Temporal Selectivity in Midbrain Electrosensory Neurons Identified by Modal Variation in Active Sensing

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Pluta SR, Kawasaki M. Temporal selectivity in midbrain electrosensory neurons identified by modal variation in active sensing. J Neurophysiol 104: 498–507, 2010. First published May 26, 2010; doi:10.1152/jn.00731.2009. Mormyrid weakly electric fish actively sense their surroundings by continuously emitting discrete pulses of electricity separated by varying intervals of silence. The temporal pattern of this pulsing behavior is related to context. While resting in the absence of an overt stimulus, baseline interpulse intervals (IPIs) mostly range 200–450 ms, and sequential variation is relatively high. Spontaneously, or following the presentation of a novel stimulus, IPIs transiently shorten during the performance of an electromotor “burst” display. We made intracellular whole cell recordings in vivo from neurons in the lateral nucleus of the torus semicircularis while the fish’s dynamic pulsing behavior modified the temporal pattern of stimulation. Stimulation was designed to simulate the spatial patterns of AM that occur during the electrolocation of a resistive object. We discovered that toral neurons selectively respond to stimulation during a particular mode of electromotor activity. Two types of temporally selective neurons were discovered: baseline-selective neurons that displayed significantly higher postsynaptic potential (PSP) amplitude and spike count per electric organ discharge (EOD) during baseline electromotor activity and burst-selective neurons that displayed significantly higher PSP amplitude and spike count per EOD during electromotor burst displays. Interval-dependent changes in the strength of excitation and inhibition contributed to their selectivity.

INTRODUCTION

Covariation between the timing of successive sensory stimuli and behavioral context is an integral component of active sensory systems. During prey interception in bats and dolphins, the interval length of sound pulses is inversely related to target distance (Akamatsu et al. 2005; Simmons et al. 1979). Similarly in pulse-type weakly electric fish, interpulse interval (IPI) greatly shortens when the animal probes novel objects in its environment (Toerring and Moller 1984). Midbrain neurons that selectively respond to the interval length of sound pulses during baseline electromotor burst displays are not confounded by changes in spectral frequency or duration of the signal (von der Emde 1992). This study is the first to describe the relationship between the temporal structure of active sensing and the response properties of midbrain neurons involved in electrolocation.

In Brevimyrus niger, interval length and sequential variation in electromotor activity are highly dependent on the behavioral context and history of stimulation. While resting stationary in the absence of an overt stimulus, a large portion of “baseline” IPIs are irregular with relatively long gaps of silence (Serrier and Moller 1989). Spontaneously, or following a brief novel stimulus, the fish will often display a momentary electromotor “burst” of short IPIs that typically lasts 2–5 s (Carlson and Hopkins 2004b; Pluta and Kawasaki 2008). If actively probing objects in the environment, IPIs become highly regular and shorten to a length typical of the peak of a electromotor burst display (Toerring and Moller 1984).

During electrolocation, objects with conductivity different from the surrounding water create a localized change in electric field amplitude (Gomez et al. 2004). Hindbrain electrosensory neurons encode changes in electric field amplitude by altering their firing rate. Some neurons increase their firing rate during the presence of objects that locally increase electric field amplitude, whereas other neurons increase their firing rate during the presence of objects that locally decrease electric field amplitude (Goenechea and von der Emde 2004). Hindbrain electrosensory efferents primarily project to neurons of the lateral toral nucleus (midbrain) (Bullock and Heiligenberg 1986; Grant et al. 1996). Neurons of the lateral toral nucleus increase or decrease their firing rate when their receptive field center is stimulated with a local electrical pulse (Sawtell et al. 2005). The lateral toral nucleus has reciprocal connections with the valvula cerebelli and tectum mesencephali (Bullock and Heiligenberg 1986; Carlson 2002). These connections make it a candidate structure for parsing the effect of temporal variation in electromotor activity on sensory processing.

The goal of the present study was to quantitatively test the relationship between the temporal structure of electromotor activity and the responsiveness of neurons in the lateral nucleus of the torus semicircularis. Intracellular whole cell recordings were made in vivo while the temporal mode of stimulation was freely determined by the fish’s dynamic sensing behavior. We discovered two distinct classes of neurons: baseline-selective neurons that displayed a significantly higher postsynaptic potential (PSP) amplitude and spike count per electric organ discharge (EOD) to stimulation during the baseline mode electromotor activity and burst-selective neurons that displayed a significantly higher PSP amplitude and spike count per EOD to stimulation during electromotor burst displays.

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METHODS

Animals and surgery

We used 57 male and female mormyrid Breivimyrus niger (formerly Briomenyurus niger) that were 6–8 cm in length, measured from the mouth to the end of the caudal peduncle excluding the fin. They were imported from West Africa through a commercial supplier and housed in 150 l glass aquaria with several other members of their cohort. They were fed live blackworms and maintained under a 12:12 h light:dark cycle.

Water conductivity and temperature in the experimental tank were kept at 80–110 μS/cm and 26–27°C. After anesthesia with tricaine methanesulfonate (MS-222, 1:5,000; Sigma, St. Louis, MO), we immobilized the fish with an intramuscular injection of gallamine triethiodide (Flaxedil: 7 μl, 0.3 mg/ml; Sigma). The fish was gently held in place with a sponged-lined clamp and aerated water was passed over its gills through a glass pipette inserted into the mouth. Approximately 90% of the fish’s body was submerged in water except for the dorsal surface of the skull and torso. Throughout the experiment, electromotor neuron (EMN) activity was monitored to ensure the health of the fish. After local application of lidocaine (2%; Hospira, Lake Forest, IL), we removed a small portion of the skull and meninges above the midbrain. The valvula cerebelli was laterally pulled in three stages to a tip diameter of 0.8 mm.

Intracellular whole cell recordings were made in vivo by following a published (Rose and Fortune 1996) methodology. Electrodes were pulled in three stages to a tip diameter of 0.8 μm and filled with a solution containing (in mM) 100 potassium acetate, 2 potassium chloride, 1 magnesium chloride, 5 EGTA, 10 HEPES, 20 potassium hydroxide, and 43 mannitol. Electrode resistance in physiological saline was 20–50 MΩ and initial seal resistances were >1GΩ. Neurons included in the study had a stable resting membrane potential less than −63 mV (including 13 mV liquid junction potential) and displayed spikes with amplitude >15 mV in response to sensory stimulation (see following text). Intracellular amplification was amplified 10-fold on an AxoClamp 2B amplifier (Molecular Devices, Palo Alto, CA) then sent to an A/D converter with a sampling rate of 20 kHz (model AD1, Tucker Davis Technologies, Gainesville, FL). Membrane potentials were saved onto a computer hard drive using custom-made software written in Matlab (The Mathworks, Natick, MA).

The pipette was advanced directly into the lateral nucleus of the torus using a microdrive (Burleigh, No. 6000) while it received positive pressure. After reaching the recording location (>50 μm below surface), the pipette was advanced in 1 μm increments while maintaining light positive pressure and passing 0.1 nA square-wave pulses (500 ms) to monitor resistance. Cell contact was indicated by a small increase in the voltage change. Negative pressure was then applied to the pipette to increase the seal resistance to giga-ohm levels. Subsequent to seal formation, negative current was briefly applied to rupture the patch. Usually, gentle bursts of positive air pressure were then applied to reduce the access resistance to <400 MΩ. There was no electrosensory stimulus while searching for neurons.

Sensory stimulation

Flexadil deactivates the EOD; therefore, the fish could no longer generate an electric field. EMN activity, which signals the timing of an EOD, was amplified 10,000 times and sent to a Schmidt Trigger to record time stamps of fictive electromotor activity (1 MHz clock, model ET1, Tucker Davis Technologies). After a neuron was successfully patched, a prerecorded global EOD mimic (G) was triggered at a delay of 3 ms from the first negative peak of each EMN volley. This time delay corresponds to the natural delay in an intact B. niger (unpublished observations). To mimic the geometry of the fish’s self-generated electric field, G was delivered through one electrode placed inside the stomach while the other was underneath the posterior end of the tail. The maximum peak-to-peak amplitude of G was set to ~65 mV per cm measured at a distance of 1.5 cm from the fish’s torso, to match the natural electric field strength. Throughout the experiment, G was continuously triggered by the EMN unless stated otherwise.

To locate each neuron’s receptive field, the fish’s body surface contralateral to the recording site was stimulated by the addition of a local EOD mimic (L) delivered at the same delay (EMN triggered) as G (Fig. 1). L was applied through a pair of silver wires that was 8 mm apart, oriented perpendicular to the skin surface, and had its nearest electrode pole ~1 cm away from the fish. The polarity and amplitude of L were controlled to adjust the polarity and strength of the summated EOD AM. Neuronal activity was monitored as the location of L was slowly scanned along the anterior-posterior or dorsal-ventral body axes. Local stimulation was tested from the posterior end of the gill cover to the tip of the nose. L was eventually positioned where it elicited the maximally observed increase or decrease in neuronal activity, relative to G alone.

During data collection, local EOD amplitude was increased (GL+ session) or decreased (GL− session), relative to G stimulation, for 10 s with pauses between sessions longer than 10 s. GL+ and GL− sessions were repetitively presented in a random order. Simultaneous recordings of membrane potential and electromotor activity were made for ≥30 s of GL+ and GL− stimuli and for 20 s with G alone. The total number of stimuli depended on electromotor rate, which averaged three to five pulses per second.

The change in local EOD amplitude due to the addition of L was measured transdermally. One pole of a fork recording electrode was inserted underneath the fish’s skin at the mid-torso while the other pole extended 4 mm perpendicular to the body surface. The amplitude of G stimulation was 148 mV (370 mV/cm). With the local stimulating wires centered on the recording fork electrode, the addition of L caused a ~20% change in peak-to-peak amplitude. To determine the width of EOD modulation, we measured EOD amplitude while the local stimulating wires were horizontally displaced 8.5 mm from the center of the recording electrode. With 8.5 mm displacement, the addition of L caused a ~3% change in peak-to-peak amplitude.

Data analysis

Action potentials were counted by locating positive derivatives in membrane potential >12 mV/ms after applying a Savitzky-Golay FIR smoothing filter (The Mathworks, Natick, MA). The filter caused a 1–3 mV reduction in action potential height while practically abolishing the electrosensory stimulus artifact. The minimum time between two action potentials was set to 1.2 ms to avoid counting the...
same spike more than once. To analyze PSP amplitude, a median filter with a time constant of 15 ms was applied to the unfiltered membrane potential. PSP amplitude was defined as the difference between the maximum/minimum potential of each sensory-evoked excitatory/inhibitory PSP (EPSP/IPSP) and the mean membrane potential of the entire 10 s trace. Plots of averaged sensory-evoked responses were temporally aligned to the onset of the electrosensory stimulus. To test the relationship among IPI, spike count and PSP amplitude, the response to the first stimulus was discarded because there was no preceding local stimulus. The response to the last stimulus was discarded because the recording often ended before the last response completed. Within-neuron differences in spike count per EOD and PSP amplitude between baseline (IPIs > 200 ms) and burst (IPIs < 200 ms) IPIs were tested for significance using a two-tailed t-test. For Figs. 3 and 7, differences in mean PSP amplitude and spike count per EOD between baseline and burst IPIs were tested with a Wilcoxon signed-ranks test.

RESULTS

Pulsing behavior

Sequences of pulse intervals could be classified into two stereotypical temporal patterns: “baseline” with long IPIs and “burst” with shorter and more regular IPIs. Ninety-eight percent of IPIs during a 40 min recording from an intact fish fell within the range of 12–450 ms (Fig. 2A). Forty-three percent ranged 200–450 ms and 55% ranged 12–200 ms (Fig. 2A). IPIs (15,785) were analyzed to determine a probabilistic boundary between “baseline” and “burst” modes of electromotor activity. The mean ($\bar{x}$) and coefficient of variation ($\sigma$) of six consecutive IPIs, IPI$_i$ to IPI$_{i+5}$ were calculated for $i = 1, 2, ..., 15,785$ where IPI$_i$ is $i$th IPI. Figure 2B shows the mean coefficient of variation as a function of the mean length of each six-IPI sequence. Pulse sequence variation was relatively high when the mean IPI length was 350–450 ms. From 200 to 300 ms IPIs, sequence variation decreased. Interestingly, as mean IPI shortened to 170 ms, a length common to electromotor burst displays, the mean coefficient of variation noticeably began to increase. We chose 200 ms as a parsimonious boundary between baseline and burst activity because of the inflection in sequence variation near this point (Fig. 2B). Figure 2C provides an example of 20 s of consecutive IPIs with several burst displays indicated by asterisks. Notice how the burst displays are generally comprised of IPIs < 200 ms.

Temporal pattern selectivity

Intracellular whole cell recordings were made from 52 neurons in the lateral nucleus of the torus semicircularis that met the inclusion criteria. Responsiveness to GL+ and GL− stimulation was tested in 34 of these neurons. Temporal selectivity was tested in 18 neurons which were recorded while the...
fish exhibited both baseline and burst modes of electromotor activity. In 17/18 cells, PSP amplitude and spike counter per EOD differed significantly between baseline and burst IPIs.

**Baseline-selective neurons**

Nine neurons responded with significantly larger PSP amplitudes and spike counts per EOD to stimulation at baseline IPIs than to stimulation during electromotor burst displays. Baseline selectivity was found in five neurons during GL− stimulation, in two neurons during GL+ stimulation, and in two neurons during G stimulation. Interval-dependent excitation (7 cells) or inhibitory summation (2 cells) appeared to underlie baseline selectivity. Figure 3 summarizes the effects of IPI length on neuronal responsiveness. Figure 3A compares the mean PSP amplitudes within each neuron between baseline (IPIs >200 ms) and burst (IPIs <200 ms) modes of electromotor activity. Mean PSP amplitude was significantly (P < 0.004, n = 9) larger during the baseline mode of electromotor activity. Figure 3B compares the mean spike counts per EOD between baseline and burst modes of electromotor activity. Mean spike count per EOD was large during baseline IPIs and significantly (P < 0.004, n = 9) decreased during electromotor burst displays. Overall, responses to baseline electromotor activity were 4.16 ± 0.57 mV (n = 9) larger in mean PSP amplitude and 1.29 ± 0.63 larger (n = 9) in mean spike count per EOD (Fig. 3C).

Figure 4 shows an example of a baseline-selective neuron that displayed an interval-dependent change in the strength of excitation. During G stimulation, each stimulus pulse elicited an EPSP-IPSP-EPSP sequence and spiking was rare across both baseline and burst modes of electromotor activity (Fig. 4A). During GL+ stimulation, excitation seemed to override the IPSP, and EPSP amplitude increased (Fig. 4A). Importantly, IPI length had a significant effect on neuronal responsiveness. Spike count per EOD and PSP amplitude were large during baseline IPIs (200–600 ms) and significantly (P < 0.01, t = 5.62 and 8.54, respectively) decreased during electromotor burst displays (Fig. 4B, top). A trace of membrane potential sampled during baseline and burst (*) modes of electromotor activity illustrates the effect of IPI length on EPSP amplitude and spike count (Fig. 4B, bottom). To clearly illustrate this effect, the averaged median-filtered EPSPs from IPIs >200 ms ( — ) were compared with the averaged EPSPs from IPIs <200 ms ( · · · ). The peak of the averaged burst EPSP was 5 mV lower than the peak of the averaged baseline EPSP (Fig. 4C, top). Notice that the IPSP present in the IPI <200 ms trace of this figure resembles the IPSP present during baseline IPIs of G stimulation in Fig. 4A. Prestimulus membrane potential increased during burst IPIs due to the temporal summation of weak PSPs. Pooled data from 40 s of GL+ stimulation show a clear, direct relationship between IPI length and EPSP amplitude (Fig. 4C, bottom). EPSP amplitude during GL− stimulation was even smaller than it was during G stimulation, and spiking was absent at all displayed IPIs (data not shown). The IPSP present during G stimulation was reversed by hyperpolarizing the cell 18.1 mV during negative current injection (data not shown).

Figure 5 shows a baseline selective neuron that responded with EPSPs to GL− and with IPSPs to GL+ stimulation. The amplitude of both EPSPs and IPSPs were diminished during electromotor burst displays. The top trace in Fig. 5A shows how interval-dependent changes in the strength of excitation had a strong effect on spike count per EOD. GL− stimulation elicited large EPSPs and high spike counts per EOD to the first pulse in a series of burst intervals (Fig. 5A). Subsequent burst IPIs within the series caused a progressive reduction in EPSP amplitude and the elimination of spiking. EPSP amplitude and spike count per EOD were significantly larger during baseline IPIs (P < 0.01, t = 5.66 and 4.3, respectively; Fig. 5B). Furthermore, when IPIs shortened to 20–30 ms, membrane potential monotonically decreased (see ↓ in A) toward baseline rather than weakly rising after each stimulus pulse, which occurred at more moderate IPIs (100–200 ms). GL+ stimulation caused the membrane to hyperpolarize after each stimulus. The trough of the averaged baseline IPSP was 2.5 mV lower than the trough of the averaged burst mode IPSP (Fig. 5C, top).

![Fig. 3. Summary data of baseline-selective neurons (n = 9). A: a comparison of each neuron’s mean postsynaptic potential (PSP) amplitude between burst (<200 ms) and baseline (>200 ms) IPIs. B: a comparison of each neuron’s mean spike count per EOD between burst and baseline IPIs. C: bar graphs of the mean difference in mean PSP amplitude and spike count per EOD between baseline and burst IPIs. Error bars equal 1 SE. One neuron was omitted from B due to high spontaneous activity.](http://jn.physiology.org/doi/abs/10.1152/jn.00265.2010)
Prestimulus membrane potential increased during burst IPIs due to the temporal summation of weak PSPs. Pooled data from 40 s of GL stimulation show a direct relationship between the magnitude of hyperpolarization and IPI length (Fig. 5C, bottom).

In two neurons, baseline selectivity appeared to be related to the summation of inhibition during electromotor burst displays. Spike count per EOD and PSP amplitude were significantly greater during baseline IPIs ($P < 0.01$, $t > 5.2$; Fig. 6, A and D). Notice how spiking was completely suppressed in Fig. 6A during stimulus intervals $<80$ ms. Both neurons were hyperpolarized during electromotor burst displays (Fig. 6, B and E). In one of two neurons (Fig. 6C), inhibition appeared to summate during burst IPIs but was weak or absent during baseline IPIs. A concomitant depression of excitation may have contributed to the weaker responses during burst displays. In the other neuron (Fig. 6F), a different mechanism may have been employed. The initial, short latency hyperpolarization appeared

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**FIG. 4.** A baseline-selective neuron that displayed a large excitatory PSP (EPSP) amplitude and spike count per EOD during baseline electromotor activity. In this and other figures, the short vertical lines below traces of membrane potential represent the times of sensory stimulation. Also, the trans-membrane resting potential is labeled in plots of the averaged sensory-evoked response. A, top: averaged, median-filtered membrane potential comparing neuronal activity during G stimulation to GL+ stimulation at IPIs $>200$ ms. Notice the strengthening of EPSP amplitude during GL+ stimulation. B, top: scatter plot of spike count per EOD as a function of IPI length. B, bottom: a sequential trace of membrane potential comparing neuronal activity during baseline pulsing behavior to neuronal activity during an electromotor burst display. C, top: averaged, median-filtered membrane potential comparing neuronal activity during IPIs $>200$ ms to IPIs $<200$ ms. C, bottom: a scatter plot of PSP amplitude as a function of IPI length. —, the linear fit between IPI length and PSP amplitude.

**FIG. 5.** A baseline-selective neuron showing interval-dependent EPSPs and inhibitory PSPs (IPSPs). A, top: a sequential trace of membrane potential sampled during GL+ stimulation. Notice the large EPSP amplitude and spike count per EOD during baseline IPIs. A, bottom: averaged, median-filtered membrane potential comparing neuronal responsiveness between baseline and burst IPIs. B: scatter plots of spike count per EOD and EPSP amplitude as a function of IPI length. C, top: averaged, median-filtered membrane potential during GL+ stimulation comparing neuronal activity between baseline and burst IPIs. C, bottom: scatter plot of PSP amplitude during GL+ stimulation as a function of IPI length. B and C, bottom: — in the PSP amplitude graphs, the linear fit between IPI length and PSP amplitude.
to align with an additional delayed hyperpolarization during burst IPIs. The temporal segregation of these hyperpolarizations is evident in the neuronal activity (see Fig. 6) of the first few baseline IPIs that occurred after an electromotor burst display. Hyperpolarizing the membrane potential of the neuron in Fig. 6C by 7.4 mV reversed the IPSP and increased EPSP amplitude (data not shown). However, a 20.3 mV hyperpolarization in membrane potential only moderately affected the neuron in Fig. 6F (data not shown). For both neurons, selectivity was tested during G stimulation only.

**Burst-selective neuron**

In a distinctly separate group of neurons, PSP amplitude and spike count per EOD were largest during electromotor burst displays. Burst selectivity was found in three neurons during GL– stimulation, in three neurons during GL+ stimulation, and in two neurons during G stimulation. Figure 7 summarizes the effect of IPI length on neuronal responsiveness in these neurons. Mean PSP amplitude was low during baseline IPIs but significantly \( P < 0.008, n = 8 \) increased during electromotor burst displays (Fig. 7A). In the majority \( (62.5\%) \) of neurons, mean spike count per EOD was \(<0.5\) during baseline IPIs but significantly \( P < 0.02, n = 8 \) increased to \(>0.9\) spikes per EOD during electromotor burst displays (Fig. 7B). Overall, stimulation during electromotor burst displays caused a 4.5 \( \pm 1.0\) mV \( n = 8 \) increase in mean PSP amplitude and a 0.87 \( \pm 0.23\) increase \( n = 8 \) in mean spike count per EOD (Fig. 3C).

Figure 8A illustrates the inverse relationship between spike count per EOD and IPI length that was typical \( n = 5/8 \) of burst-selective neurons. Figure 8B provides a sample of this neuron’s membrane potential during baseline and burst modes of electromotor activity. At baseline IPIs, the neuron exhibited an IPSP–EPSP sequence. However, during burst displays, EPSPs became the major response component, IPSPs were eliminated, and spiking was prevalent. To further illustrate this transformation, Fig. 8C plots membrane potential relative to the time of each stimulus pulse. During IPIs \( >60\) ms, IPSPs were observed that reached maximum hyperpolarization \(-17\) ms after EOD pulses. Recovery of membrane potential to the prestimulus level had a latency of \(-35\) ms. However, at IPIs \(<60\) ms, IPSPs were rare, and spiking occurred with a latency of \(20–30\) ms. A time expanded trace of membrane potential during an electromotor burst display shows the loss of IPSPs in

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**FIG. 6.** Two baseline selective neurons (left and right) displaying a reduction in baseline membrane potential during electromotor burst displays. A and D: scatter plots of spike count per EOD as a function of IPI length. B and E: sequential traces of membrane potential containing both baseline and burst modes of electromotor activity. C and F: averaged, median-filtered membrane potential comparing neuronal activity between baseline and burst IPIs.

**FIG. 7.** Summary data of burst-selective neurons \( n = 8 \). A: a comparison of each neuron’s mean PSP amplitude between burst \((<200\) ms\) and baseline \((>200\) ms\) IPIs. B: a comparison of each neuron’s mean spike count per EOD between burst and baseline IPIs. C: bar graphs of the mean increase in mean PSP amplitude and spike count per EOD at burst IPIs. Error bars equal \(1\) SE.
real time (Fig. 8D, top). The averaged, median-filtered PSPs from a 10 s recording clearly show the overall transformation from IPSPs to EPSPs during IPIs <60 ms (Fig. 8D, bottom). Prestimulus membrane potential increased during burst IPIs due to the temporal summation of EPSPs. The probability of PSP amplitude reaching the median-filtered average of 2.2 mV was 58% (n = 52/89) during burst IPIs <200 ms, while it was only 28% (n = 13/47) during baseline IPIs >200 ms. Selectivity was tested for G stimulation only.

Figure 9 shows a burst-selective neuron that displayed interval-dependent changes in the in the strength of a delayed EPSP. During GL+ stimulation, EPSP amplitude and spike count per EOD significantly increased during electromotor burst displays (P < 0.01, t = 6.8 and 7.6, respectively). However, during GL− stimulation, spike count per EOD and EPSP amplitude were approximately equal (P > 0.9 and 0.08, t = 0.09 and 1.78, respectively) between baseline and burst IPIs (Fig. 9, A and B). A comparison of neuronal activity between GL+ and GL− stimulation (at all IPIs) reveals that spike latency mirrored the temporal dynamics of the early hyperpolarization and delayed excitation (Fig. 9C). The slope of membrane depolarization was more gradual during GL+ stimulation compared with GL− stimulation. Hence the peak of EPSP amplitude had a longer latency during GL+ stimulation (112 ± 13.1 ms; n = 31) compared with GL− stimulation (63.9 ± 31.3 ms; n = 27; Fig. 9C).

Two neurons spiked selectively to a limited range of IPIs during GL+ stimulation. Interestingly, tests for temporal selectivity were most significant when the stimulus polarity (GL+) caused neuronal activity to decrease during baseline IPIs. During electromotor bursts displays of GL+ stimulation, spike count per EOD and EPSP amplitude significantly increased (P < 0.01, t = 4.27 and 4.74, respectively; Fig. 10A), but most notably at 100–200 ms IPIs. During GL− stimulation, spiking was prevalent across the entire range of IPIs. Spike count per EOD for IPIs 100–200 ms was statistically equivalent (P > 0.1, t = 1.67) to baseline IPIs >200 ms. Therefore neuronal responsiveness to 100–200 ms IPIs was consistent between stimulus polarities. However, during GL− stimulation, spike count per EOD during baseline IPIs >200 ms was statistically larger (P < 0.01, t = 3.2) than it was during burst IPIs <100 ms due to the moderate but consistent decrease in spiking at IPIs <100 ms. Therefore neuronal responsiveness decreased at IPIs <100 during both stimulus polarities. Figure 10, B and D, shows the relationship between membrane potential, IPI, and stimulus polarity. Notice that spiking in Fig. 10B (GL+) occurred exclusively during electromotor burst displays, whereas in D (GL−), spiking was prevalent for all IPIs.

**DISCUSSION**

**Behavioral relevance**

Behaviorally directed temporal modes of stimulation are an integral feature of active sensory systems (Nelson and MacIver 2006). Mormyrid weakly electric fish actively transition from long, baseline intervals of pulsing to shorter, burst intervals when
leaving rest to probe novel objects in the environment (Arnegard and Carlson 2005; Toerring and Moller 1984; von der Emde 1992). This behavioral transition can also be momentarily elicited by the delivery of a brief novel stimulus (Hall et al. 1995; Pluta and Kawasaki 2008; Post and von der Emde 1999; von der Emde and Zelick 1995) or occur spontaneously (Serrier and Moller 1989). During electrolocation, changes in the amplitude of the self-generated electric field are encoded by neurons in the hindbrain electrosensory lateral line lobe (ELL) (Goenechea and von der Emde 2004). ELL efferents primarily project to the lateral nucleus of the torus semicircularis (Grant et al. 1996). Here we show for the first time that neurons of the lateral nucleus of the torus are selective to a distinct mode of active sensing.

In our behavioral analysis, we found no definitive boundary between baseline and burst IPIs. This is expected, considering that most of the variation between the modes is explained by the sequential structure of electromotor activity (Carlson 2002). To facilitate statistical analysis, we chose 200 ms as a probabilistic cut-off, based on our quantitative and subjective assessments (Fig. 2). Because \textit{B. niger}'s pulsing behavior extends continuously over a fairly wide range of intervals (Fig. 2A), it is likely that neuronal selectivity is optimized for distinguishing more than the two broad behavioral modes defined in this paper. In fact, two neurons validated this expected variation (Fig. 10).

**Temporal selectivity and receptive field heterogeneity**

Spike count per EOD and PSP amplitude were significantly affected by the modal pattern of electromotor activity. In neurons defined as baseline-selective, EPSP amplitude and spike count per EOD were large during baseline IPIs >200 ms. However, during electromotor burst displays, EPSP amplitude significantly decreased and spike count per EOD either significantly decreased or extinguished. In neurons defined as burst-selective, EPSP amplitude and spike count per EOD were low during baseline IPIs but significantly increased during burst IPIs <200 ms.

Temporal selectivity in the torus may depend on the interaction of excitation and inhibition. Electrosensory neurons with center-surround receptive fields are well described in the hindbrain ELL (Bell and Grant 1992; Metzen et al. 2008). Therefore the IPSP-EPSP patterns of membrane potential, as displayed by Fig. 4A (during G stimulation), likely resulted from the presence of heterogeneous receptive fields. Because the self-generated electric field activates electoreceptors throughout the body surface, excitatory and inhibitory regions are stimulated simultaneously. If the excitatory and inhibitory afferents have similar salience, then stimulus amplitude in the excitatory region would need to be greater than the inhibitory surround (as the case with GL+ stimulation in Fig. 4A) to override inhibition and induce spiking. A temporal-dependent shift in the strength of either input has the capability to bias the power of excitation or inhibition and instate a filter. As was the case for most baseline-selective neurons (n = 8), excitation appeared to adapt during electromotor burst displays, thereby suppressing activity during burst IPIs. For burst-selective neurons, inhibition adapted (Fig. 8, n = 1) or excitation increased (Fig. 9, n = 7) during electromotor burst displays, thereby facilitating activity during burst IPIs. Not all temporally selective neurons displayed distinct IPSPs and EPSPs with different

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**FIG. 9.** A burst-selective neuron showing an increase in spike count per EOD and EPSP amplitude during electromotor burst displays. A: a trace of membrane potential during a burst of electromotor activity followed by baseline pulsing. B: scatter plots of spike count per EOD as a function of IPI length. C: histograms of spike latency with averaged, median-filtered traces of membrane potential of the same time window overlain. Notice how spike latency mirrors the duration of the initial hyperpolarization, which was longer during GL+ stimulation.
temporal profiles. From these results alone, the origin of temporal selectivity is not clear. Network properties involving short-term plasticity in temporally selective connections in other brain regions may be important. Further research comparing the effects of global and local stimulation patterns is needed to definitively illustrate the contribution of receptive field heterogeneity and inhibition to temporal selectivity.

In rare cases, inhibitory summation appeared to regulate temporal selectivity (Fig. 6). Inhibitory summation manifested in two distinct ways: through the accumulation of weak inhibition or by the temporal alignment of early and delayed hyperpolarizing potentials. Despite their apparent differences, both effects resulted in a net hyperpolarization of baseline neuronal activity. However, the role of EOCD input in temporal selectivity is unknown. Future experiments whereby the activity of the EOCD command nucleus is experimentally independent from sensory stimulation could provide valuable insight.

**Functional significance**

IPI shortening theoretically increases the temporal resolution of active electrolocation. Five sample/s (200 ms IPI) is likely inadequate for capturing a darting prey (Arnegard and Carlson 2005) or quickly navigating through a cluttered landscape. Therefore it is logical that “probing motor behaviors” involved in object recognition are accompanied by electromotor bursts (Toerring and Moller 1984; von der Emde 1992). Interestingly, IPI regularization, typical of probing displays, increases the sensitivity of primary afferents to EOD AM (Sawtell et al. 2005). Given this knowledge, it seems logical to speculate that most central neurons involved in electrolocation would be specialized for encoding stimuli at short, regularized intervals. However, we found that many neurons had their activity depressed during electromotor burst displays.

What advantage might be gained from having subsets of neurons differentially excited between modal patterns of sensory? On the surface, it seems illogical for a neuron involved in active electrolocation to become inactive during probing bursts of object recognition. However, in the natural world, the position of an object–stimulus relative to the electroreceptor array is rarely static. While mormyrids actively probe objects, they often swim around its perimeter (Toerring and Moller 1984; von der Emde 1992). Baseline-selective neurons are responsive to the first stimulus in a series of burst IPIs. Therefore during probing motor acts, baseline-selective neurons could be specialized for encoding the time an object eclipses their receptive field. Spike time comparisons among baseline-selective neurons at different locations in the electroreceptive field could be useful for determining the direction of an object’s movement. As such, baseline-selective neurons could project to neurons in the optic tectum that are selective to object motion (Bastian 1982). Alternatively, baseline-selective neurons could simply help regulate the initiation of electromotor burst displays by influencing the activity of the precommand nucleus (Carlson and Hopkins 2004a).

Regardless of its ultimate function, temporal selectivity has been demonstrated as an integral feature of midbrain sensory processing. The auditory system has proven to be an excellent model for investigating the excitatory and inhibitory interactions that shape interval selectivity (Crawford 1997; Edwards et al. 2007, 2008; Large and Crawford 2002; Rose and Capranica 1983). Mormyrid weakly electric fish perhaps provide an equally tractable model for understanding the importance of temporal patterning to active sensation.

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