing of the environment is a critical function for any organism to survive and is accomplished by having neurons adapt their responses to stimuli based on behavioral context in part through neuromodulators such as serotonin (5-HT). We have recently shown that one critical function of the serotonergic system in weakly electric fish is to enhance sensory pyramidal neuron responses within the electrosensory lateral line lobe (ELL) to stimuli caused by same sex conspecifics, thereby enhancing their perception. This enhancement is accomplished by making pyramidal neurons more excitable through downregulation of potassium channels. However, the nature of the 5-HT receptors that mediate this effect is not known. Here we show that the 5-HT<sub>2</sub> receptor antagonist ketanserin (ket) can effectively block the effects of 5-HT on pyramidal neuron excitability *in vitro*. Indeed, 5-HT application subsequent to ket application did not cause any significant changes in neuron excitability and responses to current injection. We further show that ket applied *in vivo* can block the effects of 5-HT on behavioral responses. Thus, our results strongly suggest that the previously observed effects of 5-HT on sensory processing within ELL and their consequences for behavior are mediated by 5-HT<sub>2</sub> receptors. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

**Key words:** neural coding, weakly electric fish, neuromodulation, neuroethology.

### INTRODUCTION

Efficient and adaptive processing of behaviorally relevant sensory stimuli is essential for survival and can be accomplished by dynamically regulating sensory neuron tuning to these stimuli (Abbott, 2005; Wark et al., 2007). Such regulation is, in part, implemented by neuromodula-

tors such as serotonin (5-HT). There are massive serotonergic projections from the raphe nuclei to almost every part of the vertebrate brain, including sensory areas such as thalamus and cortex (see Berger et al., 2009 for review). Indeed, 5-HT has been shown to significantly alter processing across several sensory systems and is thought to impose dynamic filters whose properties are tied to environmental events and/or the brain's internal state rather than the detailed information about the ongoing stimulus (Hurley et al., 2004).

Weakly electric fish generate an electric field around their body through the electric organ discharge (EOD) and can sense amplitude modulations (AMs) of this field through an array of electroreceptors scattered on their skin surface (Turner et al., 1999; Chacron et al., 2011). These electroreceptors make synaptic contact onto pyramidal neurons within the electrosensory lateral line lobe (ELL) (Bell and Maler, 2005), whose physiology has been well characterized both *in vitro* and *in vivo* (see (Maler, 2009) for review). The ELL is composed of four parallel maps of the body surface; three of which (centromedial segment (CMS), centrolateral segment (CLS), and lateral segment, (LS)) receive identical tuberous electroreceptor inputs (Carr et al., 1982; Heiligenberg and Dye, 1982; Shumway, 1989a,b). These maps have been shown to mediate different behaviors (Metzner and Juranek, 1997): in particular, the lateral map is involved in the processing of communication signals (Shumway, 1989a; Krahe et al., 2008; Marsat et al., 2009; Marsat and Maler, 2010) associated with aggressive behavior (Zakon et al., 2002; Hupe et al., 2008). Previous studies have shown that the tuning of pyramidal neurons within the maps is intrinsic (Mehaffey et al., 2008b) and originates in part from membrane conductances (Ellis et al., 2007b, 2008; Krahe et al., 2008).

Pyramidal cells also receive large amounts of neuromodulatory input (Maler et al., 1981; Johnston et al., 1990; Ellis et al., 2007a; Deemyad et al., 2011) (see (Marquez et al., 2013) for review). In particular, serotonergic innervation is densest in the lateral segment and sparsest in the CMS (Deemyad et al., 2011). Recent studies have shown that the effects of 5-HT on ELL pyramidal neurons *in vivo* is to render them more excitable, thereby increasing their responses to stimuli associated with same sex conspecifics (Deemyad et al., 2013). Remarkably, similar effects were observed *in vitro*, where it was shown that 5-HT increases pyramidal neuron excitability by downregulating both small conductance calcium-activated (SK) and M potassium channels (Deemyad

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**Abbreviations:** 5-HT, serotonin; ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; AM, amplitude modulation; CLS, centrolateral segment; CMS, centromedial segment; ELL, electrosensory lateral line lobe; EOD, electric organ discharge; ISI, interspike interval; JAR, jamming avoidance response; LS, lateral segment; ket, ketanserin.

et al., 2011). However, the nature of the 5-HT receptors that mediate this effect is not known. There are seven different families of 5-HT receptors which can be broken down further into up to 14 different subtypes, all of which are metabotropic except for 5-HT<sub>3</sub> receptors which are ionotropic. Based on previous studies showing that 5-HT<sub>2</sub> receptors downregulate potassium channels in other systems (Barnes and Sharp, 1999; Hoyer et al., 2002), we tested whether the 5-HT<sub>2</sub> receptor antagonist ketanserin (ket) can block the effects of 5-HT on ELL pyramidal neuron excitability *in vitro* and behaviorally at the organismal level.

## EXPERIMENTAL PROCEDURES

### Animals

The weakly electric fish *Apteronotus leptorhynchus* was used exclusively in this study. Animals were obtained from tropical fish suppliers and were acclimated to laboratory conditions according to published guidelines (Hitschfeld et al., 2009). All procedures were approved by McGill University's animal care committee.

### Preparation of slices

Slices were prepared as done previously (Mathieson and Maler, 1988; Ellis et al., 2007a; Deemyad et al., 2011, 2012). In preparation for surgery, fish were initially anaesthetized by placing them in a pH-buffered MS-222 solution. Fish were then quickly transferred into a holding chamber with the head firmly secured to a breathing apparatus where they were respired via a mouth tube with a well-oxygenated solution containing pH-buffered MS-222. Soft tissue covering the skull was removed using a scalpel and the skull itself was removed using forceps. Incisions severing the afferents and spinal cord were made and the brain was removed. Finally the brain was blocked at roughly a 45° angle to provide an optimal angle to slice hindbrain ELL. The hindbrain block was then placed onto the vibratome platform and fixed with super glue. The platform was then covered with an agarose solution (15%). 400- $\mu$ m ELL slices were cut by a microtome and transferred immediately to a well-infused artificial cerebrospinal fluid (ACSF) solution where they were allowed to rest for a minimum of 1 h before being transferred into a recording chamber with a constant ACSF flow rate of 4 ml/min. The ACSF was composed of (in mM): 126 NaCl, 2.5 KCl, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 1.2 MgCl<sub>2</sub>, 18 NaHCO<sub>3</sub>, 2.4 CaCl<sub>2</sub>, and 11 D-glucose) and was superfused with carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>).

### *In vitro* pharmacology

Both 5-HT and ket were obtained from Tocris and were dissolved in ACSF using concentrations of 1 mM and 100  $\mu$ M, respectively. Previous studies have shown that 5-HT at 1 mM concentration will significantly alter LS pyramidal neuron activity (Deemyad et al., 2011) while ket will be most selective for 5-HT<sub>2A</sub> receptors at a concentration of 100  $\mu$ M (Janssen et al., 2002). Both drugs were applied to the ELL Dorsal Molecular Layer (DML)

using a PicoSpritzer III (Parker Hannifin, Cleveland, OH, USA) using custom-made electrodes with a tip diameter of 1–2- $\mu$ m (Ellis et al., 2007a; Mehaffey et al., 2008a; Deemyad et al., 2011, 2012). PicoSpritzer pressure was 10<sup>5</sup> Pa with an ejection time of 140 ms. All slices were oriented so that pharmacological agents applied would, through the natural ACSF flow of the recording chamber, also pass over the ventral molecular and pyramidal cell layers (PCLs) (Ellis et al., 2007a; Deemyad et al., 2011, 2012). We used a double-barrel electrode that was advanced over the molecular layer above the recording electrode. One barrel was filled with ket while the other was filled with 5-HT. We first obtained control data from each cell as described below. In some experiments, 5-HT was then applied in order to quantify its effects on pyramidal neuron excitability. In other experiments, we first applied ket and quantified its effects on pyramidal neuron excitability before applying 5-HT.

### Recording

Sharp intracellular recordings from pyramidal neurons were obtained using previously published techniques (Ellis et al., 2007a; Deemyad et al., 2011, 2012). Glass sharp intracellular electrodes were pulled using a P-97 filament puller (Sutter Instruments, Novato, CA, USA) and were filled with a 2 M KAC solution. These electrodes had resistances between 70 and 110 M $\Omega$ . Upon entering the cell with an electrode, we injected a baseline current (typically around –0.5 nA) in order to keep the cell just under firing threshold. The recorded membrane potential difference was amplified using an Axoclamp 900A while data were recorded with a Digidata 1440A and Clampex 10.3 (Molecular Devices, Sunnyvale, CA, USA) using a 10-kHz sampling rate. Previous studies have shown that 5-HT expression is graded across the ELL segments (Deemyad et al., 2011). In particular, weak expression was found in CMS while LS displayed the strongest levels of expression. Neurophysiological studies performed *in vitro* have shown that 5-HT did not have any significant effect on CMS pyramidal neuron activity, had weak effects on CLS pyramidal neuron activity, and had the strongest effects on LS neuron activity (Deemyad et al., 2011). As we wanted to test whether ket would block the effects of 5-HT, we thus focused on recording from LS pyramidal neurons where the effects of 5-HT were strongest.

### Experiment protocols and data analysis

The spiking activities of pyramidal neurons were obtained during a current step of 0.5 nA from the baseline holding current that lasted 2 s. These were quantified by the mean firing rate (i.e. the number of spikes per unit time). Further, interspike interval (ISI) sequences were computed as the time intervals between consecutive spikes, and ISI probability densities were generated with 1 ms binwidth. The coefficient of variation (CV) was computed as the standard deviation to mean ratio of the ISI probability density. The burst fraction was defined as the proportion of ISIs that is less than 10 ms, which correspond to the range of ISIs that will give rise to

dendritic failure (Oswald et al., 2004). This value also corresponds to the trough in the bimodal ISI distribution as plotted on a log-linear scale (Turner et al., 1994) which is frequently taken as the threshold to segregate bursts from isolated spikes (Chacron et al., 2001; Doiron et al., 2003; Avila Akerberg and Chacron, 2011; Khosravi-Hashemi et al., 2011; Khosravi-Hashemi and Chacron, 2012, 2014).

Average action potential waveforms were generated in order to measure the afterhyperpolarization (AHP) following each action potential. The medium component of the AHP was measured as the differences between the baseline membrane potential value before a spike (i.e. the average membrane potential at 10–15 ms before the peak of the spike) and the membrane potential value measured at 10–40 ms after the action potential peak (Faber and Sah, 2002; Koyama and Appel, 2006). We only considered action potentials that were “isolated” (i.e. that were not immediately preceded or followed by other action potentials) in order to estimate the full length of the AHP.

We used a set of 10 current steps each lasting 1 s and ranging from 0.05 to 0.5 nA above the baseline holding current. We then measured the mean firing rate during each step. The mean firing rate was then plotted as a function of the current value minus the baseline value in order to obtain the  $f$ - $I$  curve. The gain was determined from the slope of the best linear fit to the  $f$ - $I$  curve (Mehaffey et al., 2005).

## Behavior

The procedures were the same as those used in a previous study (Deemyad et al., 2013). Briefly, 0.1–0.5 mg of Tubocurarine (Sigma, St Louis, MO, USA) was injected intramuscularly to immobilize the fish. The fish was then transferred to a recording tank and respirated via a mouth tube at a flow rate of 10 ml/min. To expose the hindbrain for recording, we first locally anaesthetized the skin on the skull by applying 2% Lidocaine. To stabilize the head, a metal post was glued to the exposed area of the skull, rostral to the opening. We drilled two small holes of  $\sim 2$  mm<sup>2</sup> over the cerebelli and the ELL areas, caudal to the T0 vein on each side of the brain. Drug application electrodes were two-barrel KG-33 glass micropipettes (OD 1.5 mm, Garner Glass Co., Claremont, CA, USA) pulled by a micropipette puller (David Kopf Instruments, Inc., Tujunga, CA, USA) to a fine tip and subsequently broken to attain a tip diameter of  $\sim 10$   $\mu$ m as done previously (Chacron and Bastian, 2008; Toporikova and Chacron, 2009). The two barrels were used for separate or simultaneous application of ket (100  $\mu$ M) and 5-HT (1 mM) in both hemispheres of the ELL using a PicoSpritzer III (Parker Hannifin, Cleveland, OH, USA). EOD frequency changes due to a 4-Hz AM were recorded previous to drug application (control), to ket alone as well as to a subsequent 5-HT application. After 45 min, behavioral responses were recorded to a sole application of 5-HT to prove the correct positioning of the electrodes. Note that previous studies have shown that saline applied in this manner does not significantly alter behavioral responses (Deemyad et al., 2013).

## Statistics and presentation of data

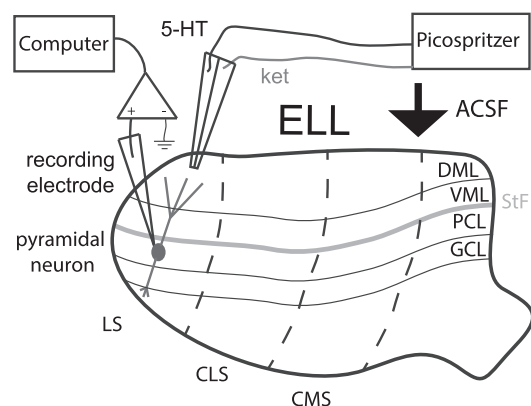
As previous studies have reported significant heterogeneities within the ELL pyramidal cell population (see (Maler, 2009) for review), we normalized all quantities with respect to control values in order to minimize the effects of such heterogeneities before performing statistical comparisons as done previously (Deemyad et al., 2011, 2012, 2013). However, we report un-normalized population-averages (mean  $\pm$  SEM) in the results throughout. Error bars in the figures represent  $\pm 1$  SEM. All statistical tests consisted of one-way analysis of variances (ANOVAs) with Tukey post hoc highly significant difference (HSD) to allow for comparison between all groups.

## RESULTS

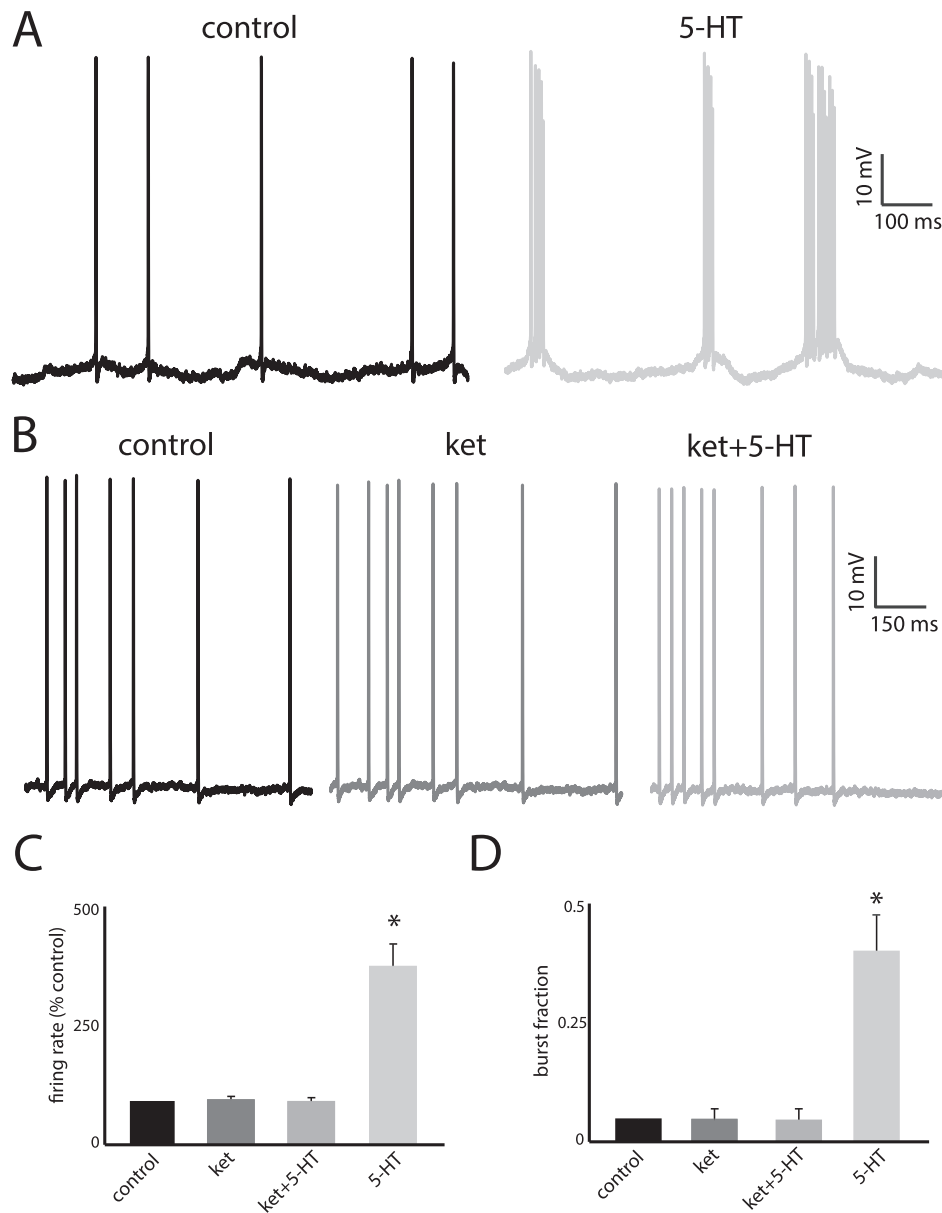
### Ket blocks the effects of 5-HT on pyramidal neuron firing *in vitro*

We first tested whether ket would block the effects of 5-HT on ELL pyramidal neuron excitability *in vitro*. To do so, we used the setup depicted in Fig. 1.

Previous studies have shown that 5-HT application will increase pyramidal neuron excitability in LS, causing increased burst firing (i.e. an increased tendency to fire packets of action potentials followed by quiescence) (Deemyad et al., 2011). Thus, we first tested whether ket would block the effects of 5-HT on increased burst firing. Fig. 2A shows the recorded transmembrane potential from a typical LS pyramidal neuron under control conditions (left) and after 5-HT application (right). It can be seen that 5-HT has a large effect on pyramidal neuron excitability by promoting the firing of packets of action potentials followed by quiescence (i.e. “bursts”), confirming previous results (Deemyad et al., 2011). In contrast, Fig. 2B shows the recorded transmembrane potential from a typical LS pyramidal neuron under control (left),



**Fig. 1.** Schematic of a typical ELL slice as it would appear in a recording chamber. The tractus stratum fibrosum (StF) is readily apparent *in vitro* and presents a landmark located just above the pyramidal cell layer (PCL). The slice is optimally placed for the artificial cerebrospinal fluid (ACSF) to flow over the molecular layer of the ELL (Dorsal Molecular Layer, DML and Ventral Molecular Layer, VML) as shown by the arrow. Accordingly, picospritzer pipettes were placed in the DML to allow for both ket and 5-HT diffusion across DML, VML, StF, PCL, GCL: granule cell layer.



**Fig. 2.** (A) Example voltage traces from a pyramidal neuron under control (left) and after 5-HT application (right). Note the transition from tonic to burst firing. (B) Example voltage traces from a pyramidal neuron under control (left), after ket application (center), and after ket + 5-HT application (right). Note the similarity between the traces. (C) Population-averaged firing rates under all conditions. (D) Population-averaged burst fractions under all conditions. “\*” indicates that the 5-HT group is significantly different from all others at the  $p = 0.05$  level using a one-way ANOVA with Tukey post hoc highly significant difference (HSD) ( $n = 7$ ). All other comparisons between group pairs were not significant ( $p > 0.1$ ).

after ket application (center), and after ket + 5-HT application (right). Comparing all traces revealed that: (1) ket did not have any noticeable effects on pyramidal neuron excitability by itself; and (2) 5-HT applied after ket did not cause a transition to burst firing. Rather, 5-HT applied after ket did not have any noticeable effects on pyramidal neuron excitability.

We quantified these data by computing the mean firing rate as well as the burst fraction (i.e. the fraction of interspike intervals less than 10 ms). 5-HT caused a large and significant increase in both firing rate (control:  $5.11 \pm 0.95$  Hz; 5-HT:  $12.45 \pm 1.42$  Hz; Fig. 2C) and burst fraction (control:  $0.06 \pm 0.06$  Hz; 5-HT:

$0.37 \pm 0.12$  Hz; Fig. 2D). In contrast, neither ket nor ket + 5-HT caused any significant change in either firing rate (control:  $5.96 \pm 1.65$  Hz; ket:  $5.45 \pm 1.30$  Hz; ket + 5-HT:  $5.42 \pm 1.62$  Hz; Fig. 2C) or burst fraction (control:  $0.07 \pm 0.02$ ; ket:  $0.06 \pm 0.01$ ; ket + 5-HT:  $0.07 \pm 0.03$ ; Fig. 2D). Thus, these data show that ket can block the effects of 5-HT on pyramidal neuron excitability.

#### Ket blocks the effects of 5-HT on pyramidal neuron AHP *in vitro*

We further tested that ket can indeed block the effects of 5-HT by quantifying the AHP after each spike. To do so, we

computed the average spike shape under each condition. Fig. 3A shows the average spike shapes under control (black) and after 5-HT application (light gray). Confirming previous results (Deemyad et al., 2011), 5-HT increases pyramidal neuron excitability by decreasing the AHP after each spike (Fig. 3A, compare black and light gray traces). In contrast, the average spike shapes obtained under control (Fig. 3B, black), after ket application (Fig. 3B, dark gray), and after ket + 5-HT application (Fig. 3B, light gray) were similar to one-another. We then quantified the AHP as done previously. While 5-HT caused a large reduction (control:  $3.12 \pm 0.72$  mV; 5-HT:  $1.62 \pm 0.43$  mV), neither ket nor ket + 5-HT caused significant changes in the AHP (control:  $3.45 \pm 0.58$  mV; ket:  $3.50 \pm 0.62$  mV; ket + 5-HT:  $3.45 \pm 0.64$  mV; Fig. 3C).

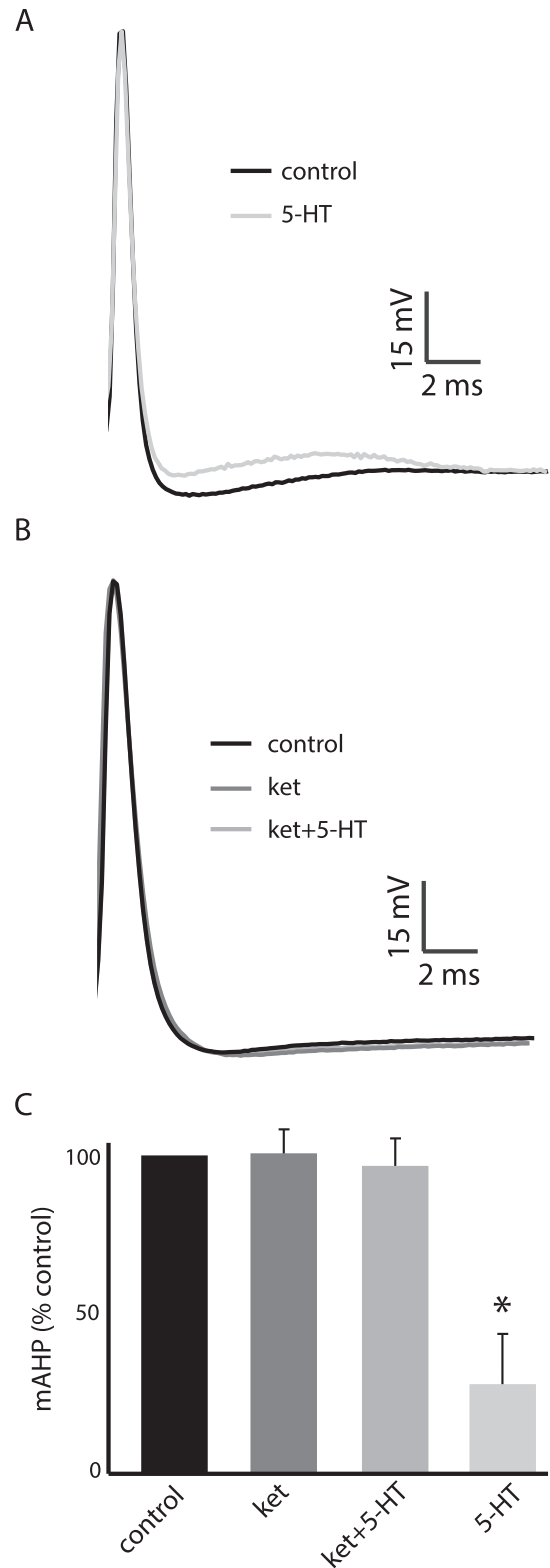
### Ket blocks the effects of 5-HT on pyramidal neuron responses to current injection

We next tested whether ket would block the effects of 5-HT on pyramidal neuron responses to different current injections. To do so, we injected different current steps and computed the firing rate as a function of current (i.e. “ $f-I$ ”) curve. Fig. 4A shows recorded voltage traces at different holding currents for control, after ket application, after ket + 5-HT application, and after 5-HT application. It can be seen that the frequency of firing increases at the same rate for control, ket, and ket + 5-HT but instead increases at a larger rate for 5-HT. We quantified these changes by computing the slope of the  $f-I$  curve (i.e. the gain) (Fig. 4B). While 5-HT significantly increased the gain (control:  $0.12 \pm 0.02$  kHz/nA; 5-HT:  $0.23 \pm 0.04$  kHz/nA), neither ket nor ket + 5-HT caused any significant changes in gain (control:  $0.13 \pm 0.02$  kHz/nA; ket:  $0.11 \pm 0.03$  kHz/nA; ket + 5-HT:  $0.13 \pm 0.02$  kHz/nA; Fig. 4C).

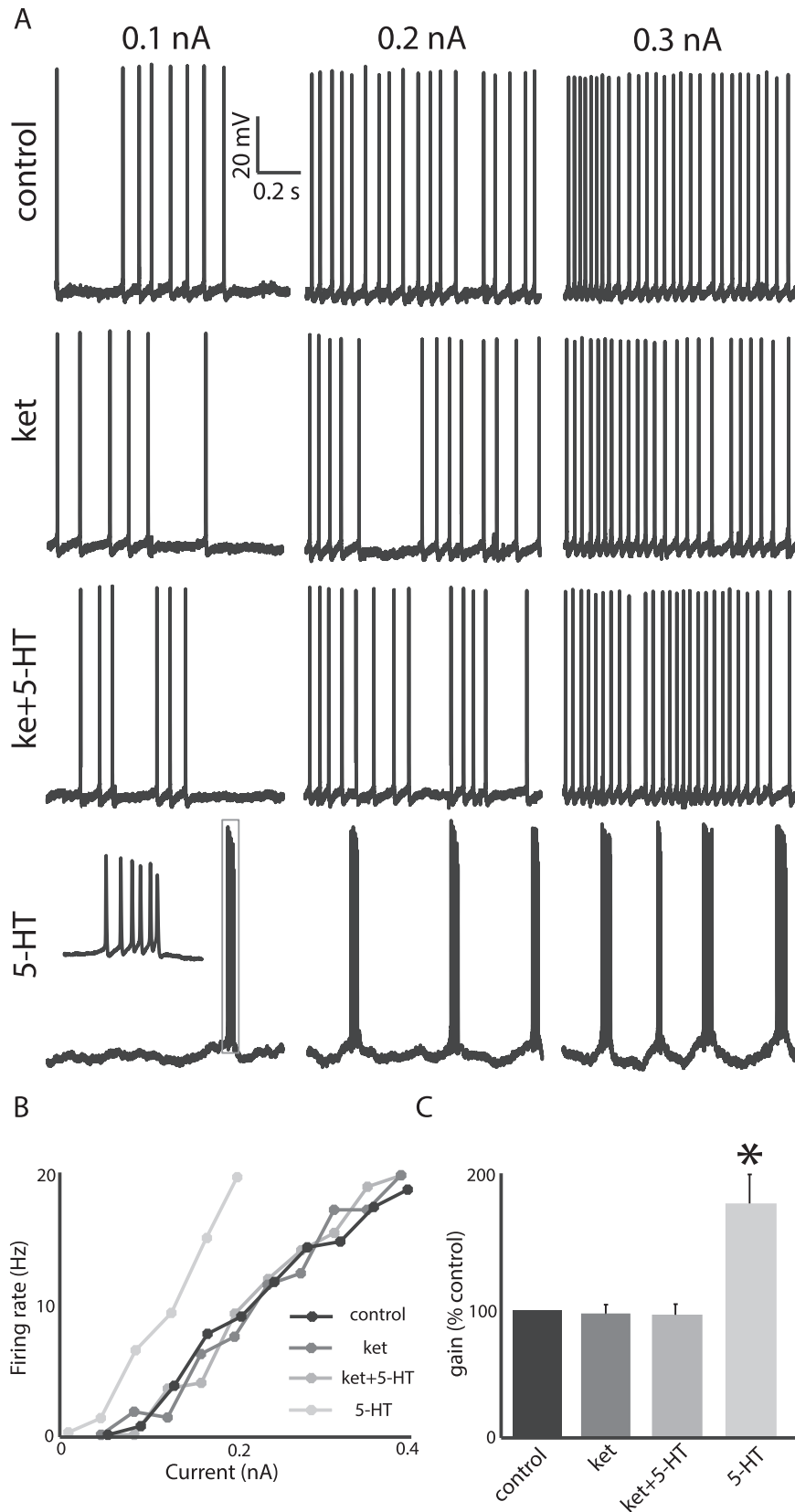
### Ket blocks the effects of 5-HT behavioral responses

We next tested whether ket blocks the effects of 5-HT injection on behavioral responses to electrosensory stimuli. In particular, we focused on two behaviors: the jamming avoidance response (JAR) as well as the production of electrocommunication stimuli called “chirps” that occur during agonistic situations (Zakon et al., 2002; Hupe et al., 2008). The JAR is a complex behavior by which the animal shifts its EOD frequency in order to increase the frequency content of jamming stimuli that occur when two conspecifics are near to one another, thereby shifting it away from the frequency range of other stimuli such as prey (Heiligenberg, 1991) and is frequently used as a measure of perception in weakly electric fish (Carlson and Kawasaki, 2006). The rate at which chirps are emitted was used as a control as previous studies have shown that 5-HT injection will reduce chirp production (Maler and Ellis, 1987; Smith and Combs, 2008; Deemyad et al., 2013).

We recorded behavioral responses of immobilized animals and injected all compounds bilaterally in the ELL (Fig. 5A). This was done in order to ensure that any changes in behavior resulting from drug injection

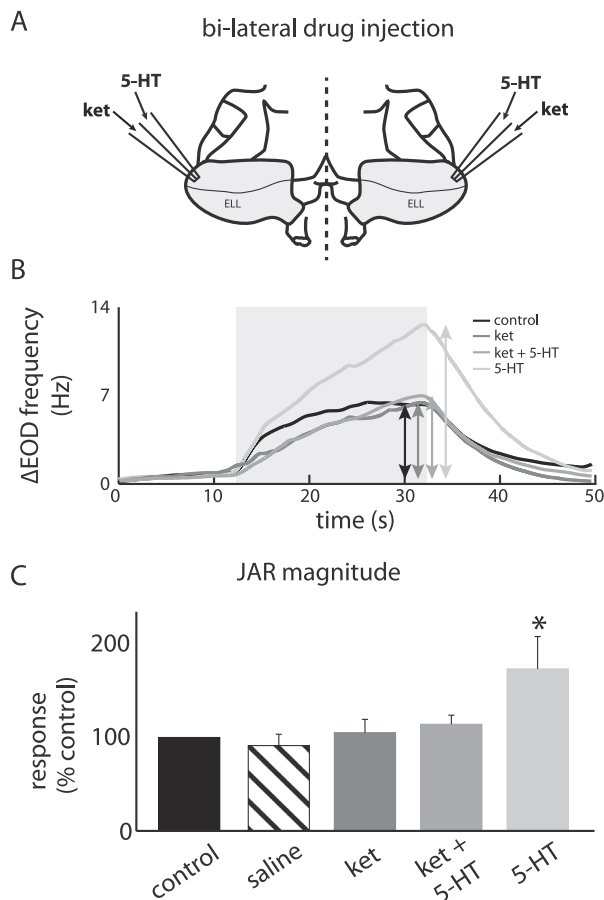


**Fig. 3.** (A) Average spike shapes under control (black) and after 5-HT application (light gray). (B) Average spike shapes under control (black), after ket application (dark gray), and after ket + 5-HT application (light gray). (C) Population-averaged AHP values under all conditions. “\*” indicates that the 5-HT group is significantly different from all others at the  $p = 0.05$  level using a one-way ANOVA with Tukey post hoc HSD ( $n = 7$ ). All other comparisons between group pairs were not significant ( $p > 0.15$ ).



**Fig. 4.** (A) Example responses from an example of a pyramidal neuron to different levels of current injection under control, after ket application, after ket + 5-HT application, and after 5-HT application. (B) *f-I* curves from this same neuron under control (black), after ket application (dark gray), and after ket + 5-HT application (medium gray), and after 5-HT application (light gray). (C) Population-averaged gain values. “\*\*\*\*” indicates that the 5-HT group is significantly different from all others at the  $p = 0.05$  level using a one-way ANOVA with Tukey post hoc HSD ( $n = 7$ ). All other comparisons between group pairs were not significant ( $p > 0.21$ ).

were solely due to altered ELL pyramidal cell output rather than the drug affecting another brain area (Deemyad et al., 2013). We found that 5-HT application significantly increased the JAR magnitude (control:  $7.02 \pm 1.34$  Hz; 5-HT:  $11.65 \pm 2.36$  Hz; Fig. 5B, C). However, ket application, which by itself did not significantly alter the JAR, did block the effects of subsequent 5-HT application (i.e. ket + 5-HT) on the JAR (ket:  $7.23 \pm 2.36$  Hz; ket + 5-HT:  $8.1 \pm 1.23$  Hz; Fig. 5B, C). Injection of saline alone did not have any significant effect on the JAR magnitude (saline:  $6.63 \pm 1.56$  Hz, Fig. 5C). We counted the number of chirps using the EOD spectrogram as done previously (Hupe and Lewis, 2008) (Fig. 6A). While 5-HT application reduced the number of chirps per unit time, ket application by itself did not significantly alter the chirp rate but blocked the effects of subsequent application of 5-HT



**Fig. 5.** (A) Schematic of the experimental setup used for bilateral exogenous ket and 5-HT application in the ELL. Two electrodes containing the drugs were placed in each ELL (*Experimental procedures*). (B) EOD frequency from an example specimen as a function of time before (black) and after drug application (ket: dark gray, ket + 5-HT: medium gray, 5-HT: light gray). The double-head arrows indicate the frequency excursion while the light gray square shows the time period during which the stimulus was applied. (C) JAR magnitude before application (black), after saline application (striped), after ket application (dark gray), after ket + 5-HT application (medium gray), and after 5-HT application (light gray). “\*” indicates that the 5-HT group is significantly different from all others at the  $p = 0.05$  level using a one-way ANOVA with Tukey post hoc HSD ( $n = 7$ ). All other comparisons between group pairs were not significant ( $p > 0.8$ ).

(Fig. 6A). These effects were confirmed across our dataset (control:  $0.51 \pm 0.18$  Hz; ket:  $0.48 \pm 0.18$  Hz; ket + 5-HT:  $0.43 \pm 0.14$  Hz; 5-HT:  $0.23 \pm 0.13$  Hz; Fig. 6B). Injecting saline alone did not have any significant effect on the chirp rate (saline:  $0.49 \pm 0.17$  Hz, Fig. 5C). We thus conclude that ket can block the effects of 5-HT on JAR and chirp rate when applied in ELL.

## DISCUSSION

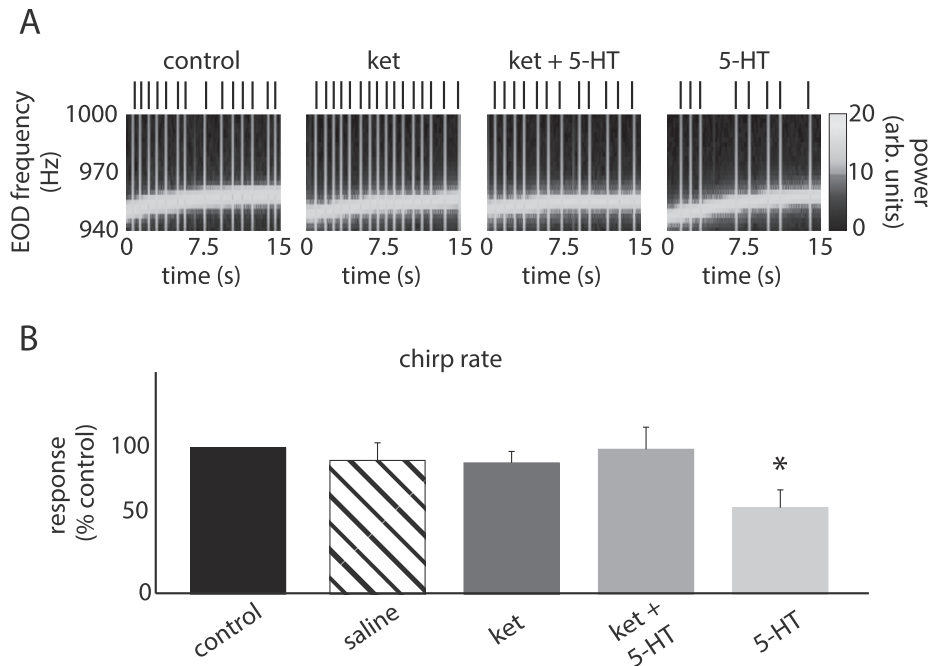
### Summary of results

We have investigated the nature of the receptors that mediate the previously observed effects of 5-HT on the excitability of ELL pyramidal cells and the resulting effects on electrosensory behaviors. First, using an *in vitro* preparation, we found that the 5-HT<sub>2</sub> antagonist ket blocked the effects of 5-HT on pyramidal cell excitability using several measures. Specifically, while 5-HT gave rise to significant increases in firing rate and burst fraction through a decreased AHP, prior ket application blocked this effect as there was no significant change in either firing rate, burst fraction, or the AHP. Further, while 5-HT application significantly increased the gain (i.e. the slope of the  $f-I$  curve), prior application of ket blocked this effect as it did not lead to significant increases in gain. Importantly, ket application did not significantly alter firing rate, burst fraction, AHP, or gain. Next, using an *in vivo* preparation, we investigated the effects of 5-HT and ket application within the ELLs on two electrosensory behaviors, namely chirp production and the JAR. While 5-HT application significantly decreased chirp rate and increased the JAR, prior ket application blocked this effect as 5-HT application then did not lead to any significant changes in chirp rate or JAR.

### 5-HT receptors present in ELL and their effects on potassium channels

Previous studies have found evidence that 5-HT can increase neural excitability by inhibiting potassium channels (Araneda and Andrade, 1991; Fagni et al., 1992; Tanaka and Nishizuka, 1994). In particular, there is strong evidence that activation of 5-HT<sub>2</sub> receptors will inhibit potassium channels (see (D’Adamo et al., 2013) for review) whereas activation of 5-HT<sub>1</sub> receptors will instead activate these (Kelly et al., 1991; Raymond et al., 2001). Previous studies have found that 5-HT increases ELL pyramidal cell excitability by inhibiting potassium conductances (Deemyad et al., 2011), which is similar to decreases in AHP seen in other systems (Araneda and Andrade, 1991). Our results showing that the 5-HT<sub>2</sub> antagonist ket can block the effects of 5-HT on ELL pyramidal cell excitability and the resulting changes in behavior thus strongly support the hypothesis that these effects are mediated by 5-HT<sub>2</sub> receptors.

Previous results have found that ket is a selective antagonist for 5-HT<sub>2</sub> with greater affinity for 5HT<sub>2A/2C</sub> receptors in weakly electric fish (Allee et al., 2008). The similarity between the effects of 5-HT agonist and antagonists used in weakly electric fish and in mammals (Smith



**Fig. 6.** (A) EOD spectrograms (i.e., EOD power spectrum as a function of time) with the vertical bands corresponding to the black vertical bars representing chirps before injection (outside left), after ket injection (left), after ket + 5-HT injection (right), and after 5-HT injection (outside right). (B) Chirp rate before injection (black), after saline injection (striped), after ket injection (dark gray), after ket + 5-HT injection (medium gray), and after 5-HT injection (light gray). “\*” indicates that the 5-HT group is significantly different from all others at the  $p = 0.05$  level using a one-way ANOVA with Tukey post hoc HSD ( $n = 7$ ). All other comparisons between group pairs were not significant ( $p > 0.8$ ).

and Combs, 2008), together with the fact that the anatomy of the 5-HT system is remarkably conserved across vertebrates (Parent, 1981), thus make it likely that the effects of 5-HT on ELL pyramidal cell activity are mediated by 5-HT<sub>2A/C</sub> receptors. Further evidence for this hypothesis comes from the fact that, although previous studies have shown that the 5-HT<sub>1A</sub> receptor antagonist WAY-100635 can affect behavioral responses in *A. leptorhynchus* when injected systemically (Smith and Combs, 2008), our preliminary data indicate that this same compound does not block the effects of 5-HT when injected into the ELLs (data not shown). It is therefore unlikely that the effects of 5-HT on ELL pyramidal neuron excitability are mediated by 5-HT<sub>1A</sub> receptors.

The hypothesis that 5HT<sub>2A/C</sub> receptors mediate the effects of 5-HT on ELL pyramidal neuron activity is interesting as previous studies have found that 5-HT increased ELL pyramidal cell excitability by inhibiting two different potassium channels: SK and M currents (Deemyad et al., 2011). While SK currents are activated through increases in the intracellular calcium concentration (Adelman et al., 2012), M currents are instead voltage-gated (Yue and Yaari, 2004; Drion et al., 2010). In ELL pyramidal cells, previous studies have found that SK channels were activated following an action potential (i.e. suprathreshold) and lead to an AHP (Ellis et al., 2007b; Toporikova and Chacron, 2009; Deemyad et al., 2011, 2012). In contrast, M currents are instead activated at membrane voltages below those necessary to elicit action potential firing (i.e. subthreshold) (Deemyad et al., 2011, 2012). A previous study has found that, despite having similar effects on the AHP and ELL

pyramidal cell excitability, these currents had opposite effects on frequency tuning (Deemyad et al., 2012). Intrinsic membrane conductances are major determinants of the frequency tuning of ELL pyramidal cells (Ellis et al., 2007a,b; Krahe et al., 2008; Mehaffey et al., 2008b; Toporikova and Chacron, 2009) and their activation/inhibition by neuromodulators is thus expected to have major effects on their responses to sensory input (Marquez et al., 2013). We thus propose that 5-HT can either increase or decrease ELL pyramidal cell responses to sensory input by inhibiting either SK or M currents through either of 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors. A recent study conducted *in vivo* has shown that the function of the serotonergic system on sensory processing by ELL pyramidal cells is to selectively enhance their responses to stimuli associated with same sex conspecifics (Deemyad et al., 2013). While these results showed that the effects of 5-HT only enhanced responses, it is important to note that they were obtained through either exogenous 5-HT application or endogenous release via raphe nuclei stimulation. Further studies using more natural behavioral contexts are needed to test whether 5-HT could either enhance or decrease ELL pyramidal cell responses to sensory input.

We note that previous studies have shown that 5-HT expression is graded across the ELL segments in *A. leptorhynchus*; expression was weakest in CMS, intermediate in CLS, and strongest in LS (Deemyad et al., 2011). *In vitro* electrophysiological results show excellent correlation with expression across segments as 5-HT did not significantly alter CMS pyramidal cell activity, weakly increased CLS pyramidal cell excitability,



and strongly increased LS pyramidal cell excitability (Deemyad et al., 2011). Previous studies have shown that, under control conditions, CMS neurons respond most strongly to stimuli causing a JAR (Shumway, 1989a; Krahe et al., 2008) and the CMS is required for JAR behavior under control conditions (Metzner and Juranek, 1997). However, the results of Deemyad et al. (2011), taken together with the fact that 5-HT injected into ELL will increase LS neuron responses to stimuli eliciting the JAR (Deemyad et al., 2013), provide strong evidence against the hypothesis that CMS neurons contribute to the observed increase in JAR magnitude caused by 5-HT. Rather, we speculate that the increase in JAR behavior is caused by the increased LS neural responses to stimuli eliciting this behavior.

### Effects of 5-HT on behavior

Previous studies have shown that 5-HT has significant influences on behavioral responses. In particular, elevated levels of 5-HT have been associated with decreased aggression and social dominance across species (Larson and Summers, 2001; Perreault et al., 2003; Sperry et al., 2003). In weakly electric fish, exogenous 5-HT systemic application decreases aggressivity as measured by the production of electro-communication signals called “chirps” that are produced during aggressive behavioral contexts (Maler and Ellis, 1987; Allee et al., 2008; Smith and Combs, 2008; Zubizarreta et al., 2012; Deemyad et al., 2013) as well as simultaneously increases the perception of low-frequency signals generated by same sex conspecifics (Deemyad et al., 2013). Different types of 5-HT receptors have been previously shown to mediate decreases in aggressivity. Indeed, previous studies using the same species of weakly electric fish have shown that activation of 5-HT<sub>1A</sub> receptor agonists/antagonists increased/decreased chirp production while 5-HT<sub>2A</sub> receptor agonists/antagonists instead decreased/increased chirp production (Smith and Combs, 2008). Qualitatively similar results were obtained in another species using measures of social dominance (Allee et al., 2008). While our results showing that the 5-HT<sub>2A/C</sub> receptor antagonist ket has no effect on chirp production appear to contradict the results of Smith and Combs (2008) at first glance, it is important to note that our injections of ket were localized to the ELLs whereas their injections were instead systemic. 5-HT receptors are located throughout the brain of weakly electric fish (Johnston et al., 1990) and, in particular, are present in brain areas controlling chirp production (Telgkamp et al., 2007). Future studies should focus on understanding how different behavioral contexts activate 5-HT receptors across different brain areas from sensory to motor in order to give rise to changes in perception and behavioral responses to stimuli arising from these contexts.

### CONCLUSION

Our results have shown that ket can block the effects of 5-HT of ELL pyramidal cell excitability and the resulting changes in behavior. While the similarity between the effects of 5-HT agonists/antagonists in weakly electric

fish and mammals and the remarkable conservation of the 5-HT system across vertebrates lend strong support to our hypothesis that 5-HT<sub>2</sub> receptors mediate the effects of 5-HT, it is important to realize that 5-HT receptors have not yet been cloned in *A. leptorhynchus*. Cloning of these receptors followed by *in situ* hybridization/immunohistochemistry is necessary to fully establish the nature of the 5-HT receptors present in ELL as well as whether they display functional homology with their mammalian counterparts.

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