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Central control of electric signaling behavior in the mormyrid *Brienomyrus brachyistius*: segregation of behavior-specific inputs and the role of modifiable recurrent inhibition

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Summary

Like all mormyrid fish, *Brienomyrus brachyistius* produces an electric organ discharge (EOD) with a constant waveform and variable sequence of pulse intervals (SPI). Periodic bursts fall into two display categories termed 'scallops' and 'accelerations', with a third category termed 'rasps' that appears to combine the two. The medullary EOD command nucleus (CN) receives excitatory input from the midbrain precommand nucleus (PCN) and the thalamic dorsal posterior nucleus (DP), both of which are regulated by a recurrent inhibitory projection from the ventroposterior nucleus of the torus semicircularis (VP). We tested the following hypotheses: (1) PCN and DP are responsible for generating different burst types (scallops and accelerations, respectively), (2) differences in the strength of recurrent inhibition are related to physiological differences between PCN and DP and (3) recurrent inhibition regulates the resting electromotor rhythm, while disinhibition releases PCN and DP, allowing them to generate bursts. Iontophoresis of the excitatory neurotransmitter L-glutamate (L-Glu) into DP led to acceleration-like output patterns, while in PCN it led to scallop-like output patterns. Iontophoresis of the inhibitory neurotransmitter γ-amino-butyric acid (GABA) into DP and PCN led to an elongation of intervals, as did iontophoresis of L-Glu into VP. Iontophoresis of the $GABA_A$ receptor blocker bicuculline methiodide (BMI) into DP and PCN induced repetitive bursting behavior and eliminated differences in the effects of L-Glu iontophoresis in the two nuclei. These results support our three hypotheses, suggesting that production of different communication behaviors may be regulated by spatially distinct groups of neurons, and recurrent inhibition and disinhibition may play an active role in driving and shaping such behaviors.

Key words: mormyrid, electric fish, *Brienomyrus brachyistius*, electric organ discharge, electromotor, central pattern generator, pacemaker, disinhibition, iontophoresis.

Introduction

The electric signaling behavior of mormyrid fish is characterized by two components: the waveform of each electric organ discharge (EOD) and the sequence of pulse intervals (SPI). While the EOD is constant and signals the sender's identity (Carlson et al., 2000; Freedman et al., 1989; Friedman and Hopkins, 1996; Hopkins, 1981), the SPI is variable and appears to play a role in signaling motivation or behavioral state (Carlson, 2002a; Hopkins, 1986; Kramer, 1993). The SPI is characterized by a relatively slow baseline rhythm, with EOD intervals typically ranging from approximately 100 ms to 300 ms (Carlson, 2002a; Teyssedre and Boudinot, 1987). This baseline rhythm may be periodically interrupted by a variety of bursts and cessations in the discharge (Carlson, 2002a; Hopkins, 1986). Such displays occur in specific behavioral contexts such as courtship and aggression, suggesting that they play an important role in social behavior (Bell et al., 1974; Bratton and Kramer, 1989; Kramer, 1974, 1976; Kramer and Bauer, 1976; Moller et al., 1989; Scheffel and Kramer, 1997, 2000).

A recent quantitative analysis of bursts in *Brienomyrus brachyistius* has revealed three modal display categories based on variation in the temporal patterning of EOD output (Fig. 1B; Carlson and Hopkins, unpublished observations). 'Scallops' are stereotyped pulse sequences in which intervals suddenly drop to 10–20 ms and then immediately return to baseline intervals of 100–300 ms. 'Accelerations' are graded decreases in interval, typically to values of 20–60 ms. Accelerations are less stereotyped than scallops, and minimum intervals for accelerations may be maintained over several EOD cycles with a high degree of regularity. Subjectively, 'rasps' appear to combine an initial scallop-like onset with an acceleration-like termination (Fig. 1B), which is supported by the quantitative characteristics of the three displays (Carlson and Hopkins, unpublished observations). Thus, rasps in this species probably result from a combination of two distinct displays.

Recent anatomical and physiological studies on the electromotor system of mormyrids have suggested a 'closed-loop' circuit that may function as a relatively simple central

pattern generator (CPG) for regulating electromotor output (Carlson, 2002b, 2003; von der Emde et al., 2000). Fig. 1A illustrates the functional connectivity of this network. Each EOD is initiated in the medullary command nucleus (CN), which activates spinal electromotor neurons (EMNs) indirectly through a projection to the medullary relay nucleus (MRN; Bell et al., 1983; Grant et al., 1986). CN integrates excitatory input from two distinct nuclei, the precommand nucleus (PCN) in the mesencephalon, and the dorsal posterior nucleus (DP) in the thalamus (Bell et al., 1983; Carlson, 2002b, 2003; von der Emde et al., 2000). DP and PCN both receive inhibitory feedback from the electric organ corollary discharge pathway (von der Emde et al., 2000), apparently *via* a projection from the dorsal subdivision of the ventroposterior nucleus (VPd) in the torus semicircularis (Bell et al., 1983; Carlson, 2002b, 2003; Carlson and Hopkins, 2001). This recurrent inhibition probably provides a rate-limiting factor to the activity of DP and PCN neurons that may be responsible for producing rhythmic resting electromotor output (Carlson, 2003; von der Emde et al., 2000). A few large neurons at the ventral edge of VP also project to DP, PCN and CN (Bell et al., 1983; Carlson, 2002b), although the functional role of these neurons has not yet been explored.

DP and PCN neurons in *B. brachyistius* show a wide diversity of firing patterns, and correlations between single unit activity and burst production suggest that distinct neuronal populations are responsible for generating scallops and accelerations (Carlson, 2003). In distantly related gymnotiform electric fish, the central posterior and prepacemaker nuclei appear analogous to DP and PCN (Carlson, 2002b), and the two are responsible for generating distinct electrical behaviors (Metzner, 1999). Based on these two lines of evidence, we hypothesized that DP and PCN are likewise responsible for driving different electrical behaviors in *B. brachyistius*. Preliminary experiments using extracellular electrical stimulation support this hypothesis, suggesting that accelerations are generated by DP, while scallops are generated by PCN (Carlson and Hopkins, unpublished observations). We tested this hypothesis using iontophoresis of the excitatory neurotransmitter L-glutamate (L-Glu) to stimulate DP and PCN neurons and observe the effects on electromotor output. It is known that L-Glu iontophoresis in PCN drives decreases in EOD interval in *Gnathonemus petersii* (von der Emde et al., 2000), although these effects have not been quantified in relation to natural signaling behavior, and the effects of stimulating DP have not been assessed.

During scallop and acceleration production, there is a decrease in the activity of VPd neurons, suggesting that disinhibition may play a role in driving these displays by releasing DP and PCN neurons from negative feedback control (Carlson, 2003). Conversely, increases in inhibition may be responsible for producing cessations in the discharge. We tested these hypotheses by several means. Preliminary immunohistochemical studies indicate that PCN is surrounded by terminals containing the inhibitory neurotransmitter γ-amino-butyric acid (GABA; Niso et al., 1989). Thus, we used iontophoresis of GABA in DP and PCN to test whether this causes increases in EOD interval to verify that DP and PCN receive GABAergic

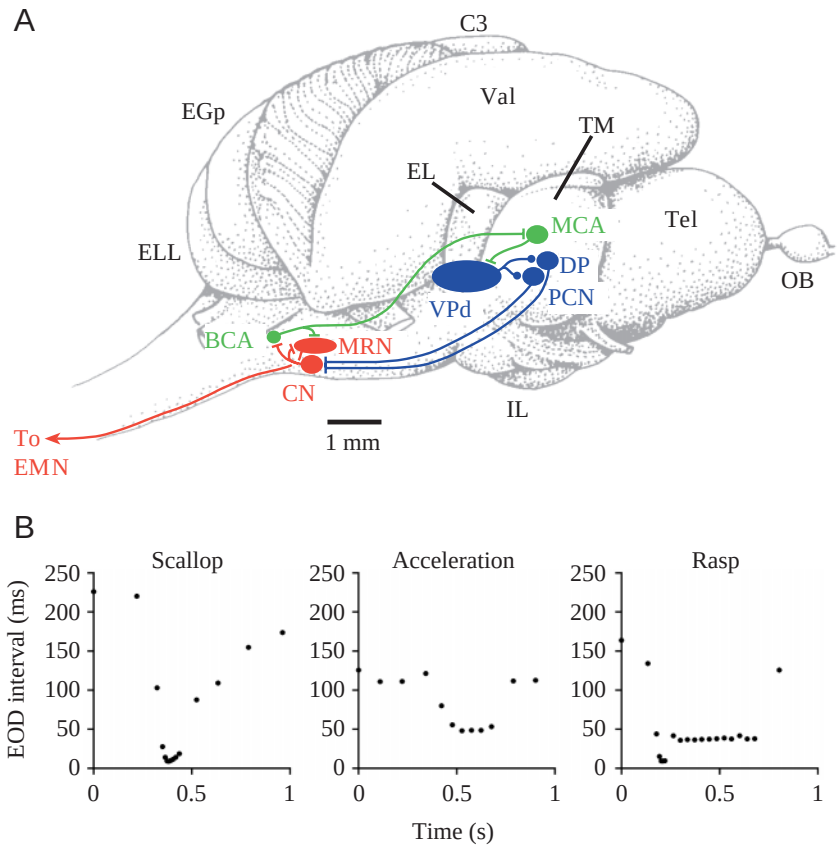

Fig. 1. (A) Sagittal schematic showing the functional neuroanatomy of the mormyrid electromotor system, based on Bell et al. (1983), Carlson (2002b, 2003) and von der Emde et al. (2000). Excitatory terminals are identified by flat lines, inhibitory terminals by solid circles. Red denotes medullary electromotor nuclei, blue denotes mesencephalic and diencephalic electromotor nuclei (topic of the current study), and green denotes corollary discharge nuclei. BCA, bulbar command-associated nucleus; C3, third cerebellar lobule; CN, command nucleus; DP, dorsal posterior nucleus of the thalamus; EGp, eminentia granularis pars posterior; EL, exterolateral nucleus of the torus semicircularis; ELL, electrosensory lateral line lobe; EMN, electromotor neurons; IL, inferior lobe of the hypothalamus; MCA, mesencephalic command-associated nucleus; MRN, medullary relay nucleus; OB, olfactory bulb; PCN, precommand nucleus; Tel, telencephalon; TM, tectum mesencephali; Val, valvula of the cerebellum; VPd, dorsal subdivision of the ventroposterior nucleus of the torus semicircularis. (B) Examples of the three burst display types produced by freely behaving *Brienomyrus brachyistius*. Quantitative analysis indicates that they fall into distinct categories based on unique temporal patterns of EOD production (Carlson and Hopkins, unpublished observations).

inhibitory input. Second, we used iontophoresis of L-Glu in VP to test whether this also causes increases in EOD interval. Finally, we used iontophoresis of the $GABA_A$ receptor blocker bicuculline methiodide (BMI) in DP and PCN to block inhibitory input and determine whether eliminating recurrent inhibition drives decreases in EOD interval.

Differences in the effects of DP and PCN on the SPI are likely to be caused by differences in their physiology, which may in turn relate to differences in the strength of recurrent inhibition from VPd neurons (Carlson, 2003). To test this hypothesis, we compared the effects of L-Glu iontophoresis in DP and PCN before and after BMI iontophoresis. If the observed differences resulting from stimulating the two nuclei with L-Glu are due to variation in inhibitory feedback, then blocking this inhibition should eliminate these differences.

Materials and methods

Animals

We used a total of 27 *Brienomyrus brachyistius* (Gill 1862), ranging in size from 8.0 g to 51.0 g in body mass and 7.9 cm to 18.2 cm in total length. Fish were either wild-caught or laboratory-bred. They were housed in 280-liter group aquaria at a temperature of 25–27°C and conductivity of 150–200 μS cm^{-1} on a 12 h:12 h light:dark cycle and fed live black worms daily. All procedures were in accordance with the guidelines established by the National Institutes of Health and were approved by the Cornell University Institutional Animal Care and Use Committee.

Surgery

Surgical procedures were identical to those described previously (Carlson, 2002b, 2003). Animals were anesthetized in a solution of 500 mg l^{-1} tricaine methanesulfonate (MS-222; Sigma Chemical Co., St Louis, MO, USA) and then respirated under a solution of 160 mg l^{-1} MS-222 during the surgery. Fish were placed on a horizontal platform with lateral supports and completely immersed in aquarium water except for the dorsal surface of the head. A flap of skin was removed from the head and the underlying tissue was scraped away to expose the dorsal surface of the skull. Lidocaine (100–200 μl of a 2% solution; Radix Laboratories, Inc., Eau Claire, WI, USA) was used as a local anesthetic. A metal post was affixed to the skull using superglue, and a small rectangular portion of the skull and meninges was removed to expose the dorsal surface of the midbrain and caudal forebrain. A reference electrode was then placed in the dorsal musculature at the posterior end of the skull. The fish were then immobilized and electrically silenced with an intramuscular injection of flaxedil (gallamine triethiodide; 100–300 μl of a 3 mg ml^{-1} solution; Sigma Chemical Co.), and the respiration was switched to freshwater for recovery.

Experimental procedure

Triple-barrel electrodes were pulled using a Sutter Flaming Brown Micropipette Puller model P-87 (Sutter Instrument Co., Novato, CA, USA) and broken to a composite diameter of approximately 10 μm, resulting in individual barrel diameters of ~2–3 μm. Each barrel was filled with one of the following solutions: (1) 3 mol l^{-1} NaCl for recording local field potentials; (2) 2% alcian blue (Sigma Chemical Co.) in Walpole acetate buffer (pH=4.0) for marking electrode locations; (3) 0.1 mol l^{-1} L-Glu (pH=8.0, adjusted with NaOH) for excitatory iontophoresis; (4) 0.5 mol l^{-1} GABA (pH=3.5, adjusted with HCl) for inhibitory iontophoresis or (5) 20 mmol l^{-1} BMI in 165 mmol l^{-1} NaCl (pH=3.2) for blocking $GABA_A$ receptors.

Electromotor output was monitored by placing a silver wire against the caudal peduncle with a reference several centimeters away. Although the electric organ is silenced by flaxedil, the EOD command can be recorded as a three-spike potential resulting from the synchronous activation of EMN (Bennett et al., 1967). The first negative peak in the EMN volley was defined as the reference time for EOD output (t_0), which in a natural situation precedes the EOD by 4–5 ms. At the start of each experiment, either DP, PCN or VP was localized initially through landmarks on the dorsal surface of the brain and then more precisely by recording characteristic field potentials that were phase-locked to the EMN volley (see Carlson, 2002b). Field potentials and EMN output were band-pass filtered from 10 Hz to 5000 Hz, amplified 10 000× on a differential AC amplifier (A-M Systems, Inc., Everett, WA, USA; model 1700) and monitored on a digital oscilloscope (Tektronix, Inc., Beaverton, OR, USA; model 5223). Iontophoretic currents were provided by a separate amplifier (A-M Systems, Inc.; Neuroprobe model 1600).

After locating a given nucleus, the horizontal position and depth of the electrode were adjusted using a microelectrode drive (Burleigh Instruments, Inc., Fishers, NY, USA; Inchworm 6000) that was held by a micromanipulator (Newport Co., Fountain Valley, CA, USA; model 462-XY-M). The position of the electrode was adjusted in 50 μm steps in all three dimensions and the location where pulsed iontophoresis of L-Glu (–0.5 μA, 500 ms pulses at 0.25 Hz) resulted in the strongest modulation in the SPI was used for all subsequent iontophoretic injection experiments in that nucleus (Fig. 2).

For all experiments, the EMN signal was sent to a Schmitt Trigger, which was output to an event timer that recorded the time of t_0 using a clock rate of 1 MHz (Tucker-Davis Technologies, Alachua, FL, USA; model ET1). Data on EOD times of occurrence were saved using custom-made software. For experiments involving L-Glu or GABA iontophoresis, 20 s of data were recorded before iontophoresis, followed by 20 s of iontophoresis (–1.0 μA for L-Glu, +1.0 μA for GABA) and an additional 20 s of recording. In most cases, opposite polarity current (+1.0 μA for L-Glu, –1.0 μA for GABA) was tested as a control, and this resulted in no observable modulation in the SPI. For experiments involving BMI iontophoresis, 1 min of data were recorded before iontophoresis, followed by 4 min of iontophoresis (+100 nA), followed by 1 min of recovery. The longer duration of BMI iontophoresis compared with L-Glu

and GABA iontophoresis was chosen based on results from previous studies in other systems in which the effects of BMI iontophoresis occurred after relatively long latencies and persisted for several minutes after termination (Fujita and Konishi, 1991; Heiligenberg et al., 1996). The physiological basis for this difference is unclear but is probably related to differences in the pharmacological effects of a receptor blocker compared with naturally occurring neurotransmitters. In some experiments, the effects of L-Glu iontophoresis in DP and PCN before and after BMI iontophoresis were determined. The

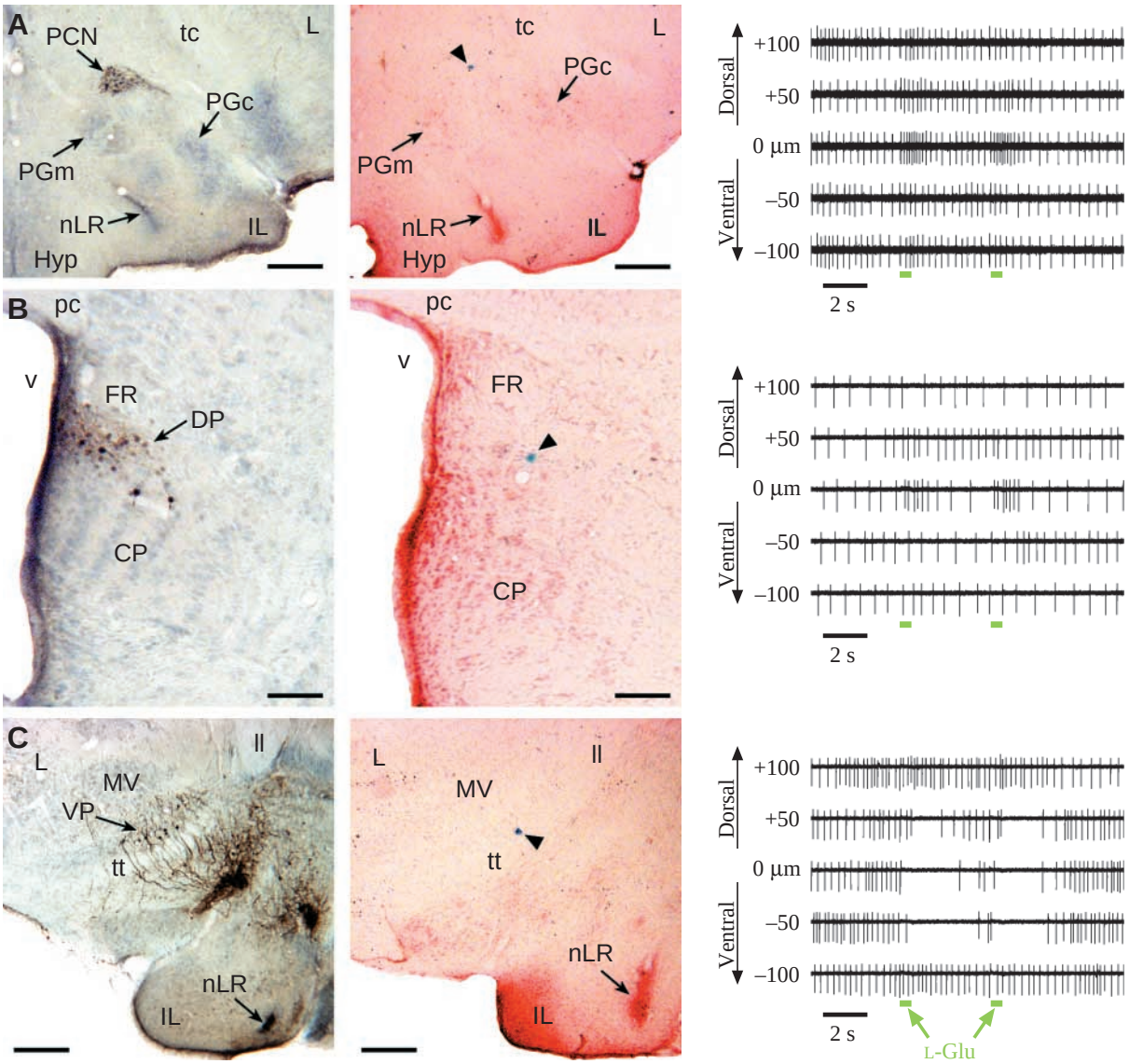

Fig. 2. Localization of iontophoresis sites. (A) The precommand nucleus (PCN). (B) The dorsal posterior nucleus (DP). (C) The ventroposterior nucleus (VP). The first column shows photomicrographs of transverse sections with retrogradely labeled neurons against a background of cresyl violet counterstain, taken from the anatomical study by Carlson (2002b). In A and B, the label results from an injection of neurobiotin into the command nucleus (CN), while in C it results from an injection of neurobiotin into PCN. The second column shows photomicrographs of transverse sections with alcian blue staining against a background of neutral red counterstain, at the same approximate rostro-caudal locations as the first column. The small blue dots provide a precise marker of electrode location, which was always accurately placed into one of the three nuclei. In the third column, continuous voltage traces of electromotor neuron (EMN) activity are shown, with each spike corresponding to a single EMN volley. Iontophoretic injections of L-glutamate (L-Glu; 500 ms pulses of –500 nA) occurred during the times represented by horizontal green lines below each series of traces. Each example is taken from the site shown in the second column, with 0 µm corresponding to the exact location of the alcian blue marker. The effects of L-Glu iontophoresis at 50 µm and 100 µm dorsal and ventral to these sites are also shown. CP, central posterior nucleus of the thalamus; FR, fasciculus retroflexus; Hyp, hypothalamus; IL, inferior lobe of the hypothalamus; L, lateral nucleus of the torus semicircularis; ll, lateral lemniscus; MV, medioventral nucleus of the torus semicircularis; nLR, nucleus of the lateral recess; pc, posterior commissure; PGc, caudal subdivision of the preglomerular nucleus; PGm, medial subdivision of the preglomerular nucleus; tc, tectocerebellar tract; tt, toro-praeeminential tract; v, ventricle. Scale bars, 200 µm in A, 50 µm in B and 200 µm in C.

procedure for L-Glu iontophoresis in these cases was identical to the normal L-Glu iontophoresis procedure, and L-Glu iontophoresis was always performed within 2 min after terminating BMI iontophoresis. Statistica 6.1 (StatSoft, Inc., Tulsa, OK, USA) was used for all statistical analyses of data on the SPI.

Histology

At the end of each experiment, we marked the location of the electrode by iontophoretic injection of alcian blue, using a 500 ms, 150 V pulse (Grass Medical Instruments, Quincy, MA, USA; model S88 stimulator). After completing the experiments, fish were placed back under general anesthesia (160 mg l^{-1} MS-222) and then perfused transcardially with Hickman's ringer solution (6.48 g l^{-1} NaCl, 0.15 g l^{-1} KCl, 0.29 g l^{-1} $CaCl_2$, 0.12 g l^{-1} $MgSO_4$, 0.084 g l^{-1} $NaHCO_3$, 0.06 g l^{-1} NaH_2PO_4) followed by ice-cold 4% paraformaldehyde/1% glutaraldehyde in 0.1 mol l^{-1} phosphate buffer (PB; pH=7.2) for fixation. The brains were removed and postfixed overnight and then transferred to 0.1 mol l^{-1} PB for storage. Brains were transferred to a solution of 30% sucrose in 0.1 mol l^{-1} PB on the night prior to sectioning. Transverse sections were cut on a freezing microtome at 50 μm, mounted on chrom-alum-subbed slides, counterstained with neutral red, dehydrated in a graded alcohol series and coverslipped with Permount (Sigma Chemical Co.).

Results

Glutamate iontophoresis

Brief pulses of L-Glu in DP (*N*=25 fish) and PCN (*N*=24 fish) led to a shortening of EMN intervals, while in VP (*N*=20 fish) it typically led to complete cessations (Fig. 2). Alcian blue marking of the locations where iontophoresis caused the greatest response led to very restricted, bright blue dots in the center of DP or PCN or in the region around VP (Fig. 2), verifying that the electrodes were located in the desired regions. In general, L-Glu iontophoresis only led to changes in EMN interval within a range of depths of 50–150 μm from the alcian blue mark (Fig. 2).

In three different fish, dose–response curves were constructed by measuring the effects of L-Glu iontophoresis on median EMN intervals with varying levels of current magnitude (from –100 nA to –900 nA in steps of –200 nA) in all three nuclei (Fig. 3A). In both DP and PCN, increasing levels of current led to greater shortening of EMN intervals, with the response beginning to saturate at approximately –500 nA and showing complete saturation at –700 nA to –900 nA (Fig. 3B). Similarly, in VP, increasing levels of current led to a greater elongation of EMN intervals, with the response saturating at –700 nA to –900 nA (Fig. 3B). Thus, for all experiments using L-Glu iontophoresis, we used current magnitudes of –1.0 μA, which was well above the level of saturation for all three nuclei and therefore provided maximal stimulation of each nucleus.

20 s injections of L-Glu into the three nuclei led to characteristic modulations in the SPI (Fig. 4). In both DP and PCN, there was a marked, maintained decrease in EMN interval that persisted throughout the duration of the stimulus, while in VP there was a complete cessation of activity for the whole period of stimulation and usually for many additional seconds after terminating the current. There was a highly significant decrease in EMN intervals during L-Glu iontophoresis in DP and PCN and a highly significant increase in EMN intervals during L-Glu iontophoresis in VP (Table 1).

Although stimulation of both DP and PCN led to a shortening of EMN intervals, the responses of the two nuclei were typically quite different. Stimulation of DP typically resulted in a smooth decrease in interval to values of ~20–50 ms that were maintained throughout the period of stimulation with a high degree of regularity (Figs 4, 5). Stimulation of PCN also led to a decrease in baseline intervals, but this baseline was typically not as low or regular as during DP stimulation (Table 1) and was punctuated by the

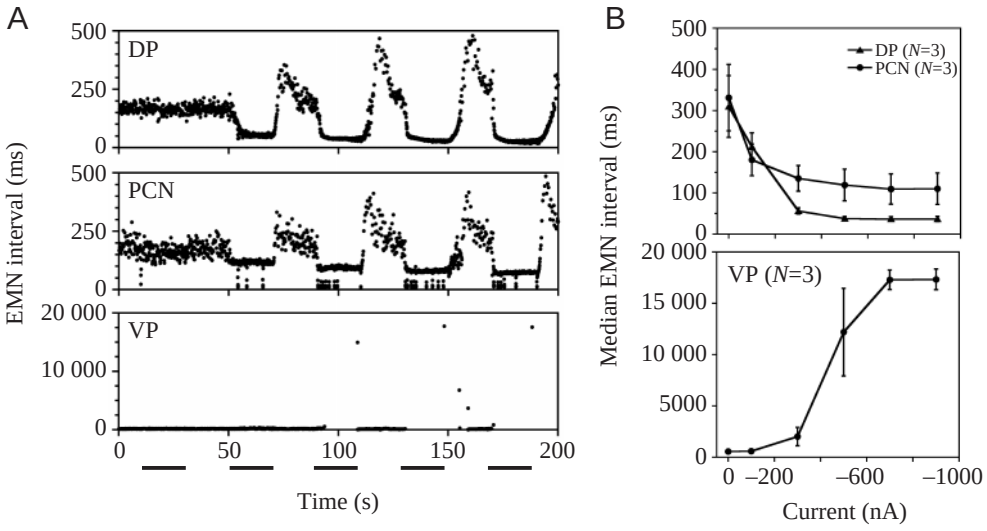

Fig. 3. Effects of varying L-glutamate (L-Glu) iontophoretic current magnitudes on electromotor neuron (EMN) intervals in the dorsal posterior (DP), precommand (PCN) and ventroposterior nuclei (VP). (A) One example from each nucleus in a single fish. EMN intervals are plotted against time. The timing of L-Glu iontophoresis is indicated by the horizontal bars beneath the plots, with the current magnitude increasing from –100 nA to –900 nA in steps of –200 nA. (B) Dose–response curves of the effects of varying current magnitude on median EMN intervals. Values shown are means ± S.E.M.

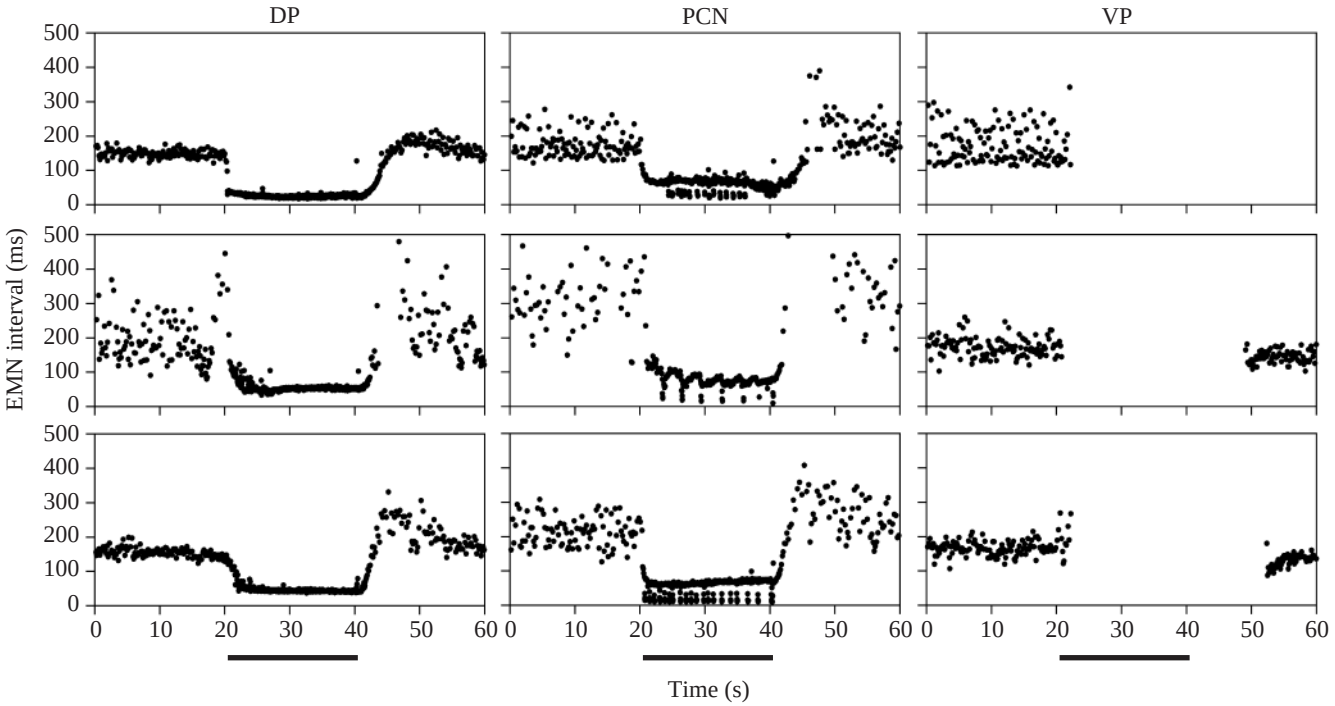

Fig. 4. Three representative examples of the effects of L-glutamate (L-Glu) iontophoresis on electromotor neuron (EMN) output in the dorsal posterior (DP), precommand (PCN) and ventroposterior nuclei (VP). Each row corresponds to a single fish. The first column shows the effects of L-Glu iontophoresis in DP, the second column shows the effects of L-Glu iontophoresis in PCN, and the third column shows the effects of L-Glu iontophoresis in VP. In each case, iontophoretic currents consisted of –1 μA for 20 s, which is indicated by the horizontal bar beneath each column.

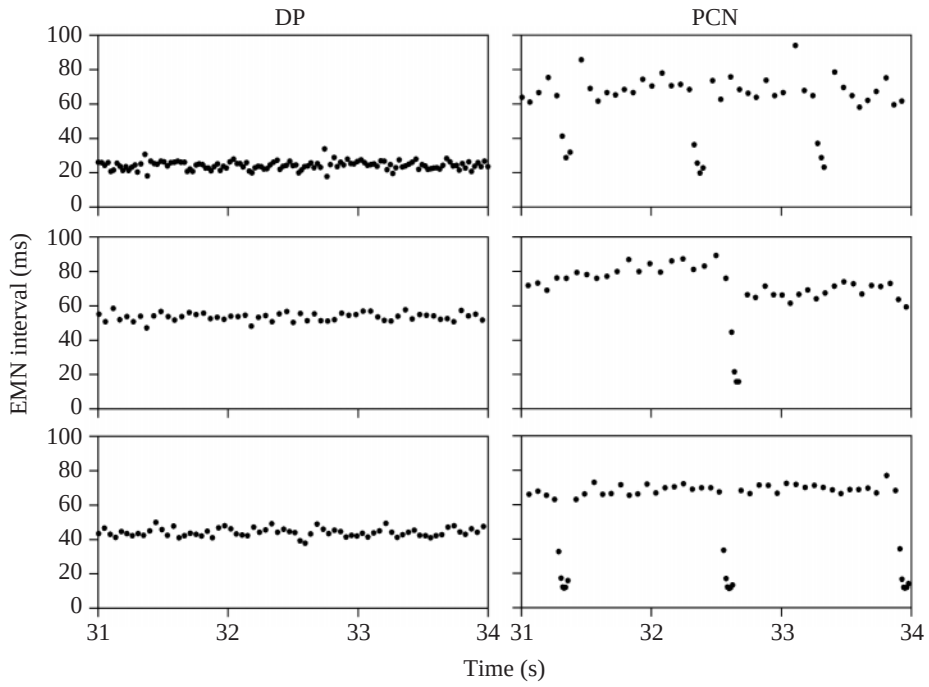

Fig. 5. Differences in electromotor neuron (EMN) output patterns in response to L-glutamate (L-Glu) iontophoresis in the dorsal posterior (DP) and precommand nuclei (PCN). Each row corresponds to a single fish. The three representative examples for each nucleus show an expanded view of the period from 31 s to 34 s of the data shown in Fig. 4.

repeated production of transient, intense bursts reaching minimum intervals of 10–25 ms (Figs 4, 5). In some cases, these transient bursts appeared identical to scallops, while in most cases, they simply appeared as non-stereotyped 'scallop-like' bursts (Fig. 5). Stimulation in PCN led to a significantly greater coefficient of variation (CV) in EMN interval (Wilcoxon matched pairs test: z_{22}=3.912, P<0.0001), smaller minimum EMN interval (z_{22}=2.354, P<0.02) and greater maximum EMN interval (z_{22}=3.360, P<0.001) compared with stimulation in DP (Fig. 6), as expected from the burst-like responses to PCN stimulation.

GABA iontophoresis

Iontophoresis of GABA in DP (N=15 fish) and PCN (N=17 fish) typically led to an elongation of EMN intervals that was maintained throughout the duration of the stimulus (Fig. 7), resulting in a

Table 1. *Changes in median EMN interval in response to iontophoresis of L-glutamate (L-Glu) for 20 s using a current magnitude of –1.0 μA in the dorsal posterior (DP), precommand (PCN) and ventroposterior (VP) nuclei*

	Median EMN interval (mean ± S.E.M.)				
Nucleus (*N*)	Pre-stimulation	L-Glu stimulation	Post-stimulation	*F*	*P*
DP (25)	381.16±48.868	37.660±2.6131	420.67±62.789	29.617	<0.000001
PCN (24)	296.70±32.242	67.229±5.9719	339.81±48.942	31.032	<0.000001
VP (20)	298.56±36.302	21371±3744.8	275.41±31.000	31.678	<0.000001

F-statistics and *P*-values from a repeated measures analysis of variance (ANOVA) for each nucleus are shown. *N* is number of fish.

significant increase in EMN intervals (Table 2). By contrast, we observed no response to GABA iontophoresis in VP (*N*=14 fish; Fig. 7) and there was no significant change in EMN intervals (Table 2).

BMI iontophoresis

Iontophoresis of BMI into DP (*N*=7 fish) and PCN (*N*=7 fish) resulted in repetitive bursting behavior that started after a relatively long latency following stimulus onset (30–150 s) and persisted for several minutes after stimulus termination (Fig. 8A). Comparing the last minute of BMI iontophoresis with the 1 min control period prior to BMI iontophoresis, there was a significant shortening of median EMN interval in both DP (Wilcoxon matched pairs test; z_7=2.3664; P<0.02) and PCN (z_7=2.2678; P<0.025). The bursts resulting from BMI iontophoresis were qualitatively similar to those produced by freely behaving animals, and included scallops, accelerations and rasps (Fig. 8B). Thus, rather than simply quantifying overall activity with general descriptors, it was possible to count the number of bursts produced. There were no significant differences in the numbers of scallops (Mann–Whitney *U* test: $z_{7,7}$=1.086; P>0.27), accelerations ($z_{7,7}$=0.192; P>0.84) or rasps ($z_{7,7}$=0.639; P>0.52) produced by BMI iontophoresis in DP compared with PCN (Fig. 8C).

Comparing the effects of L-Glu iontophoresis before and after BMI iontophoresis in both DP and PCN demonstrated a significant increase in the CV in EMN interval ($F_{1,11}$=7.848; P<0.02), a significant decrease in the minimum EMN interval ($F_{1,11}$=34.95; P<0.02) and no significant change in the maximum EMN interval ($F_{1,11}$=3.809; P>0.07). Before BMI iontophoresis, the CV was significantly greater in PCN than in DP (Fig. 9A; Mann–Whitney *U* test: $z_{7,6}$=2.571; P<0.02), although there was no significant difference after BMI ($z_{7,6}$=0.571; P>0.56). Similarly, the minimum EMN interval was significantly smaller in PCN than in DP before BMI iontophoresis (Fig. 9B; $z_{7,6}$=2.000; P<0.05), although there was no significant difference after BMI ($z_{7,6}$=0.428; P>0.66), and the maximum EMN interval was significantly greater in PCN than in DP before BMI iontophoresis (Fig. 9C; $z_{7,6}$=2.000; P<0.05), though there was no significant difference after ($z_{7,6}$=0.428; P>0.66). Thus, following BMI iontophoresis, the effects of L-Glu iontophoresis in DP and PCN were statistically identical.

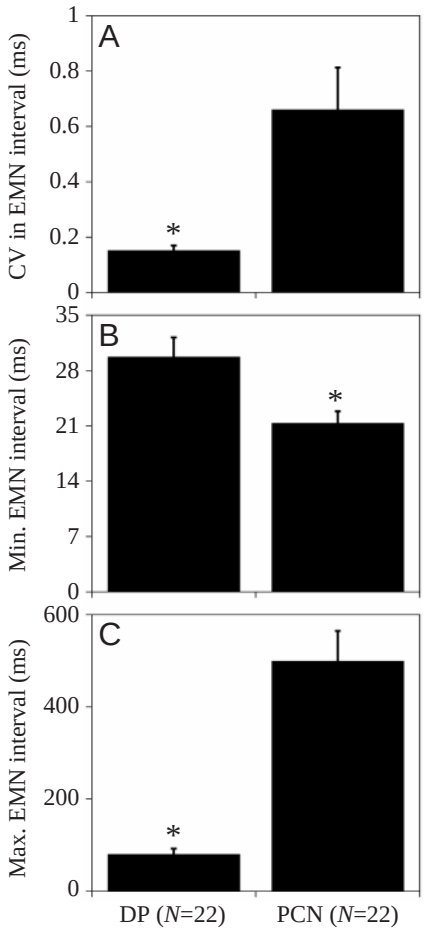

Fig. 6. The effects of L-glutamate (L-Glu) iontophoresis in the dorsal posterior (DP) and precommand nuclei (PCN) on the coefficient of variation in electromotor neuron (EMN) interval (A), the minimum EMN interval (B) and the maximum EMN interval (C). Only those fish with data from both nuclei are included. Values shown are means ± S.E.M. Asterisks represent statistically significant differences (Wilcoxon matched pairs test; P<0.05).

Discussion

We have shown that stimulation of DP and PCN neurons using the excitatory neurotransmitter L-Glu leads to a shortening of EOD intervals. This indicates that both nuclei provide excitatory input to CN and are responsible for the production of bursts, supporting previous studies that used L-

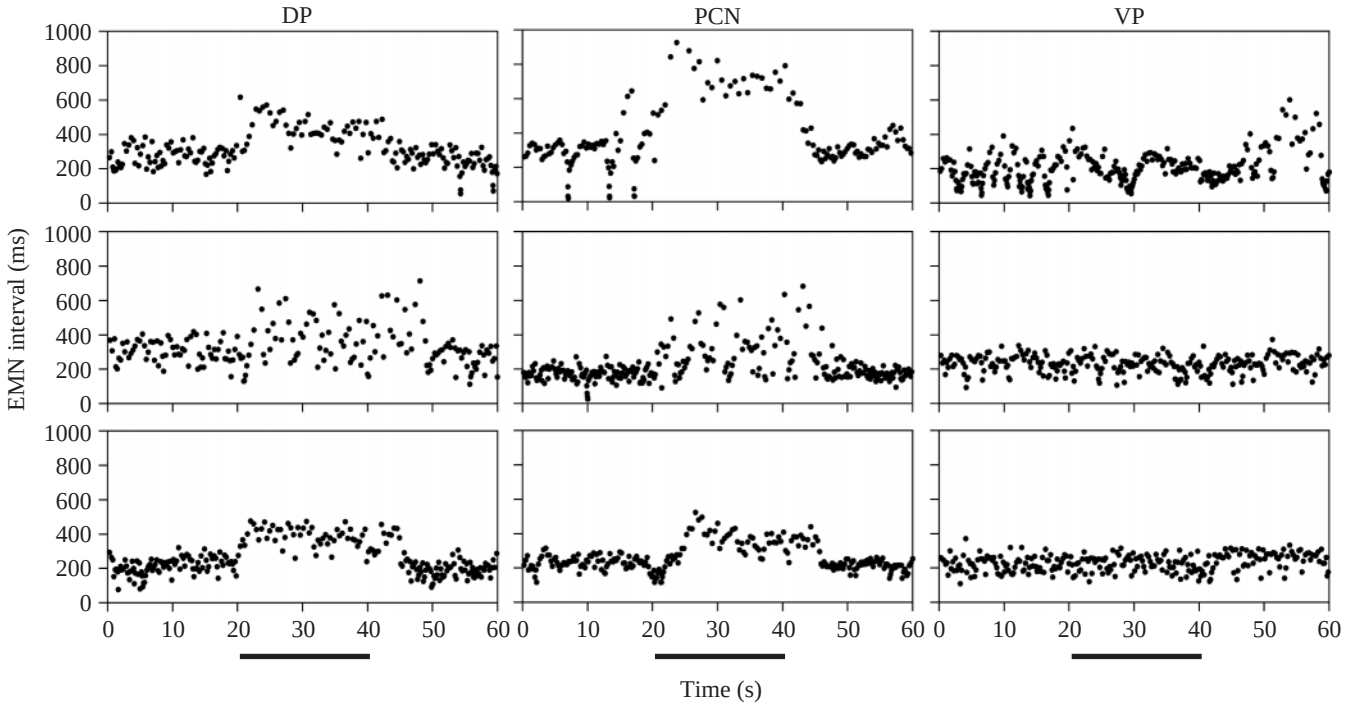

Fig. 7. Three representative examples of the effects of γ-amino-butyric acid (GABA) iontophoresis on electromotor neuron (EMN) output in the dorsal posterior (DP), precommand (PCN) and ventroposterior nuclei (VP). Each row corresponds to a single fish. In each case, iontophoretic currents consisted of +1 μA for 20 s, which is indicated by the horizontal bar beneath each column.

Glu stimulation in PCN (von der Emde et al., 2000) and recordings of single unit activity in DP and PCN (Carlson, 2003; von der Emde et al., 2000). Furthermore, electromotor output resulting from stimulating the two nuclei was significantly different. DP stimulation resulted in smooth, maintained accelerations to intervals of 20–50 ms. PCN stimulation led to a more modest decrease in baseline intervals that was interrupted by the repeated production of transient, scallop-like bursts. This supports the hypothesis that these two nuclei are responsible for generating distinct communication displays. Recent studies of single unit activity in DP and PCN have shown that units with low baseline firing rates experience an increase in activity during accelerations, while units with high baseline firing rates experience an increase in activity during scallops (Carlson, 2003). In light of the findings of the current study, this suggests that neurons within DP may correspond to the former, while neurons within PCN may correspond to the latter.

Neurons in PCN appear to be surrounded by GABAergic inhibitory terminals (Niso et al., 1989), and single units in both DP and PCN receive recurrent inhibitory feedback *via* the corollary discharge pathway (Carlson, 2003; von der Emde et al., 2000). Anatomically, DP and PCN receive a dense projection from VPd, which in turn receives input from the corollary discharge pathway (Carlson, 2002b). These three lines of evidence suggest that VPd provides recurrent, GABAergic inhibition to DP and PCN. The results of the current study support this hypothesis: iontophoresis of GABA into DP and PCN induces a significant elongation of EOD intervals, as does stimulation of VPd using L-Glu iontophoresis. In most cases, stimulation of VPd led to complete cessations in the discharge, suggesting that cessations may result from increases in the activity of VPd neurons and therefore stronger recurrent inhibition.

We hypothesized that this recurrent inhibition is responsible for regulating DP and PCN activity, and thereby maintaining

Table 2. *Changes in median EMN interval in response to iontophoresis of γ-amino-butyric acid (GABA) for 20 s using a current magnitude of +1.0 μA in the dorsal posterior (DP), precommand (PCN) and ventroposterior (VP) nuclei*

	Median EMN interval (mean ± S.E.M.)				
Nucleus (*N*)	Pre-stimulation	GABA stimulation	Post-stimulation	*F*	*P*
DP (15)	260.24±39.315	318.42±42.941	223.02±36.025	15.566	<0.00003
PCN (17)	284.67±44.115	448.04±71.982	255.12±40.670	17.185	<0.00001
VP (14)	237.82±35.102	243.60±30.572	246.94±36.056	0.8172	>0.45

F-statistics and *P*-values from a repeated measures analysis of variance (ANOVA) for each nucleus are shown. *N* is number of fish.

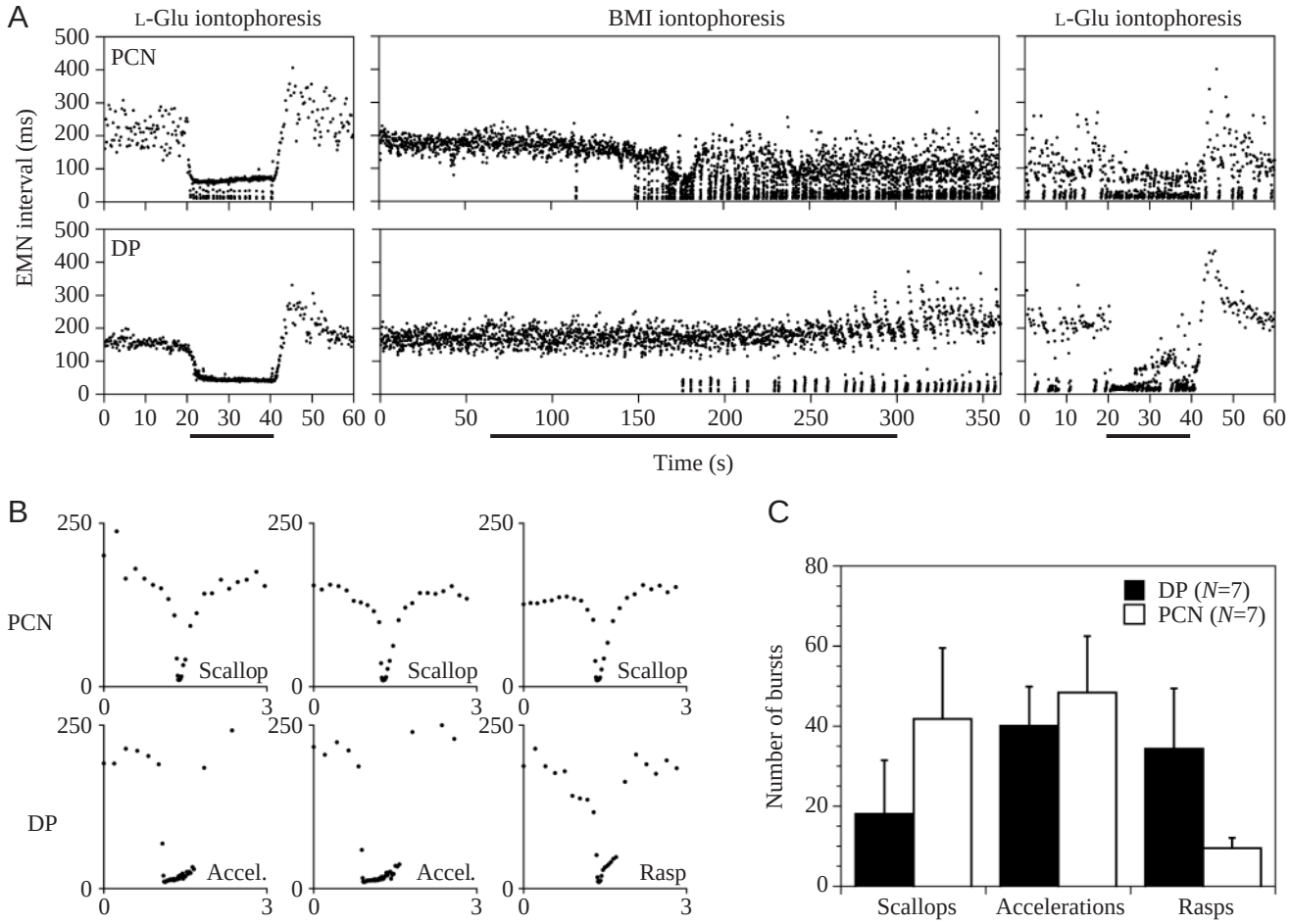

Fig. 8. (A) One example each of the effects of bicuculline methiodide (BMI) iontophoresis in the dorsal posterior (DP) and precommand nuclei (PCN), and the effects of L-glutamate (L-Glu) iontophoresis immediately before and immediately after BMI iontophoresis. L-Glu iontophoretic currents consisted of –1 μA for 20 s, while BMI iontophoretic currents consisted of +100 nA for 4 min, each of which is shown as a horizontal bar beneath each column. (B) Excerpts from A of examples of different burst types occurring during BMI iontophoresis in DP and PCN. (C) Number of different burst types induced by BMI iontophoresis in DP and PCN. Values shown are means ± S.E.M. There were no significant differences in the numbers of any of the three burst types.

the irregular, baseline rhythm of 100–300 ms EOD intervals. During burst displays, the activity of VPd neurons decreases (Carlson, 2003), so we hypothesized that disinhibition plays a role in disrupting the baseline rhythm by releasing DP and PCN from their normal rate-limiting factor, thereby allowing them to drive burst displays. In support of this hypothesis, application of the $GABA_A$ receptor blocker BMI to DP and PCN caused the SPI to go from a resting rhythm to repetitive bursting. It is unclear how the activity of VPd neurons is modulated in a natural situation, although the tectum mesencephali has a strong projection to VPd (Carlson, 2002b; Wullimann and Northcutt, 1990) and retrograde labeling suggests that VPd may also receive inputs from hypothalamic and preoptic areas (Carlson, 2002b). Application of GABA to VP did not elicit any changes in EMN activity, suggesting that a reduction in VPd activity is mediated by a different neurotransmitter or through neuromodulatory inputs.

One potential source of physiological differences between DP and PCN may be variation in recurrent inhibition from VPd neurons, which produce a stereotyped burst of action potentials starting within a few milliseconds of EOD production (Carlson, 2003; von der Emde et al., 2000). Across neurons, there is wide variation in the duration of these bursts. If different subsets of VPd neurons project to DP and PCN, this variation could lead to differences in the baseline activity of DP and PCN neurons and therefore different effects on electromotor output when stimulated. In support of this hypothesis, the effects of L-Glu iontophoresis in the two nuclei were identical following BMI iontophoresis. Furthermore, if recurrent inhibition to DP and PCN is separated into different pathways, this may provide a means for differentially activating the two nuclei and generating distinct behaviors.

Although we suggest that disinhibition plays a role in driving burst displays in a natural situation, we were able to elicit such displays solely through the application of L-Glu to DP and PCN, which presumably did not affect recurrent inhibition. Most likely, the increased excitatory input caused by L-Glu application counterbalanced the ongoing recurrent

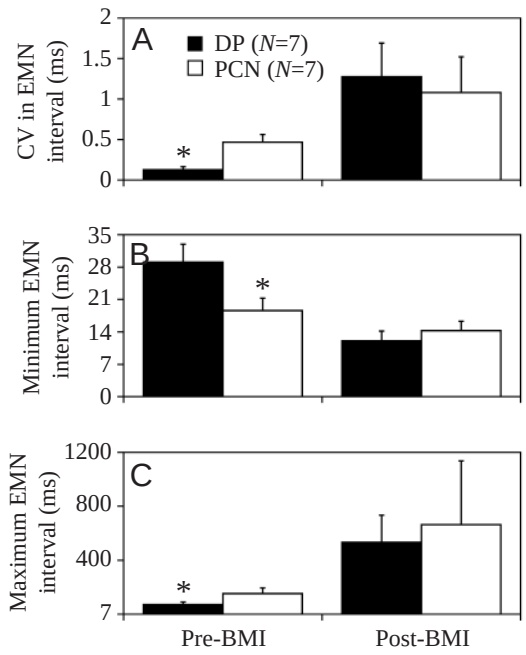

Fig. 9. The effects of L-glutamate (L-Glu) iontophoresis in the dorsal posterior (DP) and precommand nuclei (PCN) before and after bicuculline methiodide (BMI) iontophoresis on the coefficient of variation (CV) in electromotor neuron (EMN) interval (A), the minimum EMN interval (B), and the maximum EMN interval (C). Values shown are means ± S.E.M. Asterisks represent statistically significant differences (Mann–Whitney *U* test; $P<0.05$).

inhibition, resulting in increases in EMN activity. The different effects of L-Glu stimulation in DP and PCN probably relate to the different strengths of recurrent inhibition that counteract the stimulatory effects of L-Glu to varying degrees. Application of BMI to these nuclei, by contrast, leads to a maintained blockage of recurrent inhibition but no excitatory input. Not surprisingly, this also leads to increases in EMN activity. Unlike stimulation with L-Glu, however, there were no observable differences in the output patterns caused by BMI stimulation in DP and PCN, which can be explained by the fact that BMI application also removed the source of physiological differences between the two nuclei, which was not the case with L-Glu stimulation. In a natural situation, disinhibition is typified by a modest, temporary reduction in inhibitory input from the baseline level rather than a complete, maintained removal (Carlson, 2003). Unlike BMI application, this would not eliminate differences between DP and PCN but would provide a transient excitatory effect similar to the effects of L-Glu stimulation, causing the two nuclei to drive different behaviors.

Convergence in the central control of electromotor behavior

Gymnotiform electric fish from South America have electromotor and electrosensory systems that share many striking similarities with those of mormyrids (Hopkins, 1995), despite overwhelming evidence that their electrogenic and electrosensory capabilities have evolved independently (Bullock et al., 1983). EOD production in both groups of fish is controlled by a ventral midline nucleus in the medulla (CN in mormyrids and the pacemaker nucleus, or PN, in gymnotiforms), which projects to larger, adjacent relay neurons whose axons descend the spinal cord to innervate electromotor neurons (Dye and Meyer, 1986). A recent anatomical study has suggested that DP and PCN are analogous to the central posterior (CP) and prepacemaker nuclei (PPN) that provide input to PN in gymnotiforms (Carlson, 2002b). In both groups of fish, there is a rostral group of cells located within a dorsal thalamic nucleus (DP and CP) and a caudal group of cells that forms a ventrolateral extension of the dorsal thalamus (PCN and PPN). While the caudal groups of cells are relatively large with thick, extrinsic dendrites, the rostral groups of cells are small with thin, intrinsic dendrites.

Stimulating CP in gymnotiforms leads to ‘rises’ (smooth, graded increases in frequency), while stimulating PPN leads to ‘chirps’ (transient, intense bursts; Metzner, 1999). This is strikingly similar to electromotor output patterns induced by stimulation in DP and PCN, respectively, with the former driving accelerations (smooth, graded increases in frequency) and the latter driving scallops (transient, intense bursts). Thus, convergence in anatomical substrates appears to be directly linked to convergence in the behaviors they control. In gymnotiforms, the different electrical behaviors resulting from activation of CP and PPN are related to differences in the location of synapses in PN and differences in the glutamate receptor subtypes found at these synapses (Metzner, 1999). It is possible that similar differences play a role in the differential effects of DP and PCN on electromotor behavior in mormyrids, but the findings of the current study suggest that these differences may be due solely to the effects of variation in recurrent inhibition.

Diversity in mormyrid electric signaling behavior

Every species of mormyrid that has been studied produces acceleration-like displays that appear to play a role in aggression, but there is wide diversity across species in the other types of displays that may be produced (Carlson, 2002a). In *Gnathonemus petersii*, agonistic encounters are often accompanied by repetitive ‘pulse pairs’, with EOD intervals alternating between 15–16 ms and 8–9 ms (Bauer, 1972; Bell et al., 1974). Such displays have never been observed in *B. brachyistius* or any other species (Carlson, 2002a), although the number of species studied is relatively small. By contrast, scallops have never been described for *G. petersii*, although they have been described for *B. niger* (Serrier and Moller, 1989). This suggests the hypothesis that pulse pairs may be driven by PCN in species that do not produce scallops.

There is also wide diversity in the characteristics of certain displays between species. For example, scallops in *B. niger* and *B. brachyistius* are quite similar in their basic structure but differ in minimum interval (Carlson, 2002a; Carlson and Hopkins, unpublished observations; Serrier and Moller, 1989). Such differences are possibly related to differences in the

morphology and physiology of PCN neurons. Field recordings from various *Brienomyrus* species in Gabon reveal the production of rasps that differ dramatically from those in *B. brachyistius* and do not appear to result from combining a scallop and an acceleration (Hopkins, 1983; Hopkins and Bass, 1981). Scallops have not been described for these species, so it is possible that PCN controls rasp production in this group. The rich diversity of mormyrids and the signals they produce provide a rare opportunity for studying the evolution of neural circuits that govern communication behavior.

Motor networks and behavior

Much of our understanding of the mechanisms underlying stereotyped motor output comes from relatively simple networks involved in rhythmic behaviors such as locomotion, digestion, respiration and heartbeat (Marder and Bucher, 2001). The stereotyped, rhythmic output of these central pattern generators (CPGs) results from a combination of several cellular and molecular specializations, many of which are shared across different networks. The findings of the current study, as well as other recent studies (Carlson, 2002b, 2003; von der Emde et al., 2000), reveal several similarities between these networks and the mormyrid electromotor system. For instance, recurrent inhibition plays an important role in establishing rhythmic motor output in many different networks (Friesen and Stent, 1978) as well as in regulating electromotor output in mormyrids. Similarly, disinhibition serves a permissive function in activating stereotyped motor output in several motor systems (Faumont et al., 1998; Noga et al., 1988; Wang and Bieger, 1991), which also seems to be the case for generating burst displays in mormyrids.

Extracellular stimulation of restricted brain regions can elicit the production of semi-natural, species-specific communication signals in a variety of vertebrate species (Apfelbach, 1972; Demski and Gerald, 1972; Fine and Perini, 1994; Fu and Brudzynski, 1994; Goodson and Bass, 2000; Jürgens and Richter, 1986; Phillips and Youngren, 1973; Schmidt, 1966; Schuller and Radtkeschuller, 1990; Seller and Armitage, 1983; Valentine et al., 2002; Williams and Vicario, 1993). While it is clear that stereotyped signal production may be controlled by spatially distinct groups of neurons, there is often insufficient information about anatomical circuitry and how it relates to patterns of neuronal activity to gain insight into the network and cellular mechanisms involved in signal generation. Part of the reason for this is that many vertebrate communication signals are relatively complex, involving several features that vary semi-independently over time. As a result, the neural substrates underlying the generation of these signals are similarly complex, making it difficult to formulate hypotheses that directly link the activity patterns of individual neurons to specific signal characteristics.

By contrast, electric signaling behavior in mormyrids is relatively simple, consisting of two distinct components: a stereotyped EOD waveform and a variable pattern of EOD production (the SPI). The characteristics of the former are controlled by the morphophysiological characteristics of the electric organ (Bennett, 1971), while the latter is determined by patterns of activity in CN (Grant et al., 1986). Thus, unraveling the mechanisms involved in regulating the SPI breaks down to a problem of understanding the generation of spike times in CN. As this and other recent studies have shown (Carlson, 2002b, 2003; von der Emde et al., 2000), this relative simplicity makes the mormyrid electromotor network an excellent model system for studying the mechanisms of generating stereotyped temporal patterns in vertebrate communication. The many similarities between this system and the gymnotiform electromotor network, as well as with CPGs in general, suggest that insights gained into the functioning of this network are likely to be instructive towards general issues in the motor control of behavior.

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