Sensory Specializations of Mormyrid Fish Are Associated with Species Differences in Electric Signal Localization Behavior

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Abstract

The ability to localize communication signals plays a fundamental role in social interactions. For signal localization to take place, the sensory system of the receiver must extract information about distance and direction to the sender from physical characteristics of the signal. In many sensory systems, information from multiple peripheral receptors must be integrated by central sensory pathways to determine the sender location. Here, we asked whether evolutionary divergence in the electrosensory and visual systems of mormyrid fish is associated with signal localization behavior. In mormyrids, differences in the distribution of electroreceptors on the surface of the skin are associated with differences in the midbrain exterolateral nucleus (EL). Species with electroreceptors clustered in three rosettes on both sides of the head have a small and undifferentiated EL. In contrast, EL is enlarged and subdivided into anterior (ELa) and posterior (ELp) regions in species that have electroreceptors broadly distributed throughout the body. Interestingly, species with EL and clustered electroreceptors also have larger visual systems and higher visual acuity than species with ELa/ELp and broadly distributed electroreceptors. Species with broadly distributed electroreceptors and ELa/ELp approached a simulated conspecific by following the curved electric field lines generated by the electrosensory stimulus. In contrast, a species with small EL and clustered electroreceptors, but an enlarged visual system, followed shorter and straighter paths to the stimulus source. In the central electrosensory system, evoked field potentials in response to stimuli delivered from the left versus the right differed more in EL than in ELa/ELp. Our results suggest that signal localization behavior is associated with differences in sensory specializations. We propose that the distribution of electroreceptors on the body affects the ability of individuals to align parallel to electric field lines and maintain such alignment while approaching the signal source. The spatial resolution of sensory information relayed from the periphery to the midbrain in species with clustered electroreceptors may allow for gross, but not fine, processing of sender location. Furthermore, visual information may play an important role in localizing signaling individuals in species with small EL and clustered electroreceptors. In line with previous studies, we suggest that the

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physiological and behavioral differences associated with signal localization reflect adaptations to different habitats and social environments.

Introduction

Animal communication mediates biologically important social behaviors like attracting mates and defending resources [Bradbury and Vehrencamp, 2011]. In such contexts, receivers must be able to detect, recognize, and localize the senders. To locate the sender, the sensory system of the receiver must extract information about directionality and distance from physical characteristics of communication signals. For instance, the ability to use small differences in arrival time at both ears is a textbook example of how the auditory systems of birds and mammals localize acoustic signal sources in space [Carr and Konishi, 1990; Grothe, 2003; Brand et al., 2002]. Not all communication modalities, however, can use changes in signal properties due to propagation through the environment for signal localization.

Weakly electric fish of the family Mormyridae communicate using pulse-type electric organ discharges (EODs). Importantly, EODs form electrostatic fields that, contrary to acoustic signals, do not propagate as waves through the environment (Fig. 1a) [Hopkins, 1986]. Because these signals exist only as electrostatic fields, there are no delays or changes in signal waveform due to propagation that might provide information about the direction to the source [Hopkins, 1986]. Nonetheless, EOD electrostatic fields resemble those of a dipole for distances greater than one body length and the potential and gradient of the electric field drop in a predictable manner with distance from the fish (Fig. 1a) [Knudsen, 1975]. The electrosensory system of weakly electric fish can potentially use this variation to obtain information about sender location.

Mormyrids have an electrosensory pathway devoted to processing communication signals [reviewed in Baker et al., 2013]. EODs produced by other fish are detected by a specific type of electroreceptor on the surface of the skin called knollenorgans [Derbin and Szabo, 1968]. Interestingly, knollenorgan anatomy and physiology vary among mormyrid species and are related to the ability of some species to detect subtle variations in EOD waveform (Fig. 1b) [Harder, 1968; Carlson et al., 2011; Baker et al., 2015]. Knollenorgans are distributed throughout the surface of the head, back, and belly in species sensitive to EOD waveform variation [Carlson et al., 2011; Baker et al., 2015]. These electroreceptors fire single time-locked spikes in response to electrosensory stimuli [Carlson et al., 2011; Baker et al., 2015]. Importantly, these spiking electroreceptors respond to inward current transients [Bennett, 1965]. Because electric current from an external stimulus enters the body on one side and leaves the body on the opposite side, electroreceptors on opposite sides of the body experience opposite stimulus polarities and respond to different phases of electrosensory stimuli [Hopkins and Bass, 1981]. This means that knollenorgans on one side of the body respond to rising edges of a stimulus, while knollenorgans on the opposite side of the body respond to falling edges [Bennett, 1965]. Spike timing differences among electroreceptors on opposite sides of the body encode signal duration and waveform, and may provide information about sender location [Friedman and Hopkins, 1998; Lyons-Warren et al., 2013a].

In contrast, species insensitive to EOD waveform have knollenorgans clustered in three rosettes on both sides of the head (Fig. 1b) [Harder, 1968; Carlson et al., 2011; Baker et al., 2015]. These knollenorgans produce spontaneous oscillating potentials and respond to electrosensory stimuli with an increase in oscillatory amplitude and a phase reset that results in transient oscillatory synchrony among receptors [Harder, 1968; Baker et al., 2015]. Importantly, knollenorgans on opposite sides of the body reset to phases that are 180º out of phase with each other [Baker et al., 2015]. This difference in phase does not encode information about signal duration or waveform, but may provide information about stimulus location [Baker et al., 2015].

Species differences in knollenorgan anatomy and physiology are also associated with differences in the central electrosensory system [Carlson et al., 2011]. The midbrain exterolateral nucleus (EL), a brain region devoted to processing communication signals [Bennett and Steinbach, 1969; Bell and Grant, 1989; Amagai, 1998; Vélez and Carlson, 2016], is enlarged and subdivided into anterior (ELa) and posterior (ELp) regions in species that have distributed electroreceptors and are sensitive to EOD waveform variation [Carlson et al., 2011]. In these species, EOD sensitivity arises in ELa and is achieved by comparing spike timing differences among electroreceptors on opposite sides of the body in an excitatory-inhibitory circuit [Friedman and Hopkins, 1998; Lyons-Warren et al., 2013a]. In contrast, EL is small and undifferentiated in species with clustered electroreceptors that are insensitive to EOD waveform variation [Carlson et al., 2011]. A homologous excitatory-inhibitory neural circuit
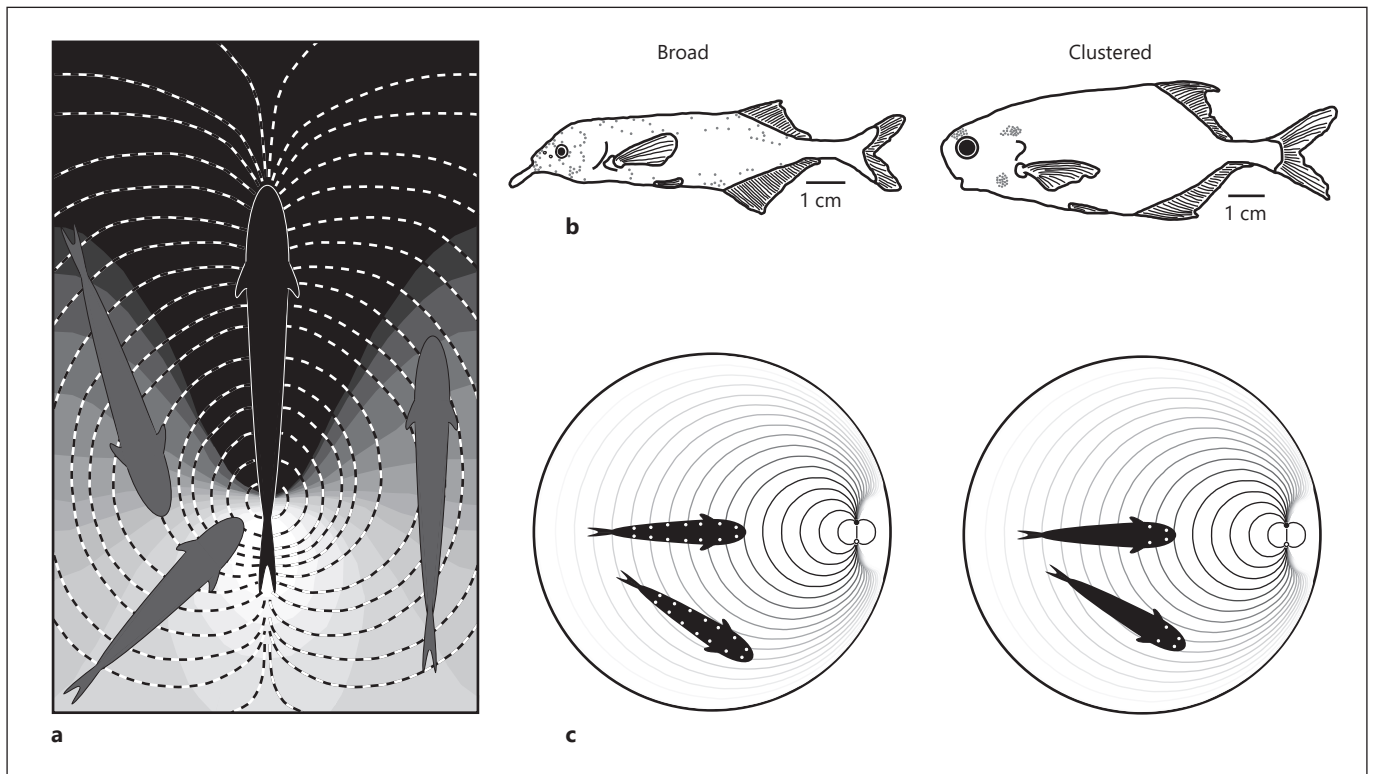
Fig. 1. Species differences in electroreceptor distribution along the body may affect sampling of the electrostatic field produced by other individuals and may relate to the ability to localize the source of a communication signal. **a** Schematic of a social interaction between mormyrid fish showing the electrostatic field generated by the EOD of 1 fish (black) and how different fish sample the field depending on their position relative to the signaling fish (gray). The field potential (color map, black: positive, white: negative) and gradient (current lines in black and white) vary with distance from the fish and resemble those of a dipole for distances larger than a body length. **b** There are species differences in how knollenorgans, the electroreceptors that sense communication signals produced by other individuals, are distributed along the body. Some species have knollenorgans (gray dots) broadly distributed throughout the head, back, and belly (left), while other species have knollenorgans clustered in three rosettes on both sides of the head (right). Species with clustered electroreceptors on both sides of the head also tend to have relatively larger eyes than species with broadly distributed electroreceptors. **c** Differences in knollenorgan distribution along the body may affect how much information about the electric field is sampled. The electric field lines produced by a tangential dipole electrode in a circular tank are curved and the amplitude of the electric current decreases with distance from the source. When oriented perpendicular to the electric field lines, species with broadly distributed electroreceptors (left, white dots) may sample a wider range of electric current amplitudes than species with clustered knollenorgans (right, white dots). When oriented parallel to the electric field lines, electroreceptors on the head and tail experience stimuli of similar current in species with broadly distributed knollenorgans; this source of information is not available for species with clustered electroreceptors.

in EL is incapable of analyzing EOD waveform, but it may process information about signal location by comparing responses from knollenorgans on opposite sides of the body [Vélez et al., 2017].

Interestingly, species differences in electrosensory specializations are associated with differences in the visual system [Stevens et al., 2013]. Species with small EL and clustered electroreceptors have an enlarged optic tectum (OT) and relatively larger eyes than species with ELa/ELp and broadly distributed electroreceptors [Stevens et al., 2013]. Importantly, the enlargement of the visual system is associated with higher visual acuity in species with small EL and clustered electroreceptors [Stevens et al., 2013]. Thus, different lineages of mormyrids appear to have evolved visual or electrosensory specializations, but not both.

A previous study on passive electrolocation by mormyrid fish with electrosensory specializations concluded that mormyrids localize signal sources by first orienting parallel to an electric current line and then swimming

forward while maintaining such alignment [Schluger and Hopkins, 1987]. This alignment may be achieved by comparing the potential and gradient of the electrostatic field across the population of electroreceptors [Schluger and Hopkins, 1987; Davis and Hopkins, 1988; Shieh et al., 1996; Hopkins et al., 1997]. Therefore, differences in electroreceptor distribution along the body may lead to differences in signal localization behavior. Species with broadly distributed electroreceptors may sample the electric field over a greater region than species with clustered electroreceptors (Fig. 1c), which may provide them with more opportunities to compare signal properties in space and, ultimately, facilitate signal localization. In contrast, species with clustered electroreceptors and visual specializations may rely more heavily on visual cues during signal localization behavior. Here, we tested the hypothesis that evolved differences in electrosensory and visual processing relate to signal localization behavior. We investigated passive electrolocation abilities and midbrain directional sensitivity in species with clustered and broadly distributed electroreceptors.

Materials and Methods

Our protocols for testing, handling, and housing animals were approved by the Institutional Animal Care and Use Committee at Washington University in St. Louis and fulfill the guidelines established by the National Institutes of Health. Details about housing and handling individuals can be found in Baker et al. [2015].

We used five mormyrid species in this study. One species, *Petrocephalus tenuicauda*, belongs to the subfamily Petrocephalinae, has oscillatory knollenorgans clustered in three rosettes on both sides of the head, has a small and undifferentiated EL, and is insensitive to EOD waveform [Carlson et al., 2011; Baker et al., 2015]. Three species belong to a monophyletic group in the subfamily Mormyrinae known as "clade A", have spiking knollenorgans distributed throughout the head, back, and belly, have an enlarged and subdivided ELa/ELp, and are sensitive to EOD waveform variation: *Brienomyrus brachyistius*, *Brevimyrus niger*, and *Pollimyrus adspersus* [Carlson et al., 2011]. Our fifth species, *P. microphthalmus*, is an interesting addition because it belongs to the subfamily Petrocephalinae but it is the only known species in the subfamily that has broadly distributed electroreceptors and an enlarged ELa/ELp, and is sensitive to EOD waveform variation [Carlson et al., 2011]. Compared to clade-A species and *P. microphthalmus*, *P. tenuicauda* has an enlarged visual system and higher visual acuity [Stevens et al., 2013]. While the small and undifferentiated EL is the most likely ancestral state of this brain region, it is unclear whether broadly distributed electroreceptors is the ancestral state or whether it evolved independently in the clade-A and *P. microphthalmus* lineages [Carlson et al., 2011]. A larger visual system and better visual acuity likely evolved only once, in the lineage containing *P. tenuicauda* [Stevens et al., 2013].

Experiment 1: Passive Electrolocation

We replicated the behavioral experiments of Schluger and Hopkins [1987] to study passive electrolocation of a simulated conspecific and determine how differences in sensory processing relate to signal localization behavior. We obtained data from 6 *P. tenuicauda* (standard length, SL: 6.2–8.4 cm), 9 *B. brachyistius* (SL: 7.0–11.7 cm), 7 *B. niger* (SL: 5.6–8.0 cm), 7 *P. adspersus* (SL: 6.6–8.0 cm), and 5 *P. microphthalmus* (SL: 6.6–8.7 cm). Given the high overlap across species in the size of the subjects tested, variation in the amount of electrosensory information available at the periphery likely depends on electroreceptor phenotype rather than body size: it is higher in species with broadly distributed electroreceptors and lower in *P. tenuicauda*, the species with clustered electroreceptors on both sides of the head.

Experimental Apparatus

Experiments of passive electrolocation were conducted in a circular test arena that consisted of a cylindrical plastic tank of 56 cm in diameter and 36.5 cm in height. The tank was filled to a height of approximately 10 cm with approximately 25 L of water (water conductivity range: 175–225 μS/cm). At the center of the test arena, we placed a shelter for the fish consisting of three plastic posts (8 cm tall) and a circular ceiling made of opaque plastic (14 cm in diameter). Four white-light lamps were equidistantly placed around the circumference of the top edge of the tank. We placed eight dipole stimulus electrode pairs around the test arena, 28 cm from the center, separated by 45°. The positive and negative leads of the dipole electrode pairs were separated by 4.5 cm and oriented tangentially to the tank wall. The tank was placed inside a dark, sound-proof walk-in chamber. The intensity of the white-light lamps on top of the tank was controlled with a rheostat from outside the chamber. The temperature inside the chamber was kept between 25 and 28 °C with a room heater (Optimus) and monitored from outside the chamber with a digital thermometer (Coralife) coupled to a probe placed inside the chamber. The chamber was illuminated with infrared (IR) light and all experiments were recorded with an IR-sensitive video camera (Eclipse) mounted 1 m above the test arena and centered on the central fish shelter. The video feed of the camera was sent to a monitor and 4-channel DVR (G-Pro, Eclipse) outside the chamber used to observe behavior in real-time and to save the video files for subsequent analyses (video frame rate: 30 frames/s).

Stimuli

All species tested in this study communicate using pulse-type EODs that have a species-specific waveform and are delivered with variable inter-pulse intervals (IPIs). We used natural EODs as stimuli for our behavioral experiments. For each subject, we randomly chose a conspecific natural EOD from a library of 10–15 EODs previously recorded from different individuals. Natural EODs were presented with either normal or reversed polarity (randomly chosen) and delivered with a random sequence of IPIs obtained from a uniform distribution between 50 and 100 ms. This range of synthetic IPIs spans the range of natural IPIs used by mormyrids in different social contexts [Carlson, 2002]. The sequence of EODs was generated digitally (19.5-kHz sampling rate) in Matlab (Mathworks, Natick, MA, USA), converted to analog with a Model RX 8 signal processor (Tucker-Davis), attenuated with a PA5 attenuator (Tucker-Davis), and isolated from the ground with a stimulus isolation unit (A-M Systems, Model 2200). One of the

eight dipole electrode pairs around the perimeter of the arena was randomly chosen and selected with a switch box for stimulus delivery. An intermittent, low-intensity LED, placed just above and outside of the tank, was active during stimulus presentation and used for timing calibration during video analyses.

Experimental Protocol

At the beginning of each trial, we placed a subject in the test arena and gave it 10 min to acclimate under only IR illumination. After the acclimation period, we turned on the overhead lamps and set the light intensity to the minimum level on the rheostat. We then incremented the light intensity every 30 s by sliding the rheostat knob a step equal to 5% of the knob's range. We stopped increasing the light intensity when we reached a level bright enough for the subject to seek shelter but low enough such that it would sporadically leave the shelter to explore [Schluger and Hopkins, 1987]. Once the subject was consistently shifting between sheltering and exploring behaviors, we gave the fish an additional 5 min of acclimation in the corresponding light setting. After this 5-min acclimation period, we started the stimulus playback after the fish completed 10 s under the shelter.

For the first playback trial, we set the amplitude of the stimulus to 0.75 mV/cm, as measured at the center of the arena. We played back the stimulus for 60 s, during which the subject was allowed to move freely in the tank. We scored a positive response when the fish entered a "response zone" consisting of a region around the stimulus electrode of 6.5 cm radially and 13 cm tangentially. To avoid overstimulation, we stopped stimulus playback as soon as the subject entered the response zone or after 60 s of stimulus presentation, whichever came first. After the first trial, we gave the subject a 5-min time-out period without electrosensory stimulation. After the time-out period, we chose the signal polarity and stimulating electrode at random and started a second trial. If the subject responded to the preceding trial, the stimulus was delivered at the same level. If, on the other hand, the subject failed to respond in the previous trial, the level of the stimulus was increased by 5 dB. We continued this pattern until the subject failed to respond four consecutive times or until signal amplitude was set to 4.1 mV/cm at the center of the arena and did not elicit a response. When either of these two criteria were met, testing for that subject was terminated. We used stimulus levels between 0.75 and 4.1 mV/cm because this range is above reported behavioral thresholds and within the range of amplitudes at which mormyrids have been observed to interact [Moller, 1970; Moller et al., 1989; Lyons-Warren et al., 2012].

Video Analysis

We recorded and saved digital videos of all tracks obtained. We used the plug-in MtrackJ [Meijering et al., 2012] in Fiji (Fiji Is Just Image-J) [Schindelin et al., 2012] to track the path of the fish and to measure a set of linear and angular variables of the tracks. We tracked the head of the fish manually from the first frame in which the head was outside of the shelter until the first frame in which the head entered the response zone, advancing the video three frames at a time (0.1-s steps). From each track, we measured: (i) the latency to leave the shelter (in s), (ii) the length of the track from the shelter to the response zone (in cm), (iii) the duration of the track (in s), (iv) the average speed (in cm/s), and (v) the turn angles. The turn angles were obtained every three consecutive samples and measured as the angular change (from 0° to 179°)

between the vector formed by the first two samples and the vector formed by the second and third samples. Higher values of turn angles represent movements that deviate from a straight line, while turn angles equal to zero represent sequential track segments on a straight line. For each track, we calculated the mode and coefficient of variation (CV) of the turn angles. In Matlab (Mathworks), we then normalized all measurements so that the stimulating electrode was positioned at 0°. We also normalized responses to the same stimulus polarity by reflecting the tracks in response to reverse-polarity stimuli along the 0° axis. We then measured the angles at which subjects left the shelter and entered the response zone. Exit and entrance angles were measured relative to the line between the center of the shelter and the stimulus electrode in the normalized tracks.

Statistical Analysis

We asked whether swimming behavior to localize a source of electrosensory stimuli varied with the different phenotypes of electroreceptor distribution along the body: broadly distributed versus clustered. To answer this question, we grouped data from all species with broadly distributed electroreceptors and compared it to the data obtained from *P. tenuicauda*, the species with clustered electroreceptors. Because distributed electroreceptors may have evolved independently in clade A and *P. microphthalmus* [Carlson et al., 2011], and because *P. microphthalmus* is more closely related to *P. tenuicauda* than to clade A, we also explored potential species differences in tracking behavior. We performed a principal components (PCs) analysis on the six variables measured: (i) latency to leave shelter, (ii) path duration, (iii) path length, (iv) speed, (v) mode turn angle, and (vi) CV turn angle. We compared PCs among electroreceptor phenotype and species using linear mixed models in R [R Core Team, 2014] with the package lme4 [Bates et al., 2012]. We started with a null model that only included subject ID as a random effect. To this null model, we added either electroreceptor phenotype or species as predictor variables to determine whether the model was improved. We assessed model fit by using χ^2 distribution to obtain p values on likelihood ratio tests comparing the null model with the full models.

We also asked whether the angles at which subjects left the shelter and entered the response zone varied across electrosensory/visual phenotypes and species. We first used V tests [Zar, 1999] to test the null hypothesis that exit and entrance angles were uniformly distributed against the alternative hypothesis that subjects were oriented towards the stimulus electrode (0°). We then used Mardia-Watson-Wheeler tests to compare the distribution of exit and entrance angles between phenotypes and species. This analysis tests whether two or more samples from circular data differ significantly from each other in mean angle, angular variance, or both [Mardia and Jupp, 2000]. For the purpose of our study, this second analysis is particularly important because all species may show nonuniform angular distributions and significant orientation towards the stimulus electrode, but the angular variance may differ between species or phenotypes. For instance, the distribution of exit angles in species that track current lines may have a high variance, while the distribution of exit angles of species that swim straight to the stimulus electrode may have a low variance. All analyses of circular statistics were performed in Oriana v.4.0 (Kovach Computing Services, Anglesey, UK).

Experiment 2: Directional Sensitivity in the Central Electrosensory System

Differences in electroreceptor distribution along the body are associated with gross anatomical differences in the central electrosensory system. Species with clustered electroreceptors have a small and undifferentiated EL; in contrast, EL is enlarged and subdivided into ELa and ELp regions in species with broadly distributed electroreceptors [Carlson et al., 2011]. We asked whether directional sensitivity in the midbrain varies between species with small and undifferentiated EL and species with enlarged and subdivided ELa/ELp. We obtained in vivo evoked potentials from EL of *P. tenuicauda* (6 subjects) and from ELa and ELp of *P. microphthalmus* (3 subjects) and *B. niger* (2 subjects) in response to electrosensory stimulation delivered with two different orientations.

Evoked Potential Recordings

We followed previously described protocols for obtaining evoked field potentials from the midbrain EL and readers are referred to those studies for additional details not reported here [Carlson, 2009; Lyons-Warren et al., 2013b; Vélez and Carlson 2016]. Briefly, we anesthetized the fish with 300 mg/L of tricaine methanesulfonate (MS-222, Sigma-Aldrich, St. Louis, MO, USA) and paralyzed it with 100–150 µL of 3 mg/mL solution of gallamine triethiodide (Flaxedil, Sigma-Aldrich). The fish was then transferred to a recording chamber (20 × 12.5 × 45 cm) filled with fresh water. We left a small region of the head above water level and used a pipette in the mouth to respirate the fish with an aerated solution of 100 mg/L MS-222 to maintain general anesthesia for surgery. We applied 0.4% Lidocaine at the surgery site as local anesthetic. We first removed the skin and secured a pole on the skull. We then removed part of the bone to expose the left ELa and ELp in *B. niger* and *P. microphthalmus*. The midbrain EL of *P. tenuicauda* is not exposed as in the other two species; therefore, we used two retractors made from borosilicate capillary glass to separate the OT and the valvula cerebelli to expose the left EL. We brought the fish out of general anesthesia after surgery by switching to aerated fresh water respiration and we monitored the fish's electromotor output with a pair of electrodes next to the tail of the fish [Carlson, 2002]. Flaxedil silences the EOD, but the pair of electrodes next to the tail can record the EOD command from the spinal electromotor neurons. EOD commands were amplified 1,000 times (A-M Systems, Model 1700) and sent to a window discriminator for time-stamping (World Precision Instruments, SYS-121). We recorded evoked potentials after the fish had completely recovered from anesthesia.

To record evoked field potentials, we used electrodes made of borosilicate capillary glass (o.d. = 1.0 mm, i.d. = 0.5 mm; A-M Systems, Model 626000) pulled on a Flaming/Brown micropipette puller (Sutter Instruments Company, Model P-97). We broke the tip of the micropipette to a diameter of 10–15 µm and filled it with 3 M of NaCl. We used a differential AC amplifier (A-M Systems, Model 1700) to amplify (1,000 times) and bandpass filter (0.01–5 kHz) the evoked potentials. Evoked potentials were then digitized at a rate of 97.6 kHz (Tucker Davis, Model RX 8) and saved to drive using custom software in Matlab. For each stimulus, we calculated a mean evoked potential by recording and averaging the responses to 10 repetitions of the stimulus. The midbrain EL and ELa/ELp are brain regions devoted to processing communication signals produced by other individuals.

When an EOD command is generated, responses to the fish's own EOD are blocked in the hindbrain by an inhibitory corollary discharge and no information reaches the midbrain EL [Bennett and Steinbach, 1969; Bell and Grant, 1989; Amagai, 1998; Vélez and Carlson, 2016]. For this reason, all repetitions in which a fish produced an EOD command 2–5 ms before the stimulus were ignored and retaken.

Stimuli and Analysis of Evoked Potentials

We investigated evoked potentials in response to stimuli delivered from the left and the right sides of the fish. For each species, the stimulus used was a natural conspecific EOD. For each fish, one natural EOD was randomly chosen from a library of 10–15 prerecorded conspecific EODs. The parameters for stimulus delivery were set digitally in Matlab (Mathworks); the stimulus was then converted to analog with a Tucker-Davis RX 8 signal processor, attenuated with a Tucker-Davis PA5 attenuator, isolated from ground with an A-M systems 2200 stimulus isolation unit, and delivered through vertical electrodes on the walls of the recording chamber. A uniform, transverse electric field was delivered via a linear array of three anodal electrodes on one side of the fish and a linear array of three cathodal electrodes on the other side of the fish [Lyons-Warren et al., 2013b]. Each array traversed the longitudinal extent of the fish so that the entire body received a homogenous transverse field. The anodal stimulus electrodes were set to the left or the right side of the fish's body for stimuli delivered from the left and the right sides, respectively. The stimulus amplitude was set to 31 mV/cm at the position of the fish.

From the mean evoked potential for each stimulus orientation, we measured the peak-to-peak amplitude, the width of the evoked potential, and the area of the evoked potential. The width was measured as the time between the zero-crossings of the negative portion of the evoked potential. Also from the negative portion of the evoked potential, we calculated the area as the sum of the absolute value of the evoked potential amplitude at each time sample, multiplied by the sampling rate. We ran a PCs analysis on these three variables and, for each individual, we calculated the Euclidian distance in two-dimensional PC space between the PC scores for evoked potentials obtained for each stimulus orientation. Our rationale was that, if information about signal orientation is present in the neural responses of the midbrain, then evoked potentials should vary with stimulus orientation. Such variation would be evidenced as greater distances between PC scores.

We analyzed variation in neural responses for each species separately. In *B. niger* and *P. microphthalmus*, we analyzed evoked potentials from ELa and ELp separately. In *P. tenuicauda*, we used the latency to the negative peak as a proxy for the recording position along an anterior-posterior axis in EL [Vélez and Carlson, 2016]. We have previously shown that the shape and latency of EL evoked potentials vary along this axis and span the range of variation observed in ELa/ELp of clade-A species and *P. microphthalmus* [Vélez and Carlson, 2016]. We used repeated measures ANOVA to compare the Euclidian distances between PC scores across ELa and ELp in *B. niger* and *P. microphthalmus*. We used a linear regression model to investigate Euclidian distances between PC scores as a function of latency to the evoked potential in *P. tenuicauda*. All analyses were conducted in R [R Core Team, 2014].

Results

Experiment 1: Passive Electrolocation

The rate of test success, calculated as the number of subjects that showed one or more responses to the stimulus electrode divided by the total number of subjects tested, was close to 0.5 for all species: *B. brachyistius* (9/20), *B. niger* (7/12), *P. adspersus* (7/14), *P. microphthalmus* (5/11), and *P. tenuicauda* (6/11). The maximum number of trials obtained from a single individual were 4 for *B. brachyistius*, 3 for *B. niger*, 4 for *P. adspersus*, 6 for *P. microphthalmus*, and 4 for *P. tenuicauda*. The position of the rheostat knob at which subjects were tested ranged between 5 and 40% in *B. brachyistius* and *P. adspersus*, and was always 20% in *B. niger*, *P. microphthalmus*, and *P. tenuicauda*. The stimulus levels at which subjects showed positive responses ranged from 0.75 to 4.1 mV/cm in all species. Figure 2 depicts the raw tracks in response to conspecific EODs for each species. Sample videos of passive electrolocation behavior are available as online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000496493).

The raw tracks from each trial for each species show that *P. tenuicauda*, the species with clustered electroreceptors and an enlarged visual system, took straighter paths to the stimulus electrode than species with broadly distributed electroreceptors (Fig. 2). We ran a PCs analysis on the six variables measured for each track: (i) the latency to leave the shelter (in s), (ii) the length of the track (in cm), (iii) the duration of the track (in s), (iv) the average speed (in cm/s), (v) the mode turn angle, and (vi) the CV in turn angles (Table 1). The first PC (PC1) had an eigenvalue of 2.2, explained 37% of the variance, and loaded heavily (>0.4) on the speed and the length and duration of the track. With an eigenvalue of 1.5, PC2 explained an additional 25% of the variance and loaded heavily on the mode and CV of turn angles, and to a lesser extent on the speed. PC3 loaded heavily (0.86) on the latency to leave the shelter, explained an additional 16% of the variance, and had an eigenvalue of 0.96.

Species with clustered electroreceptors and an enlarged visual system had faster movements and shorter tracks in length and duration than species with distributed electroreceptors, as evidenced by differences across electroreceptor phenotypes in PC1 ($\chi^2_1 = 11.189$; $p < 0.001$; Fig. 3a). Specifically, PC1 varied among species ($\chi^2_4 = 17.44$; $p = 0.0016$), and *P. tenuicauda*, the species with clustered electroreceptors, had significantly higher values of PC1 than *B. brachyistius* (post hoc Tukey test: $p = 0.004$) and *B. niger* ($p = 0.042$), and

Table 1. Loadings of track measurements on the three PCs

	PC1	PC2	PC3
Duration	**−0.635**	0.121	−0.022
Path length	**−0.575**	−0.009	0.241
Speed	**0.408**	**−0.418**	0.322
Mode turn angle	0.284	**0.506**	0.024
CV turn angle	−0.135	**−0.652**	0.2844
Time to leave shelter	−0.028	−0.359	**−0.870**
Eigenvalue	2.202	1.516	0.959
Proportion of variance	0.367	0.253	0.16
Cumulative proportion	0.367	0.620	0.780

Values in bold highlight variables that load heavily (>0.4) on each PC.

marginally higher than *P. adspersus* ($p = 0.067$). Values of PC1 did not differ significantly between *P. tenuicauda* and *P. microphthalmus* ($p = 0.518$), or among species with distributed electroreceptors (all $p > 0.210$). Note, however, that differences in PC1 could be influenced by species differences in swimming behavior unrelated to signal localization. For example, if overall swimming speed is faster in some species, such species would have higher values of velocity and lower values of duration, even if they swam the same length. Thus, to further investigate differences in tracking behavior, we specifically looked at differences in the length of the path. Species with clustered electroreceptors followed shorter paths to the stimulus electrode than species with distributed electroreceptors ($\chi^2_1 = 6.102$; $p = 0.013$; Fig. 3b). When comparing across species, however, we found no significant differences in path lengths ($\chi^2_4 = 8.551$; $p = 0.073$).

We found no differences in PC2 (Fig. 3c), which loaded on the mode and CV turn angles, between electroreceptor phenotypes ($\chi^2_1 = 1.802$; $p = 0.179$) or among species ($\chi^2_4 = 7.444$; $p = 0.114$). Values of PC3, which loaded heavily on the latency to leave the shelter, differed between electroreceptor phenotype with longer latencies in species with distributed electroreceptors ($\chi^2_1 = 4.009$; $p = 0.045$; Fig. 3d). When comparing among species, however, we found no significant differences in PC3 values ($\chi^2_4 = 6.879$; $p = 0.142$).

Table 2 summarizes the circular descriptive statistics of exit and entrance angles for each species and phenotype. The angles at which subjects exited the shelter were significantly oriented towards the stimulus electrode in both electroreceptor phenotypes, in all clade-A

Fig. 2. Species with broadly distributed electroreceptors tend to follow curved electric current lines while approaching simulated conspecifics, while species with clustered electroreceptors follow straighter paths. **a** Diagram of the passive electrolocation playback experiment setup. A tangential dipole electrode (positive lead: open circle, negative lead: closed circle) was used to playback a conspecific EOD. The shade of grays of the electric field lines portray a schematic representation of how the amplitude of the electric current varies in the experimental setup. **b–f** Individual tracks followed to approach the stimulus electrode by species with broadly distributed electroreceptors (**b–e**) and clustered electroreceptors (**f**). The circle in the center represents the shelter from which fish started a playback experiment and the gray box represents the response zone around the stimulus electrode. *B. brachyistius*: $n = 9$ subjects, 12 tracks; *B. niger*: $n = 7$ subjects, 13 tracks; *P. adspersus*: $n = 7$ subjects, 12 tracks; *P. microphthalmus*: $n = 5$ subjects, 11 tracks; *P. tenuicauda*: $n = 6$ subjects, 10 tracks.
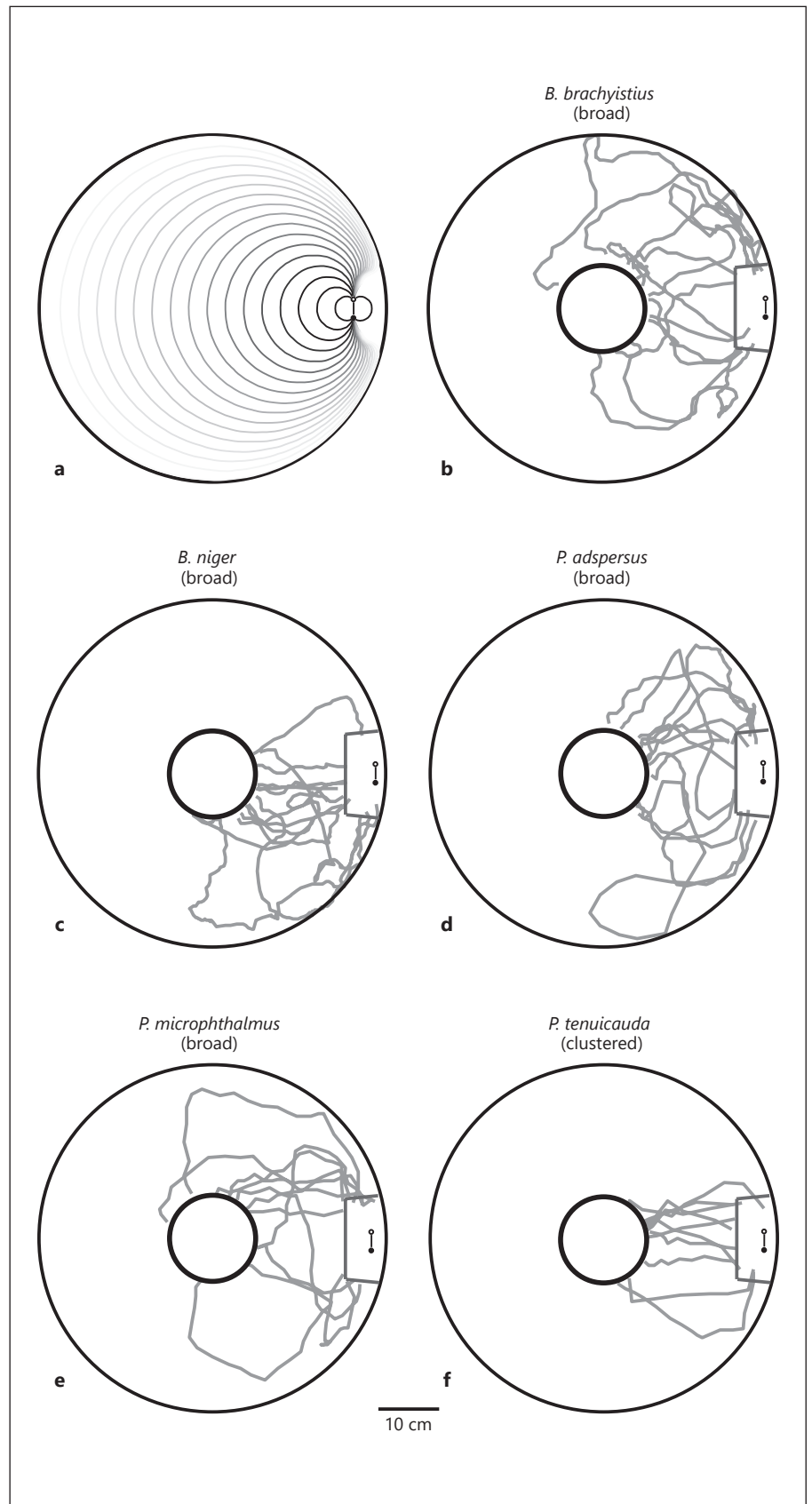
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Table 2. Descriptive statistics of the distribution of angles at which subjects exited the shelter and entered the response zone, and results of circular statistical analyses testing the null hypothesis of uniform angular distribution against the alternative hypothesis of orientation towards the stimulus electrode

	Exit angles					Angles at response zone				
	mean vector ($\mu°$)	length of mean vector (r)	angular variance	V test (u)	V test (p)	mean vector ($\mu°$)	length of mean vector (r)	angular variance	V test (u)	V test (p)
B. brachyistius (broad)	6.6	0.50	0.50	2.442	0.006	3.5	0.65	0.35	3.181	<0.001
B. niger (broad)	328.7	0.76	0.24	3.298	<0.001	331.8	0.84	0.16	3.79	<0.001
P. adspersus (broad)	15.4	0.77	0.24	3.615	<0.001	5.4	0.74	0.26	3.629	<0.001
P. microphthalmus (broad)	41.4	0.48	0.55	1.574	0.058	18.9	0.64	0.36	2.829	0.002
P. tenuicauda (clustered)	358.9	0.82	0.18	3.647	<0.001	0.7	0.76	0.24	3.386	<0.001
Broad phenotype	2.7	0.56	0.44	5.499	<0.001	357.2	0.69	0.31	6.732	<0.001
Clustered phenotype	358.9	0.82	0.18	3.647	<0.001	0.7	0.76	0.24	3.386	<0.001

The line between the stimulus electrode and the center of the shelter is designated as 0°.

species and *P. tenuicauda* (the species with clustered electroreceptors), and marginally so in *P. microphthalmus* (Table 2). Similarly, entrance angles to the response zone were significantly oriented towards the stimulus electrode in all species and both electroreceptor phenotypes (Table 2). The distribution of exit angles did not vary in mean angle and/or angular variance between electroreceptor phenotypes (Mardia-Watson-Wheeler test: $W = 2.61$, $p = 0.27$), or among species ($W = 10.68$, $p = 0.22$; Fig. 4). Similarly, there were no significant differences in the distributions of angles at which subjects entered the response zone between electroreceptor phenotypes ($W = 1.3$, $p = 0.52$), or among species ($W = 8.9$, $p = 0.35$; Fig. 4).

Experiment 2: Directional Sensitivity in the Central Electrosensory System

The three measurements we obtained from the evoked potentials (i.e., peak-to-peak amplitude, width, and area) were summarized by two PCs (Table 3; Fig. 5a). The first PC (PC1) had an eigenvalue of 1.69, loaded heavily (–0.72) on the area of the evoked potential, and explained 56% of the variance. The second PC (PC2) had an eigenvalue of 1.04, explained an additional 35% of the variance, and loaded heavily on the peak-to-peak amplitude of the evoked potential (0.77) and the area of the evoked potential (–0.64).

We quantified variation in neural responses as the Euclidian distance between PC scores of evoked potentials in response to stimuli delivered from the left and the right sides of the fish. Variation in evoked potentials was small

in *B. niger*, with mean (±SE) Euclidian distances between PC scores of 0.07 (±0.04) in ELa and 0.18 (±0.04) in ELp (Fig. 5b). In *P. microphthalmus*, mean (±SE) Euclidian distances between PC scores were 0.66 (±0.12) in ELa and 0.33 (±0.13) in ELp. Euclidian distances between PC scores were significantly higher in ELp than ELa in *B. niger* ($F_{1,1} = 14224$, $p = 0.005$), but not different between nuclei in *P. microphthalmus* ($F_{1,2} = 3.34$, $p = 0.21$). In *P. tenuicauda*, Euclidian distances between PC scores ranged between 0.6 and 2.1 (Fig. 5b). Variation in neural responses depended on the latency to the evoked potential, with larger Euclidian distances at shorter latencies (Fig. 5b; $F_{1,4} = 11.14$, $p = 0.029$; slope = –0.58, adjusted $r^2 = 0.67$).

Discussion

We asked whether differences in sensory specializations relate to localization behavior of electric communication signal sources. We have shown differences in signal tracking behavior that relate to electroreceptor distribution along the body and size of the visual system. Consistent with previous findings from a study focused on *B. brachyistius* [Schluger and Hopkins, 1987], species with distributed electroreceptors, enlarged ELa/ELp, and small visual system tended to follow the curved electric field lines to locate the signal source (Fig. 2b–e). In contrast, a species with clustered electroreceptors, small EL, and enlarged visual system followed shorter paths and had faster movements towards the source of

Table 3. Loadings of evoked potential measurements on the two PCs

	PC1	PC2
Peak-to-peak amplitude	−0.4356	**0.7721**
Area	**−0.7179**	0.0121
Width (at zero-crossings)	−0.5430	**−0.6354**
Eigenvalue	1.693	1.044
Proportion of variance	0.565	0.348
Cumulative proportion	0.565	0.913

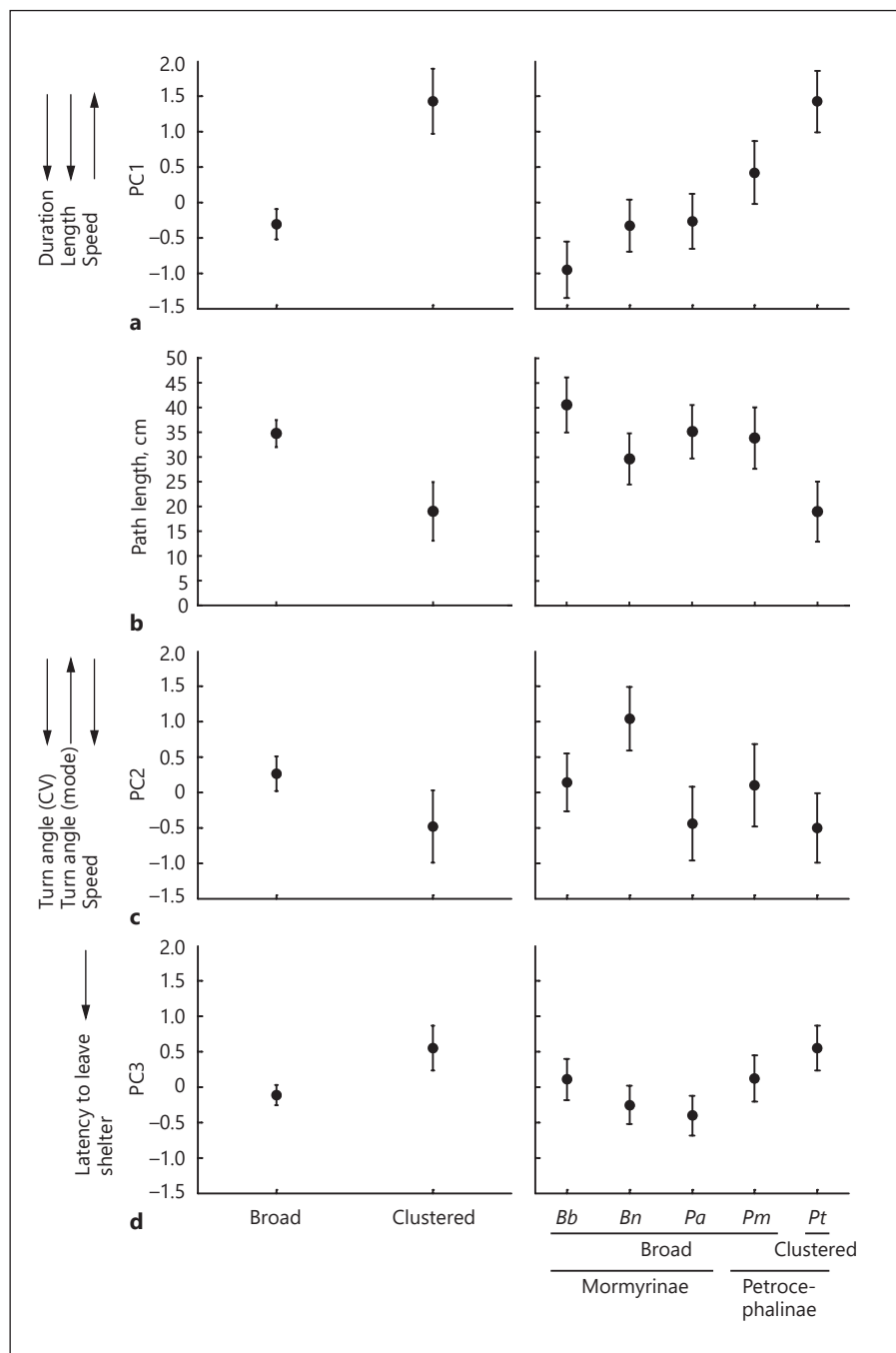Values in bold highlight variables that load heavily (>0.6) on each PC

electrosensory stimuli. Rather than following the electric field lines, species with clustered electroreceptors take straighter paths to the stimulus source. These straighter paths were sometimes directed to the stimulus electrode; at other times, these initial paths were slightly off target, and were corrected by large turns towards the stimulus once the fish was closer to the signal source (Fig. 2f). These differences in signal localization behavior do not imply that some species are better electrolocators than others; we propose that these differences reveal different strategies to localize signal sources that reflect adaptations to different habitats and social environments, and are likely related to species differences in how visual and electrosensory stimuli are analyzed. We also asked whether midbrain neural activity in response to electrosensory stimuli delivered from different locations differed between species with clustered electroreceptors and small EL as compared to species with broadly distributed electroreceptors and enlarged ELa/ELp. Evoked potentials were more dependent on stimulus location in the anterior end of the small EL as compared to the posterior end of EL, ELa, and ELp (Fig. 5b). Importantly, our results with *P. microphthalmus*, the only known species in the subfamily Petrocephalinae that has broadly distributed electroreceptors and an enlarged ELa/ELp, provide strong support for an association between the knollenorgan electrosensory pathway and signal localization behavior. We discuss our results in relation to potential underlying mechanisms for the observed patterns, differences in peripheral and central processing of electrosensory stimuli, and how these behavioral and physiological differences relate to differences in life history traits.

From their study focused on a species with broadly distributed electroreceptors, Schluger and Hopkins [1987] concluded that weakly electric fish localize signal sources by first orienting parallel to an electric current line and then swimming forward while maintaining such alignment. Because these fish followed the curved current lines instead of taking a direct approach to the signal source, they proposed that weakly electric fish may not be able to determine the distance or the direction of the stimulus source from a single point without following a current line [Schluger and Hopkins, 1987]. We followed the same experimental protocol and obtained similar results from additional species with broadly distributed electroreceptors. Furthermore, our results show that species with clustered electroreceptors do not follow electric current lines to localize sources of electrosensory stimuli and instead take shorter paths to the signal source. Such behavior could be interpreted as if species with clustered electroreceptors obtain enough information about distance and direction to the stimulus source before starting an approach. Note, however, that neither our study nor the study by Schluger and Hopkins were designed to determine whether mormyrids can analyze distance and direction to a source of electrosensory stimuli from a single point in space. Thus, the observed differences in signal localization behavior cannot be attributed to species differences in this ability. Although species differences in this perceptual ability are possible, we propose that differences in signal localization behavior reflect differences in how visual and electrosensory stimuli are encoded and processed by species with visual and electrosensory specializations.

In our experimental setup, information about the spatial location of the stimulus source could have been obtained by the electrosensory system or by both the visual and electrosensory systems. Previous studies on *Gnathonemus petersii*, a species with small visual and large electrosensory systems [Stevens et al., 2013], show that both vision and passive electrolocation are used when localizing food [von der Emde and Bleckmann, 1998], shelters [Rojas and Moller, 2002], and other individuals [Moller et al., 1982]. Thus, it is likely that all species in our study used visual and electrosensory cues while approaching the signal source. Importantly, species with small EL and clustered electroreceptors have bigger eyes, an enlarged OT, and higher visual acuity than species with ELa/ELp and distributed electroreceptors [Stevens et al., 2013]. Such visual specializations may allow species with small EL and clustered electroreceptors to locate the signal source visually at greater distances than species with ELa/ELp, or even before starting an approach. The shorter paths taken by *P. tenuicauda* (Fig. 2, 3) suggest that species with visual specializations and clustered elec-

Fig. 3. Species with clustered electroreceptors approach electrosensory stimuli faster and follow shorter paths than species with broadly distributed electroreceptors. **a** Values of PC1 (which loads positively on speed, and negatively on path duration and length) differed across electroreceptor phenotype (left) and species (right), with lower values in species with broadly distributed electroreceptors. **b** Species with broadly distributed electroreceptors took longer paths to the stimulus electrode than species with clustered electroreceptors. **c** There were no differences across electroreceptor phenotype or species in values of PC2, which loads heavily on the mode and CV of the turn angles during approach. **d** The latency to leave the shelter tended to be longer for species with broadly distributed electroreceptors, as evidence by lower values of PC3. Broad: broadly distributed electroreceptors; Clustered: clustered electroreceptors in three rosettes on both sides of the head; *Bb*: *B. brachyistius*; *Bn*: *B. niger*; *Pa*: *P. adspersus*; *Pm*: *P. microphthalmus*; *Pt*: *P. tenuicauda*. Bars indicate the phenotype (broad or clustered) and subfamily (Mormyrinae or Petrocephalinae) of each species.
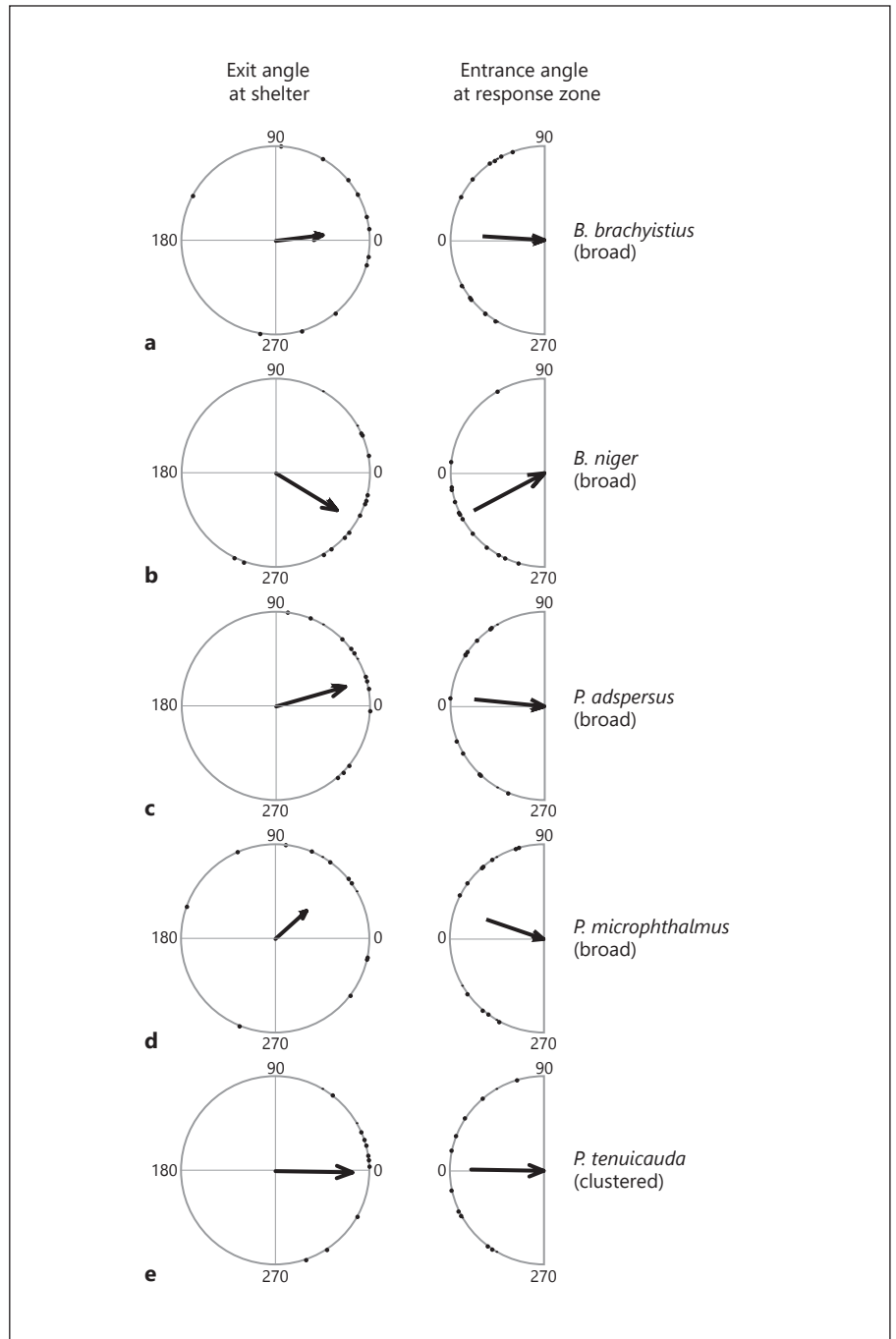
troreceptors do not follow electric current lines, but may instead integrate visual and electrosensory stimuli to localize a signal source. The longer paths taken by species with enlarged ELa/ELp and distributed electroreceptors (Fig. 2, 3) suggest that these species may rely more heavily on tracking electric current lines than on visual cues to locate a signal source. How mormyrids with different sensory specializations integrate visual and electrosensory stimuli to locate signal sources, throughout an approach or from a single point in space, is an open question that deserves further investigation.

The mechanisms by which species with clustered electroreceptors analyze electric fields to take shorter and straighter paths to a signal source are currently unknown. We have recently shown that the peripheral electrosensory system is more sensitive in species with clustered

Fig. 4. The angles at which subjects left the shelter and entered the response zone were similar across species with broadly distributed electroreceptors (**a–d**) and clustered electroreceptors (**e**). Points depict the angles at which subjects exited the shelter (left) and entered the response zone (right). The direction and length of the arrows represent the mean angle (μ) and the length of the mean vector (r) in circles and semicircles with a radius of 1.0 (see Table 2). Analyses of entrance angles were conducted with raw data and with corrected data to account for the half-circle nature of entrance angles; both analyses yielded nonsignificant differences between phenotypes and among species.

oscillatory receptors than in species with broadly distributed spiking receptors [Vélez and Carlson, 2016]. Such higher sensitivity may arise because weak signals are more efficiently detected and amplified by coherent summation in networks of resonating oscillators [Kandel and Buzsáki, 1997; Steriade and Timofeev, 2003; Buzsáki and Draguhn, 2004]. Furthermore, coupling of several os-

cillators could provide enhanced opportunities to process complex patterns of neural activity [Buzsáki and Draguhn, 2004]. These properties of resonating oscillator networks may allow clustered oscillatory electroreceptors to integrate electrosensory information in a way that provides a more robust estimate of directionality and distance to the signal source than broadly distributed

Vélez/Ryoo/Carlson

spiking electroreceptors. Alternatively, the pattern of signal localization behavior observed from species with clustered electroreceptors may reflect limitations of the electrosensory system to follow current lines rather than an ability to determine distance and direction to the stimulus source. We propose that differences in the spatial resolution of electrosensory information relayed form the periphery to the midbrain underlie the differences in signal localization behavior between mormyrids with clustered and broadly distributed electroreceptors. The ability to align parallel to the electric field lines may be facilitated by broadly distributed electroreceptors along the body: greater variation in signal properties is sampled throughout the body providing more spatial information about the signal source. In contrast, the reduced amount of spatial information obtained by clustered electroreceptors hinders the ability to orient parallel to the electric field lines. Fish with clustered electroreceptors may instead rely on a bilateral comparison of receptors on opposite sides of the head to determine the signal's general direction. Below, we describe how these two different strategies for signal localization may be achieved based on our current knowledge of the peripheral and central electrosensory system of mormyrids.

Electric current from an external stimulus enters the body on one side and leaves the body on the opposite side. Therefore, electroreceptors on opposite sides of the body experience opposite-polarity stimuli. This change in polarity occurs somewhere midway through the body of the fish. When a fish is oriented perpendicular to the electric field lines (Fig. 1c), electroreceptors on the left and right sides of the body experience opposite-polarity stimuli. This information is available to both fish with broadly distributed and clustered electroreceptors. In contrast, when a fish is oriented parallel to the electric field lines, the amount of information available varies between fish with clustered and broadly distributed electroreceptors. Knollenorgans on the anterior and posterior halves of the body of species with broadly distributed electroreceptors experience opposite-polarity stimuli. Knollenorgans clustered in three rosettes on both sides of the head experience stimuli with the same polarity. Thus, by comparing stimulus polarity on opposite sides of the transverse, sagittal, and frontal planes of the body, fish with distributed knollenorgans may maintain fixed orientations with respect to the electric field lines. In species with clustered electroreceptors, the amount of information that can be encoded at the peripheral level may be enough to analyze the general direction to the stimulus source based on stimulus polarity (i.e., left vs. right), but not enough to precisely align to the electric field lines.

The amplitude of the current lines also varies among the electroreceptor population, with a greater variation among knollenorgans of fish with broadly distributed electroreceptors than those of fish with clustered electroreceptors (Fig. 1c). In species with clustered electroreceptors, the amount of variation in amplitude among knollenorgans changes little with the receiver's spatial orientation with respect to the signal source. In contrast, in species with broadly distributed knollenorgans, amplitude variation among the electroreceptor population is much higher when the receiver is oriented perpendicular to the electric current lines than when it is oriented parallel to them (Fig. 1c). Thus, the amount of variation in signal amplitude among the knollenorgan population can also be exploited by species with broadly distributed electroreceptors to orient parallel to an electric field line and maintain this orientation while swimming to approach the signal source.

Differences in knollenorgan physiological responses to electrosensory stimuli between species with clustered and distributed electroreceptors may also have implications for directional sensitivity. In species with broadly distributed electroreceptors, spiking knollenorgans fire single time-locked spikes in response to inward current transients [Bennett, 1965; Baker et al., 2015]. Thus, knollenorgans on one side of the body respond to rising edges of the EOD while knollenorgans on the opposite side respond to falling edges of the EOD. These small spike time differences among knollenorgans distributed throughout the body may allow for fine-scale analysis of signal location and enable the fish to maintain specific orientations relative to the electric field lines. In contrast, oscillating knollenorgans of species with electroreceptors clustered in rosettes respond to electrosensory stimuli with a phase reset that results in transient oscillatory synchrony [Baker et al., 2015]. Electroreceptors on opposite sides of the head reset to phases that are 180° apart; therefore, knollenorgans on one side reset to a peak first and knollenorgans on the opposite side reset to a trough first [Baker et al., 2015]. At the peripheral level, this difference in phase reset may encode signal location [Baker et al., 2015]. However, the resolution of sensory information relayed from the periphery to the midbrain may allow for gross (i.e., left vs. right), but not fine, processing of sender location in species with clustered electroreceptors.

Little is known about directional sensitivity of the knollenorgan pathway in the central electrosensory system. We show here differences in directional information available in the EL of species with clustered electroreceptors and the ELa/ELp of species with distributed elec-
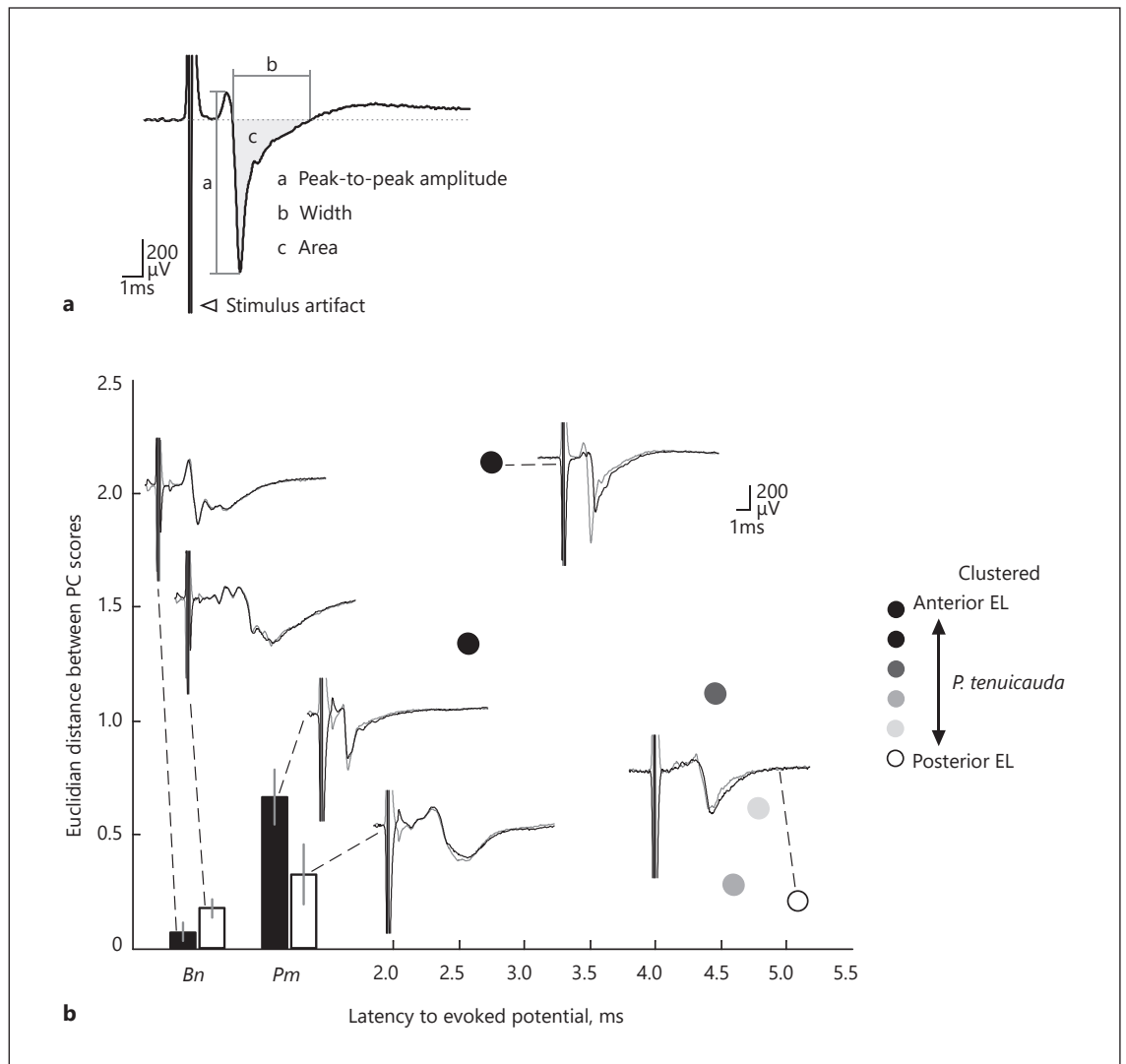
Fig. 5. Evoked potential responses vary more with signal location in the EL of species with clustered electroreceptors than in the ELa/ELp of species with broadly distributed electroreceptors. **a** We obtained evoked field potentials from EL and ELa/ELp in response to electrosensory stimuli delivered from the left and right. We measured the peak-to-peak amplitude, the width of the evoked potential, and the area of the evoked potential (shaded region). These three variables can be summarized by two PCs (Table 2). **b** Variation in PC scores across stimulus orientations was higher for EL of *P. tenuicauda* (circles) than ELa (black bars) and ELp (white bars) of *B. niger* (*Bn*) and *P. microphthalmus* (*Pm*). In *P. tenuicauda*, variation in PC scores was higher for evoked potentials with shorter latencies (darker colors and lower latency values), which represent evoked potentials obtained towards the anterior end of EL. The line traces are representative examples of evoked potential traces in response to stimuli delivered from opposite sides of the body for each species (black: right side, gray: left side).

troreceptors (Fig. 5). We have recently shown that the neural circuits in EL and in ELa/ELp of clade A and *P. microphthalmus* include the same building blocks and follow the same general motif [Vélez et al., 2017]. Cells in the hindbrain nucleus of the electrosensory lateral line lobe project ipsi-, contra-, and bi-laterally to the anterior end of EL and to ELa of clade A and *P. microphthalmus*,

with a contralateral bias. These projections from the hindbrain synapse onto two types of cells: large cells and small cells. Large cells are GABAergic and synapse onto small cells. Small cells project to multipolar cells in the posterior end of EL and in ELp of clade-A species and *P. microphthalmus*. The main difference between the neural circuits in EL and ELa/ELp appears to be that hindbrain

Vélez/Ryoo/Carlson

axonal projections follow a long and tortuous path after they enter ELa, but not EL. This difference between the EL and ELa neural circuits has important implications for sensory processing of communication signals and likely results in differences in the type of information analyzed: EOD waveform in ELa and EOD source localization in EL.

In species with ELa/ELp, small cells in ELa analyze EOD waveform by comparing the small spike time differences among knollenorgans on opposite sides of the body [reviewed in Baker et al., 2013]. Small cells in the ELa circuit receive inhibition thorough large cells elicited by knollenorgans on one side of the body and excitation from hindbrain cells elicited by knollenorgans on the opposite side of the body. Excitatory input onto small cells is delayed due to the long and convoluted path that hindbrain axonal projections take upon entering ELa. Variation in the length of the axonal projections onto small cells establishes variation in small-cell selectivity for duration between edges of the EOD, which enables the analysis of EOD waveform. Thus, small cells in ELa convert spike timing differences among electroreceptors on opposite sides of the body into a population code for signal waveform [Lyons-Warren et al., 2013a]. Signal location might also be represented as a population code among small cells in ELa [Lyons-Warren et al., 2013a], but such information might be too fine to be detected by evoked potentials (Fig. 5b). It has also been suggested that signal location in mormyrids is analyzed in the medioventral nucleus (MV) and the OT [Friedman and Hopkins, 1998].

In species with EL, small cells may use the 180° difference in phase reset of knollenorgans on opposite sides of the head to analyze EOD source localization, but not EOD waveform. Small cells in EL receive direct inhibition from large cells and excitation from hindbrain cells [Vélez et al., 2017]. Small cells could determine EOD location through a subtraction mechanism if knollenorgans from one side of the body trigger inhibition, and knollenorgans from the opposite side trigger excitation. An EOD coming from one direction would elicit maximal excitation from one side of the body and minimal inhibition from the opposite side, whereas an EOD coming from the opposite direction would elicit maximal inhibition and minimal excitation. In the other hemisphere of the brain, responses would likely mirror this pattern. Indeed, our results provide physiological evidence that information about EOD location is available and potentially analyzed in the anterior end of EL. The strong variation in evoked potentials suggests that a significant amount of information about signal location is available in the anterior end of EL (Fig. 5b). In addition, the change in the amount of variation in evoked potentials between the anterior and the posterior ends of EL suggest that signal location is analyzed in these brain regions. It remains unknown whether and how other brain regions, like the MV and OT, play a role in signal localization in species with clustered electroreceptors and small and undifferentiated EL. Furthermore, how visual and electrosensory stimuli are integrated in the central nervous system to analyze signal source location in species with different sensory specializations is also an open question. Nevertheless, our physiological and behavioral results suggest that fish with clustered electroreceptors analyze whether the signal comes from the left or the right, swim in that general direction and, if they are swimming off target, correct their direction based on visual and/or electrosensory cues.

Recent studies suggest that physiological and behavioral differences between species with different sensory specializations reflect adaptations to different social environments [Carlson et al., 2011; Stevens et al., 2013; Baker et al., 2015; Carlson, 2016; Vélez and Carlson, 2016]. Species with a small visual system but enlarged ELa/ELp and distributed electroreceptors tend to be solitary, seek shelter, defend territories, and show social competition [Hopkins, 1980; Lavoué et al., 2004; Carlson, 2016]. Species with ELa/ELp and distributed electroreceptors follow the electric field lines generated by a simulated conspecific (Fig. 2) [Schluger and Hopkins, 1987]. The ability to follow electric current lines may allow individuals to obtain more information about the sender during the approach. An enlarged and subdivided ELa/ELp is associated with the perceptual ability to detect EOD waveform variation [Carlson et al., 2011]. The waveform of the EOD is species specific, highly stereotyped, and provides information about sender identity [Hopkins, 1981]. For territorial species, obtaining information about the identity of a potential intruder might have important fitness consequences. Thus, territorial species might benefit from obtaining information about who the intruder is while approaching it.

In contrast, species with small EL and clustered electroreceptors tend to form shoals and to show social affiliation [Hopkins, 1980; Lavoué et al., 2004; Carlson, 2016]. Our results show that species with clustered electroreceptors do not follow electric field lines, but rather take faster and shorter paths to approach a simulated conspecific. For shoaling species, the ability to quickly localize and join the shoal when separated might be more important than identifying a signaling individual. There-

fore, shoaling species may benefit from obtaining information about where the sender is and approaching it quickly. Furthermore, given their larger visual systems and higher visual acuity compared to mormyrids with ELa/ELp [Stevens et al., 2013], petrocephalines with small EL may also rely on visual information while localizing signaling individuals. An interesting exception, however, is *P. microphthalmus* because they have broadly distributed electroreceptors, an enlarged ELa/ELp, perceive variations in EOD waveform, and tend to follow electric field lines, but form shoals like other petrocephalines [Lavoué et al., 2004; Baker et al., 2015]. Nevertheless, field and lab observations suggest that *P. microphthalmus* inhabits deeper waters and are less visual than other petrocephalines [Stevens et al., 2013]. Therefore, habitat, and not only social behavior, may drive the physiological and behavioral differences between species with ELa/ELp and distributed electroreceptors and species with EL and clustered electroreceptors.

Our results add to a growing line of research showing how evolutionary changes in sensory systems relate to changes in perception and behavior. Here, we have shown differences in signal tracking behavior that relate to differences in the anatomy and physiology of the peripheral and central electrosensory and visual systems. In turn, these physiological and behavioral differences likely reflect adaptations to different habitats and social environments. Our results set the groundwork for future comparative studies on the cellular and network mechanisms for signal localization within the knollenorgan sensory pathway of mormyrids, on how visual and electrosensory cues are integrated to analyze signal source location, and on the ecology and social behavior of species that differ in electrosensory and visual specializations [Carlson et al., 2011; Stevens et al., 2013; Baker et al., 2015; Vélez et al., 2017].

Statement of Ethics

The experimental protocols to test animals were approved by the Institutional Animal Care and Use Committee at Washington University in St. Louis and conform the standards established by the National Institutes of Health.

Disclosure Statement

The authors declare that no competing interests exist.

Author Contributions

B.A.C. and A.V. contributed to conceptualization and design of the study. A.V. and D.Y.R. performed experiments and analyses. The manuscript was drafted by A.V. and B.A.C., and edited, revised, and approved by A.V., D.Y.R., and B.A.C.

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