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Different Classes of Glutamate Receptors and GABA Mediate Distinct Modulations of a Neuronal Oscillator, the Medullary Pacemaker of a Gymnotiform Electric Fish

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Gymnotiform electric fish generate distinct communicatory signals by modulating the rate of their electric organ discharges (EODs). Each EOD is triggered by a command pulse from the medullary pacemaker nucleus (PN), which contains pacemaker cells and relay cells. The firing rate of this nucleus is modulated by inputs from the diencephalic prepacemaker nucleus (PPN). The NMDA receptor blocker APV and the kainate/quisqualate receptor blocker CNQX, administered to the PN, suppress different types of modulations, indicating that different classes of glutamate receptors mediate the generation of different modulations. A comparison of the 2 genera, *Hypopomus* and *Eigenmannia*, reveals that sustained modulations, such as smooth rises in the rate of pacemaker cell firing and the selective silencing of the relay cells (only observed in *Hypopomus*), are mediated by NMDA receptors, whereas the brief and rapid acceleration, called "chirp" or "decrement burst," is mediated by kainate/quisqualate receptors.

Application of the GABA blocker bicuculline reveals that the 2 genera differ in the mechanism by which they slow the firing rate of their pacemaker. Whereas *Hypopomus* uses GABAergic inhibition to slow down and ultimately silence its pacemaker cells, *Eigenmannia* reduces tonic, APV-sensitive excitation originating from its PPN and lacks GABAergic inhibition in the PN.

Studies on pattern-generating neural systems have shown that specific transmitters and neural modulators are able to induce different modes of activities in anatomically fixed neuronal networks. Through control of such modulators, an animal can thus utilize the same neuronal hardware to generate qualitatively different behaviors (Marder, 1987; Harris-Warrick, 1988; Getting, 1989; Harris-Warrick and Marder, 1991). This notion has emerged mainly from studies of invertebrate systems, but it also holds for vertebrate systems: Gymnotiform electric fish provide convenient vertebrate preparations in which a variety of electrical behaviors can be induced through modulations of a simple neuronal network in their pacemaker nucleus (PN).

A gymnotiform fish, *Hypopomus*, generates "pulse-type" electric organ discharges (EODs) at mostly regular frequencies between a few and several tens per second. While the wave form of the pulses is highly stereotyped, with a duration of a few milliseconds, the frequency of EOD pulses exhibits various modulations in different behavioral contexts (Westby, 1975; Hagedorn, 1983, 1986). The behavioral repertoire of *Hypopomus brevirostris* includes rises and falls of EOD frequency, 2 types of interruptions of EODs, and chirps (also referred to as "decrement bursts"; Hopkins and Heiligenberg, 1978; Heiligenberg and Bastian, 1980; Hopkins and Westby, 1986; Kawasaki and Heiligenberg, 1989). These modulations originate from the prepacemaker nucleus (PPN) in the diencephalon, which provides modulatory inputs to the PN in the medulla. Neurons in the pacemaker nucleus normally fire in synchrony, producing command pulses that trigger individual EOD pulses. Under the influence of modulatory inputs from the PPN, a simple neuronal circuit within the PN, which consists of only 2 types of neurons, operates in different modes and produces a variety of behavioral outputs (Kawasaki and Heiligenberg, 1989).

Similar, though less numerous, types of modulations are performed by the related genus, *Eigenmannia*, which, in contrast to *Hypopomus*, discharges its electric organ in a continual, wave-type manner. Recent neuropharmacological studies on the PN of wave-type genera have revealed that smooth rises in EOD frequency are mediated by NMDA receptors, whereas chirps are mediated by kainate/quisqualate receptor subtypes (Dye et al., 1989). This discovery encouraged us to explore the neuropharmacological basis of the richer repertoire of pacemaker modulations in *Hypopomus*.

In this study, we applied glutamate and its agonists, as well as blockers for different receptor subtypes, to the PN of *Hypopomus* and *Eigenmannia*, while various behaviors were induced either naturally or artificially by stimulating the PPN. We also applied GABA and its blocker bicuculline to test our earlier hypothesis that frequency falls are mediated by different mechanisms in pulse- and wave-type species.

Materials and Methods

We followed the methods applied in our earlier studies on *Hypopomus* (Kawasaki and Heiligenberg, 1989) and on *Eigenmannia* and *Apteronotus* (Dye et al., 1989). Animals were curarized by injection of 10–20 μl of a 2% Flaxedil solution in Ringer's solution and were gently suspended in a sponge-lined clamp with only the dorsal surface of their head protruding above the water line. They were respirated by a constant flow of aquarium water, approximately 2–4 drops per sec, through a tube inserted in their mouth. The residual EOD signal was recorded by a suction electrode placed over the tip of the tail.

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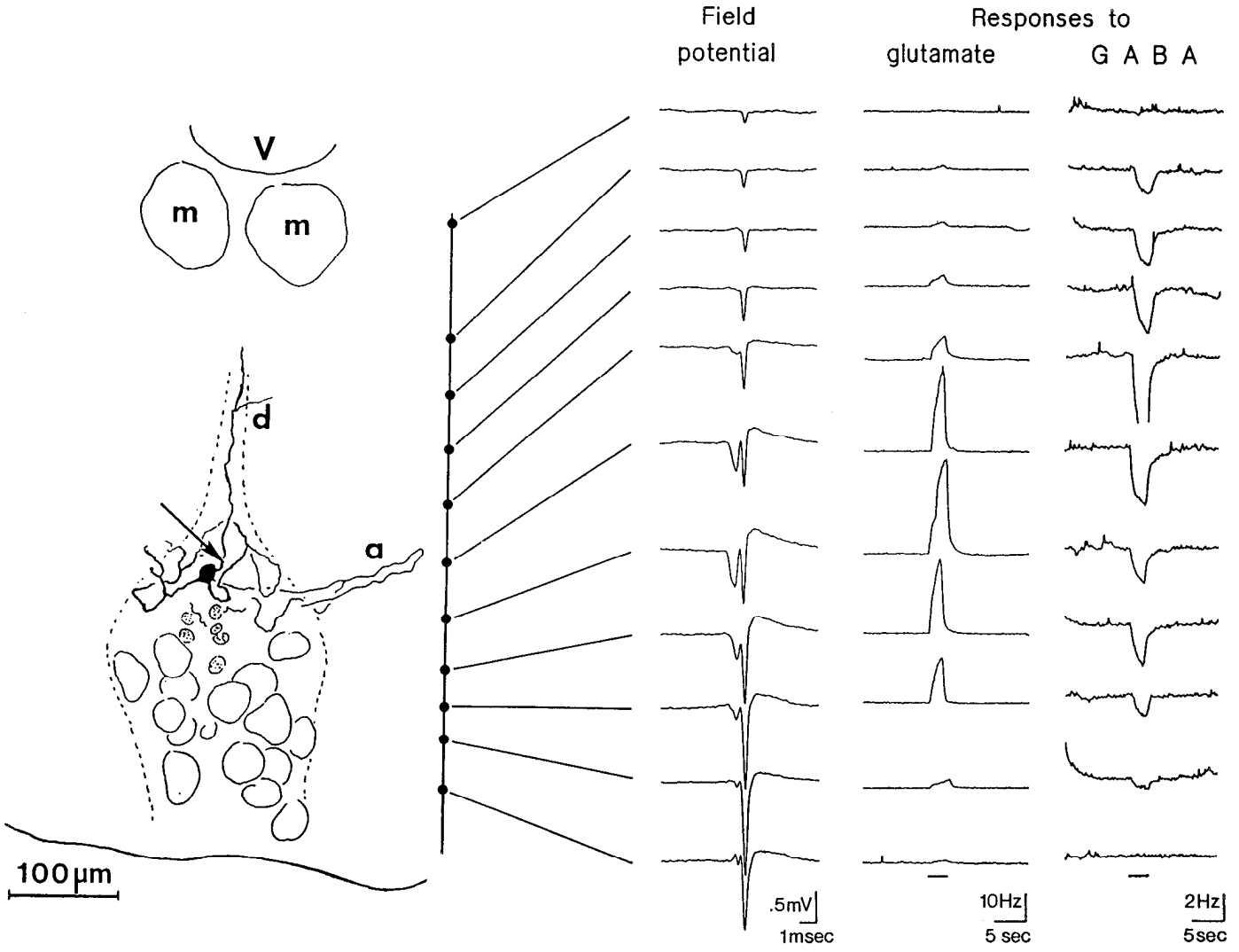


Figure 1. *Left,* Camera lucida drawing of the medullary PN of *Hypopomus brevirostris,* showing large relay cell somata ventrally and small pacemaker cell somata (*stippled,* one labeled *black*) dorsally. This pacemaker neuron was labeled by intracellular injection of HRP to reveal the structure of its dendrites (*d*) and axon (*a*). All labeled pacemaker cells showed the looped axon outside the nucleus. The PN rests on the ventral surface of the brain. *m,* Mauthner axons; *V,* ventricle. *Right,* Field potentials were recorded (*left column*), and iontophoretic effects of glutamate (*center column*) and GABA (*right column*) on EOD frequency were measured at different depths in the nucleus. Note that the first peak of the field potential is largest at the dorsal part of the nucleus, and the second peak is largest at the ventral part of the nucleus. The *penetration line,* which actually passed vertically through the center of the nucleus, indicates corresponding depths of recording and iontophoresis sites. These depths were determined in reference to the location of an alcian blue dye mark deposited at the tip of the *arrow.* This location corresponds to the *sixth dot* along the penetration line. As a result of a progressive frequency fall, the EOD was interrupted in the *fifth trace* in the *right column.* By using the timing of EODs as a reference, field potential data were averaged 50 times to reduce noise. The resting frequency of the EOD was 11 Hz.

After local application of Xylocaine, a small hole, approximately 1 mm in diameter, was drilled above a lateral boundary of the tectal commissure in order to reach the area of the PPN 1200–1600 μm beneath. A small piece of cartilage connecting the parietal bones was excised above the caudal end of the cerebellum in order to reach the PN in the central medulla at a depth of 2200–2800 μm from the cerebellar surface. A small area of a parietal bone was then glued with Histoacryl to a Plexiglas holder for mechanical stabilization.

The PN, which is unpaired, was penetrated with a glass capillary filled with 3 M NaCl for recording of its field potential. The exact location of the nucleus could be determined by the wave form of its potential (Fig. 1, right). By means of a separate microdrive, the PPN, which is paired, was penetrated unilaterally with a glass capillary of a tip diameter of 5–10 μm filled with 0.1 M L-glutamate (pH, 8.0) for iontophoretic stimulation of its neurons. Depending upon the location of this stimulating electrode, each of 4 distinct types of pacemaker modulation could be elicited: frequency rises, frequency drops, chirps, or sudden interruptions (Kawasaki and Heiligenberg, 1989). After localization of the PN, the NaCl capillary was removed, and a triple-barrel electrode was advanced to the same location. One barrel was again filled with 3 M NaCl to record the field potential of the PN for accurate placement of the electrode, whereas the 2 remaining barrels were filled with solutions of glutamate, GABA, or their agonists, antagonists, or control solutions to be injected into the PN either by iontophoresis (glutamate, GABA, NMDA, kainate, and quisqualate) or by pressure (APV, CNQX, and bicuculline).

The pharmacological agents tested were glutamate (Sigma), GABA (γ-amino-*n*-butyric acid, Sigma), the NMDA receptor blocker D(−)2-amino-5-phosphonovaleric acid (APV; Cambridge Research Biochemicals), the kainate/quisqualate receptor blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Cambridge Research Biochemicals), the GABA blocker bicuculline methiodide (Sigma), the glutamate agonist NMDA (*N*-methyl-D-aspartate, Sigma), kainic acid (Sigma), and quisqualic acid (Sigma). Although an initial batch of γ-D-glutamylaminomethylsulphonic acid (GAMS; Cambridge Research Biochemicals) had shown consistent effects when applied to the pacemakers of the wave-type

species *Eigenmannia* and *Apteronotus* (Dye et al., 1989), subsequent batches of this drug from the same company failed to produce reliable effects in *Eigenmannia* and *Hypopomus*. The more recently developed kainate/quisqualate blocker CNQX yielded consistent effects in both species and, therefore, was employed in these experiments. All concentrations expressed in this report refer to the concentrations of agents in the capillary electrodes.

For pressure injection, we pushed a tightly fitting polyethylene tube into the open end of the barrel and connected the other end via a T joint to a pressure line. By manually closing the open end of the T joint, we could increase the pressure inside the barrel and force small quantities of solution out of the tip of the barrel (inner diameter, 5–10 μm). On the basis of visual inspection of the size of a droplet extruded in air, we calculated that a quantity of approximately 0.1 nl was ejected over a period of 1–5 sec. Even at threshold concentrations of the agent, this quantity was sufficient to cause noticeable effects when applied to the optimal location within the PN. An injection of this quantity of any solution, even an artificial cerebrospinal fluid (in mM: 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 1.1 $MgSO_4$, 1.1 $CaCl_2$, 16 $NaHCO_3$), which served as a control, could be detected by an immediate, brief rise of the pacemaker frequency, and auditory monitoring of this rise helped to limit the volume of the injection by manual control of the pressure. Normally, we tested 2 different agents at the same location in the PN to determine the specificity of their effects. In addition, we then moved the stimulating electrode to a different location within the area of the PPN to monitor the effects upon different forms of pacemaker modulations.

By on-line use of a PDP 11/73 computer, we monitored and plotted the instantaneous frequency of the pacemaker signal recorded from the tip of each fish's tail. A simultaneous tape recording of this signal and of the schedules of stimulations and pressure injections served for later analysis of these data.

We performed experiments on 32 individuals of *Hypopomus brevirostris,* the same species used in our previous study (Kawasaki and Heiligenberg, 1989), and 5 individuals of *Eigenmannia lineata.* In most instances, we tested more than 1 drug in 1 fish, often using one as a control for the action of the other. Each drug was tested in at least 3 individuals of *Hypopomus* and at least 2 individuals of *Eigenmannia.*

Results

An overview of pacemaker modulations in gymnotiforms

The PN of *Hypopomus* consists of electrotonically coupled pacemaker cells, which generate the discharge cycle, and relay cells, which are driven by the pacemaker cells and innervate the spinal motor neurons of the electric organ (Bennett et al., 1967; Bennett, 1971). Whereas the pacemaker cells and their extensive dendrites occupy the dorsal portion of the nucleus, the relay cells fill its ventral portion (Fig. 1, left). As described earlier (Kawasaki and Heiligenberg, 1989), various modulations of the EOD frequency are observed in the context of courtship and can be elicited by stimulating distinct sites in the PPN. Frequency rises are caused by acceleration of the pacemaker cells. They are shown not only in the context of courtship, but also in response to novelties in sensory inputs of any modality. Frequency drops, which can lead to a gradual interruption of EODs, are caused by deceleration of the pacemaker cells. In addition to interruptions following frequency drops, we also observed sudden interruptions which, however, are caused by sustained depolarization of the relay cells and hardly affect the cycle of the pacemaker cells. Chirps are sudden, brief accelerations of the EOD cycle that, in their stronger form, lead to a progressive attenuation of the EOD amplitude. Therefore, chirps are also referred to as "decrement bursts" (Hagedorn, 1983). Chirps are generated by selective recruitment of the relay cells and interfere little with the pacemaker-cell cycle.

In the context of its jamming avoidance response (JAR), *Eigenmannia* gradually raises its EOD frequency in response to an interfering signal of slightly lower frequency and lowers its EOD frequency in response to a signal of a slightly higher frequency. Gradual rises in frequency are caused by enhanced activity of the small cells in the PPN (Kawasaki et al., 1988) and are reversibly blocked by application of the NMDA receptor blocker APV to the PN (Dye et al., 1989). Gradual decrements in EOD frequency appear to be caused by tonic inhibition of the small cells in the PPN (Rose et al., 1988; Keller and Heiligenberg, 1989). In the context of aggression and courtship, *Eigenmannia* may abruptly and briefly raise its EOD frequency for several cycles, and a sufficiently strong rise in frequency may lead to a short cessation of the EOD. This behavior, called chirping ("interruptions" by Hopkins, 1974) is triggered by the large cells of the PPN (Kawasaki et al., 1988) and can reversibly be blocked by the kainate/quisqualate receptor blockers GAMS (Dye et al., 1989) and CNQX (present study).

Iontophoresis of glutamate and GABA in the PN

Glutamate and GABA were iontophoresed in the PN without stimulating the PPN to explore their modulatory effects upon the pacemaker cycle.

In *Hypopomus,* glutamate and GABA were alternately iontophoresed through the first and second barrel of a triple-barrel microcapillary, while the field potential of the nucleus was recorded through the third barrel containing NaCl (3 M). The depth of the electrode tip within the PN was estimated by the wave form of the field potential (Fig. 1, right). The first negative peak of the field potential, which reflects the activity of the pacemaker cells, is more prominent at the dorsal locations in the PN. The second negative peak, which reflects the activity of the relay cells, is larger at deeper locations in the PN (Kawasaki and Heiligenberg, 1989). In some penetrations, the first negative peak was further divided into 2 subcomponents. In the case shown in Figure 1, the second negative peak was larger than normal throughout the nucleus, probably reflecting individual variation.

Iontophoresis of glutamate (100 mM, −50 nA, 3 sec) induced frequency rises in the EOD most strongly when applied to the dorsal part of the PN, where the first peak of the field potential was maximal. Iontophoresis of GABA (100 mM, +50 nA) induced frequency falls when applied to the dorsal part of the nucleus, though the most sensitive location was located slightly more dorsal than the location giving strongest responses to glutamate (Fig. 1, right). At the most effective site, GABA caused a complete interruption of the EOD.

At slightly deeper locations, sudden frequency modulations that were indistinguishable from naturally occurring chirps could be induced by glutamate, provided that a much stronger iontophoretic current (−200 nA) was used (Fig. 2*A*). Chirps are generated by synchronized firing of relay cells (Kawasaki and Heiligenberg, 1989). The induction of chirps by steady iontophoresis of glutamate in the PN implies that no synchronized input from the PPN is required for the synchronization of relay cells. At even deeper locations, sudden interruptions could be induced by glutamate, but again, only with a large iontophoretic current (−200 nA; Fig. 2*B*). These modulations, induced by the direct application of glutamate to the PN, were indistinguishable from those induced by the stimulation of the PPN (Kawasaki and Heiligenberg, 1989, their figs. 7, 10). While frequency rises could readily be induced with weak iontophoretic currents, chirps and sudden interruptions could not be induced reliably even with strong currents.

In *Eigenmannia,* iontophoresis of glutamate (100 mM, −200 nA, 20 sec) into the PN induced minor rises in EOD frequency (1–2 Hz). Prolonged iontophoresis failed to induce a further

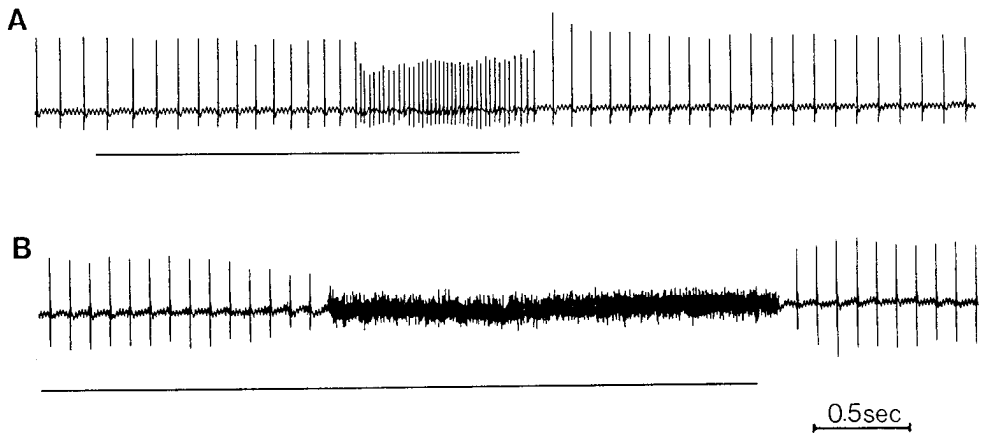


Figure 2. *A*, A chirp (decrement burst) was induced by iontophoresis of glutamate (100 mM) in the pacemaker nucleus of *Hypopomus*. The iontophoretic current (−200 nA) was applied over the period indicated by the *bar* underneath the record. *B*, A sudden interruption was induced by iontophoresis of glutamate to the PN. The iontophoretic current (−200 nA) was started 8 sec before the beginning of the trace and continued over the period indicated by the *bar*.

increment in frequency. The elevated frequency rapidly returned to the resting level within several seconds after the iontophoretic current was turned off. When a much larger current was applied (−2 μA, 10 sec), EODs were interrupted after a rapid frequency rise (10 Hz). Glutamate was effective throughout the nucleus, though the effects were strongest where the field potential, which in this genus consists of a single peak, was largest. GABA (100 mM, +2 μA, 30 sec) was iontophoresed in all parts of the PN, but there was no detectable effect at all.

APV blocks EOD frequency rises in Hypopomus *and* Eigenmannia

The existence of natural excitation by glutamate in the PN of *Hypopomus* was tested by injecting pharmacological blockers into the PN by pressure, while stimulating corresponding sites in the PPN to induce behaviors. APV was injected in the PN of *Hypopomus*, while frequency rises were induced by stimulation of a site in the PPN. As shown in Figure 3, top, APV (500 μM) reversibly blocked frequency rises. The blocking effect of APV was observed in 17 nuclei at 33 injection sites. Control injection of artificial cerebrospinal fluid had no effect. At low concentrations of APV (50–500 μM), injections at the dorsal part of the PN appeared more effective than injections at the ventral portion. Frequency rises induced by presenting novelties in sensory stimuli were also reversibly blocked by injection of APV (not shown). In agreement with our earlier studies (Dye et al., 1989), APV also blocked EOD frequency rises associated with the JAR in *Eigenmannia* and lowered the resting frequency of the EOD (Fig. 4, left). As previously suggested (Rose et al., 1988), PPN neurons that control the JAR are spontaneously active, tonically excite the PN, and maintain its resting frequency. Further excitation of the PPN neurons raises the pace-

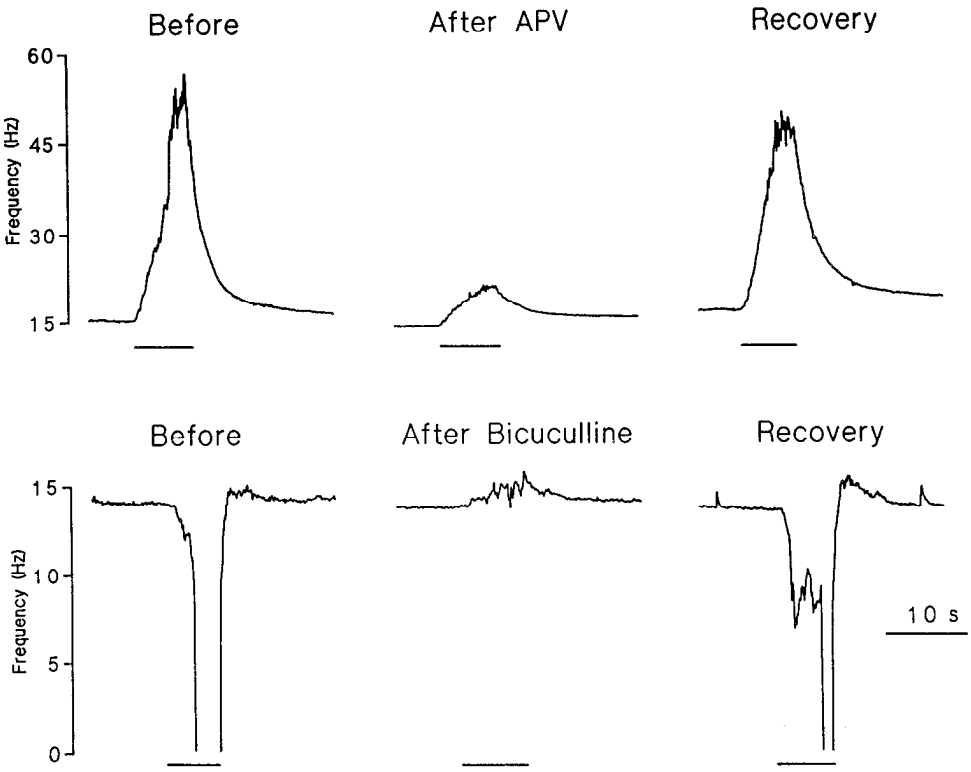


Figure 3. Pacemaker frequency rises (*top*) and frequency drops (*bottom*) can be induced selectively by stimulating different sites of PPN in *Hypopomus*. While application of APV to the PN blocks frequency rises, application of bicuculline blocks frequency drops. *Bars* underneath the frequency records indicate the period of iontophoretic stimulation of the PPN with L-glutamate. The concentrations of APV and bicuculline were 500 and 200 μM, respectively. In the *upper row*, the record in the *center* was taken 20 sec after pressure injection of APV, and recovery was tested 110 sec later. In the *lower row*, the record in the *center* was taken 15 sec after pressure injection of bicuculline, and recovery was tested 261 sec later. The *small rise* in frequency after the injection of bicuculline is due to a weak, simultaneous stimulation of a neighboring site in the PPN causing frequency rises. This response is normally masked by a much stronger frequency drop but, as a result of its longer time course, often appears as the pacemaker recovers from the frequency drop.

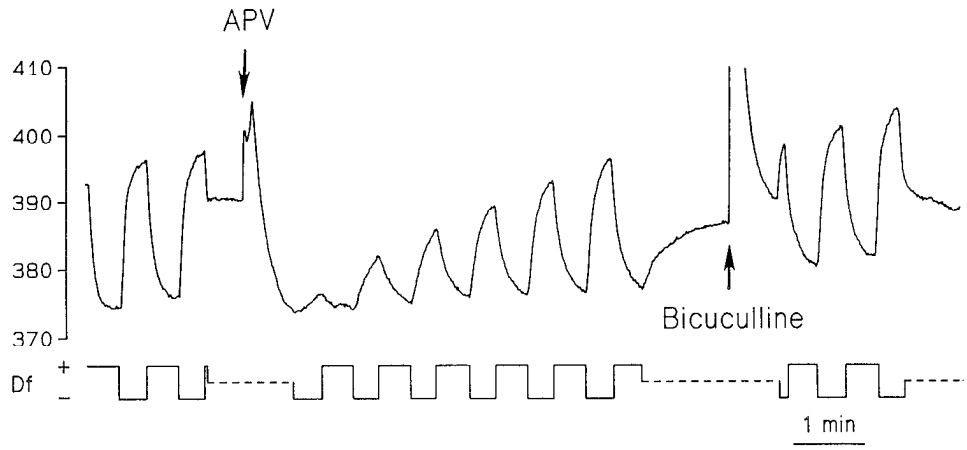


Figure 4. While application of *APV* (500 μM) to PN of *Eigenmannia* lowers resting level of pacemaker frequency and suppresses rises in frequency in context of JAR, application of *bicuculline* (2 mM) has no effect in this genus. The transient rise in response to the injection of bicuculline was not due to the effect of bicuculline because similar rises were observed when a control solution (artificial cerebrospinal fluid) was injected. The *bottom trace* indicates the sign of the frequency difference, *Df*, between a jamming signal and the fish's EOD substitute, with positive Dfs causing a drop in pacemaker frequency and negative Dfs causing a rise. *Dashed lines* indicate absence of the jamming signal to assess the resting frequency of the pacemaker.

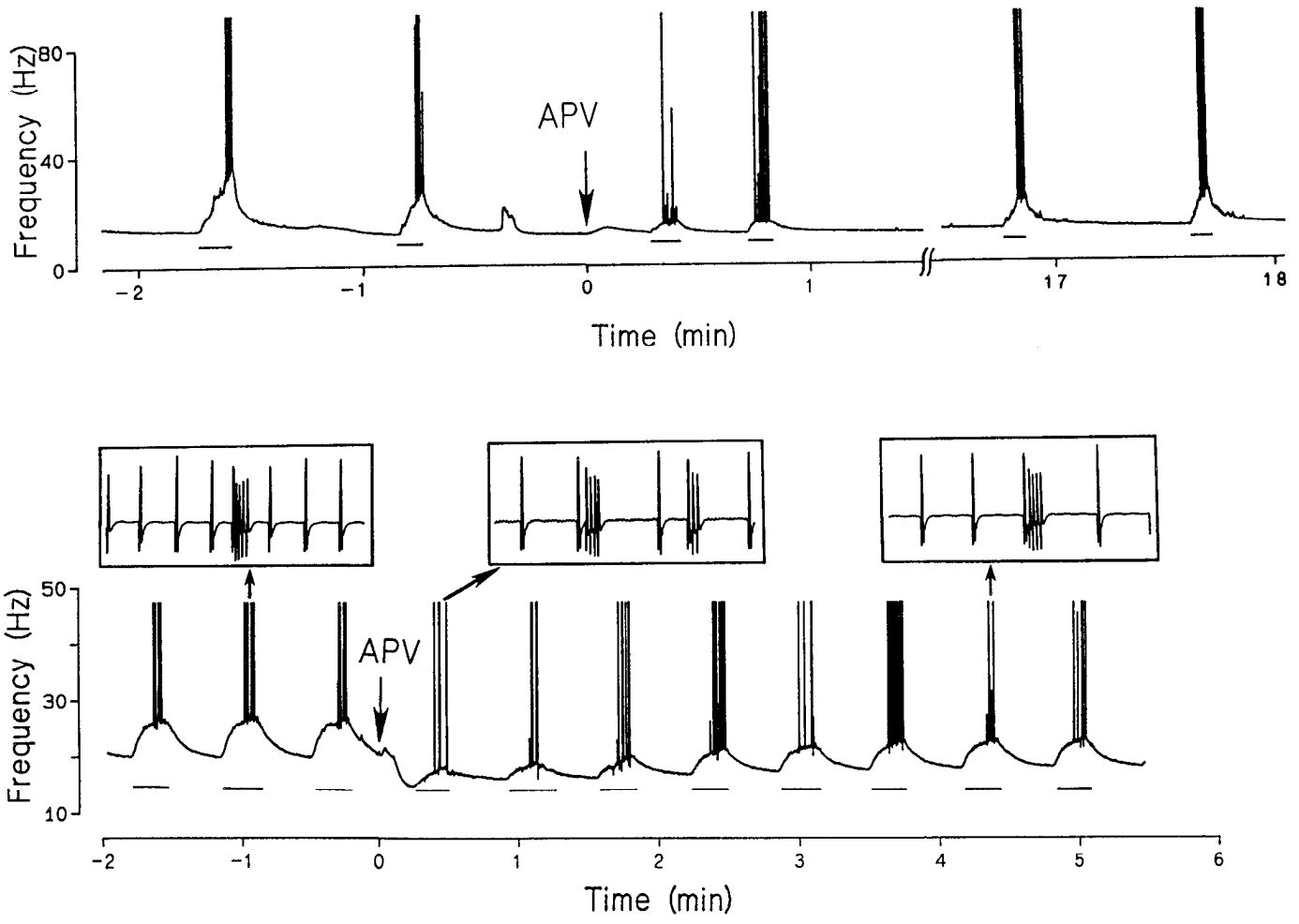


Figure 5. Following injection of *APV* into PN of *Hypopomus*, frequency rises are greatly attenuated, whereas chirping is little affected. The *upper* and *lower graphs* represent experiments conducted on different individuals. In both cases, a site in the PPN was chosen that, upon iontophoresis of L-glutamate (*bars* underneath frequency records), induced frequency rises as well as chirping in the pacemaker. The concentrations of APV were 5 and 60 μM in the *upper* and *lower records*, respectively. In the case of the *lower record*, injection of APV lowered the resting frequency of the EOD, as well. This appears to be due to blocking of tonic inputs from the PPN that had been elevated by a long series of glutamate stimulation. *Boxes* show expanded records of individual chirps embedded in the regular rhythm of EODs. Although chirping seems to occur less frequently immediately following the APV application, its form is not affected by this treatment. *Time span* of each *box* is 270 msec.

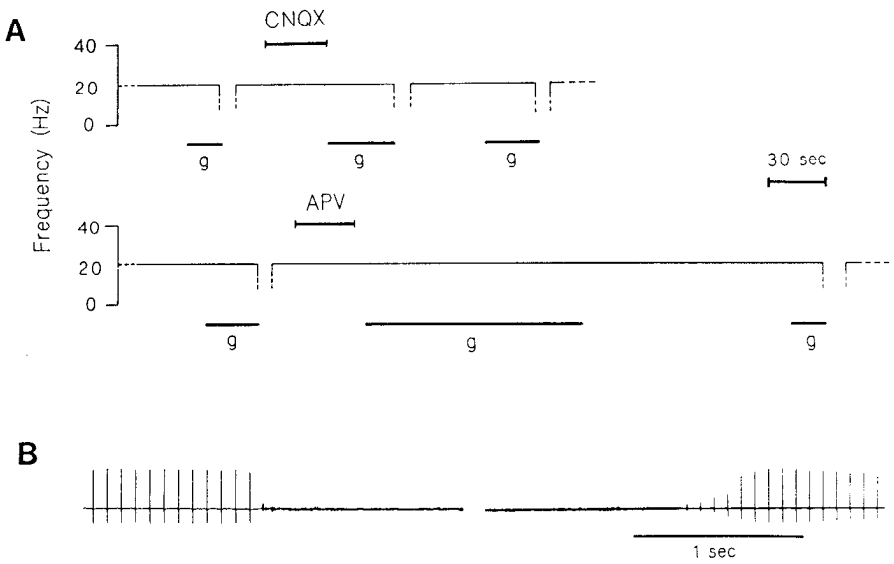


Figure 6. In *Hypopomus, APV* also suppresses interruptions of EOD caused by sustained depolarization of its relay cells. Interruptions of this kind, shown as *breaks* in the EOD frequency records in *A*, can be elicited by iontophoresis of L-glutamate (*g*) in the vicinity of the PPN (*bars* underlining records in *A*). In contrast, application of *CNQX* to the pacemaker has no effect upon interruptions. The *left* and *right traces* in *B* represent the beginning and the end of an interruption that lasted approximately 5 sec. The absence of EOD pulses is marked by elevated noise caused by desynchronized ringing of relay cells.

maker frequency, whereas inhibition of the spontaneous activity lowers the pacemaker frequency. Accordingly, after the injection of APV, the resting frequency fell and remained at a low level, and the magnitude of the JAR was diminished. Resting frequency and magnitude of JAR then recovered over the course of several minutes. Control injection of cerebrospinal fluid had no effect on either resting frequency or frequency rises, except for the transient increase of the EOD frequency probably due to mechanical disturbance of the nucleus.

In *Hypopomus,* a stimulation site in the PPN could be chosen such that frequency rises and chirps were elicited simultaneously. Injection of APV (50–500 μM) attenuated frequency rises without significant impairment of chirping. The selective blocking of frequency rises by APV was confirmed in 4 nuclei at 8 injection sites. At higher concentration (5 mM), however, APV also suppressed chirping to a minor extent (Fig. 5). This agrees with a similar selectivity in the action of APV reported earlier for *Eigenmannia* (Dye et al., 1989). The threshold concentration of APV, necessary for a significant attenuation of frequency rises in *Hypopomus,* was 50 μM.

APV blocks the sustained depolarization of relay cells in Hypopomus

Hypopomus can suddenly silence its EOD by sustained and selective depolarization of its relay cells, and this response can be elicited by stimulating various locations in the caudal PPN (Kawasaki and Heiligenberg, 1989). APV (50 μM) blocked this response reversibly (Fig. 6). The blocking effects were observed with 7 injections made in 6 nuclei. It was not clear whether the injection into the ventral part of the PN was more effective. Injection of either bicuculline (2 mM), CNQX (500 mM), or the control solution had no effect on this type of interruption.

CNQX selectively blocks chirping in Hypopomus *and* Eigenmannia

CNQX (500 μM) was injected in the PN of *Hypopomus* while gradual rises and chirps were induced simultaneously through stimulation of the PPN. While its effect upon gradual frequency rises was minimal, chirping was significantly and reversibly suppressed (Fig. 7). Applications of CNQX appeared to be most effective at the deeper location in the nucleus. We tested APV and CNQX at the same injection site within the PN by using separate barrels of the triple-barrel electrode. By alternately injecting APV and CNQX at concentrations of 50 and 500 μM, respectively, we could demonstrate the selective effects of these 2 agents. For example, the data in the lower half of Figure 5 and in Figure 7 were obtained with consecutive injections at the same location. This selectivity persisted through successive applications of the 2 drugs over the course of hours. We observed that CNQX selectively blocked chirps but not frequency rises in 12 cases. Injection of the control solution had no effect on chirping.

We also applied CNQX to the PN of *Eigenmannia* and found that it selectively suppressed chirping, without affecting the JAR. This supports our earlier conclusion (Dye et al., 1989), based on the application of GAMS, that chirping is mediated by a kainate/quisqualate receptor.

Iontophoresis of NMDA, kainate, and quisqualate in the PN

NMDA, kainate, and quisqualate were iontophoresed into the PN without stimulating the PPN to assess their direct effects upon the pacemaker cycle.

In *Hypopomus,* NMDA (2–5 mM), kainate (2–10 mM), and quisqualate (10 mM) were iontophoresed at different depths in the pacemaker using the same method as used for the glutamate iontophoresis. All these drugs strongly induced frequency rises at the dorsal part of the nucleus. These drugs were 10–50 times more potent than glutamate: A concentration of 2–10 mM, applied with −50 nA, evoked frequency rises of the EOD that were stronger and longer lasting than those evoked by 100 mM glutamate and with the same amount of current. The long-lasting aftereffects are probably due to the lack or the weakness of natural uptake mechanisms for these agonists. At an injection site near the center of the nucleus, where NMDA induced only smooth frequency rises, kainate induced a chirplike jitter in the EOD pattern, in addition to smooth frequency rises (Fig. 8). Sudden interruptions, which are characterized by desynchronized EODs, could be induced by iontophoresis of NMDA at deeper locations in the nucleus. The onset, offset, and quality of the desynchronized EOD trace during the interruption were

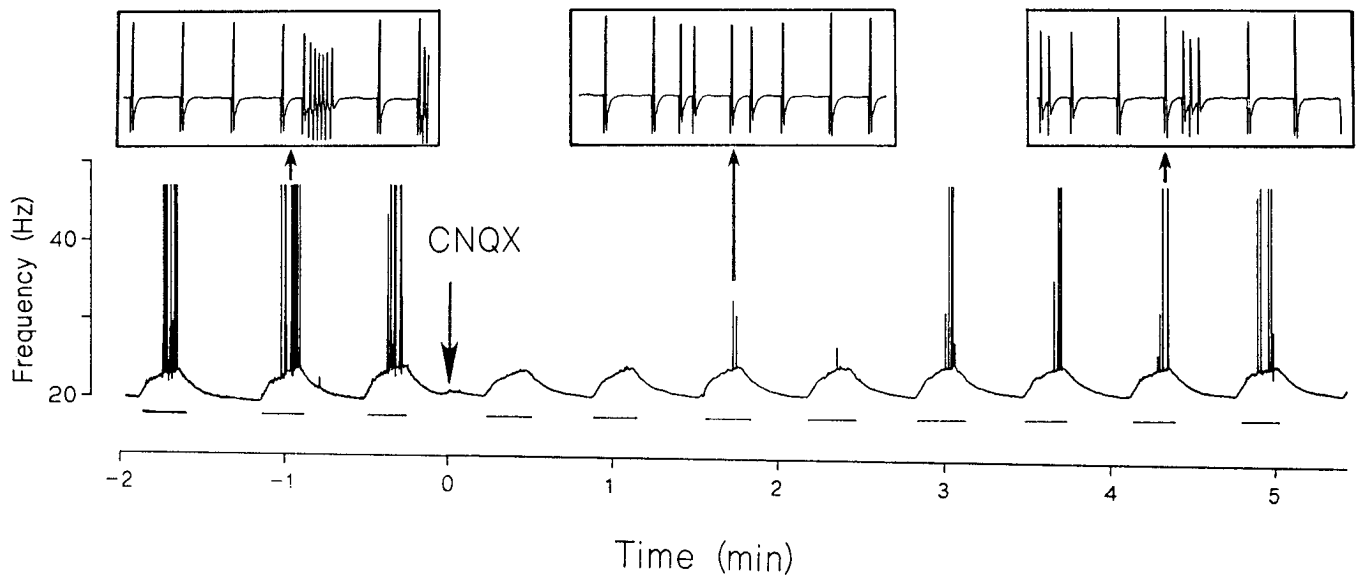


Figure 7. Following injection of *CNQX* (250 μM) to PN of *Hypopomus*, chirping is largely suppressed, whereas frequency rises are not affected. As in Figure 5, a site in the PPN was chosen that induced chirping as well as frequency rises in response to L-glutamate (*bars* underneath frequency record). This record was taken 8 min after the lower record in Figure 5 in the same fish, with the triple-barrel pipettes remaining at the same location within the pacemaker, demonstrating that the selectivity of the action of the 2 drugs is not an artifact of the application site. The expanded traces (*boxes*) of individual chirps from the longer record underneath show that the intensity of chirping, that is, the rate of pulses within a burst, is also reduced by CNQX. *Time span* of each *box* is 270 msec.

indistinguishable from those in the interruptions presented in Figure 2*B*. Although less effective than alternately iontophoresed NMDA (2 mM, −50 nA), kainate (2 mM, −50 nA) and quisqualate (10 mM, −50 nA) could also induce sudden interruptions in some penetration. As was the case with glutamate iontophoreses, chirps and sudden interruptions were difficult to induce consistently by iontophoresis of NMDA, kainate, or quisqualate.

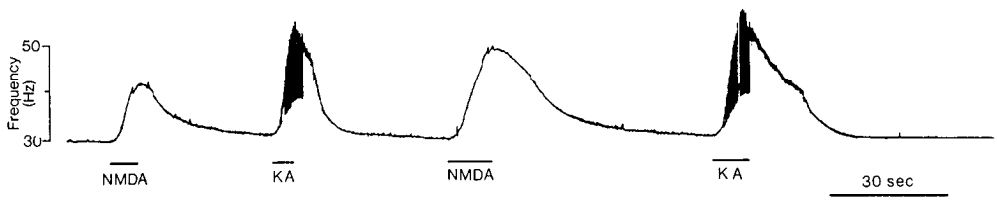
In *Eigenmannia,* iontophoresis of NMDA (10 mM, −60 nA) caused gradual acceleration in EOD frequency. As long as the iontophoresis was continued (tested up to 80 sec), the EOD frequency was gradually increased to 50 Hz above the resting frequency. The recovery was very slow: It took more than 100 sec to return to the resting level after the current was turned off. Kainate (5 mM, −30 nA) iontophoresed at the same location caused a short-term acceleration and a following interruption of the EOD: During the first 10 sec of iontophoresis, the EOD frequency increased to 20 Hz above the resting frequency; then, the frequency rapidly fell, and the EODs were interrupted. The EODs resumed about 30 sec after the current was turned off. Quisqualate (5 mM, −30 nA) had exactly the same effects. Such differential effects of NMDA and kainate/quisqualate were seen over a depth of 350 μm.

GABA inhibits the pacemaker cycle in Hypopomus *but not in* Eigenmannia

The existence of physiological inhibition by GABA in the PN of *Hypopomus* was tested by injecting bicuculline into the PN by pressure, while stimulating an appropriate site in the PPN to induce frequency falls and interruptions. As shown in the lower half of Figure 3, bicuculline (200 μM) reversibly blocked frequency falls and interruptions. Control solution of artificial cerebrospinal fluid injected through a neighboring barrel had no effect. The blocking effects of bicuculline were observed in 6 nuclei with 12 injections. Bicuculline appeared most effective when applied to the dorsal part of the PN, suggesting that GABAergic inhibition acts upon the pacemaker cells (Kawasaki and Heiligenberg, 1989). Injection of APV (5 mM) had no effect on frequency falls and subsequent interruptions in *Hypopomus.*

Injections of bicuculline (2 mM) were made at 7 different sites within the PN of an *Eigenmannia.* Rises and falls of the EOD frequency observed during the JAR of *Eigenmannia* were not affected at any injection site (Fig. 4, right), supporting our earlier interpretation that this genus maintains its resting EOD frequency by tonic activity of the small cells in the PPN and lowers its EOD frequency by tonic inhibition of these cells. In 2 other

Figure 8. *NMDA* (5 mM) and kainate (*KA*; 10 mM) were alternately iontophoresed at same location in PN of *Hypopomus.* While NMDA induced smooth frequency rises, kainate induced a strong jitter in the timing of the EODs. The wave form of the field potential recorded at this location was similar to the one in the fourth row from the bottom in Figure 1, right.



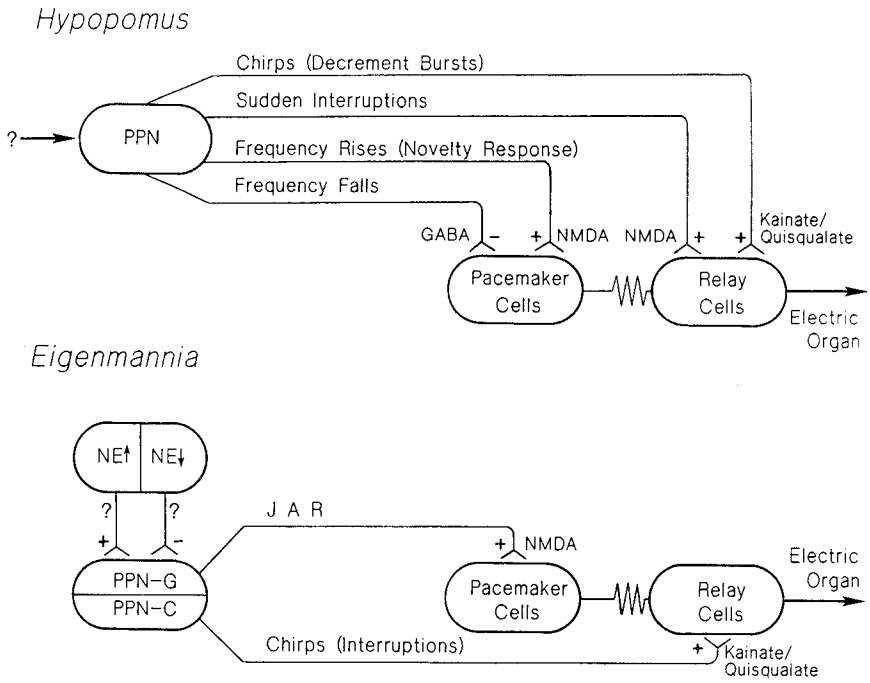


Figure 9. Prepacemaker–pacemaker organization in *Hypopomus* and *Eigenmannia.* In *Eigenmannia,* the *PPN* is subdivided into *PPN-G* and *PPN-C,* which, when stimulated, cause frequency rises and chirps, respectively (Kawasaki et al., 1988). *NE*↑ and *NE*↓ are subdivisions in the nucleus electrosensorius that, respectively, cause frequency rises and frequency falls when stimulated (Keller and Heiligenberg, 1989). Substructures of and inputs to the prepacemaker nucleus of *Hypopomus* are unknown.

nuclei, bicuculline and APV were alternately injected through different barrels of an electrode. Again, bicuculline had no effect, while APV blocked frequency rises.

Discussion

This study demonstrates that 2 genera of gymnotiform electric fish, *Hypopomus* and *Eigenmannia,* appear to exploit the kinetically distinct properties of different glutamate receptors to generate different forms of pacemaker accelerations, whereas they differ in their means of decelerating the pacemaker cycle. The specific and selective blocking effects of APV and CNQX indicate that NMDA receptors mediate gradual accelerations of pacemaker cells and the sustained depolarization of relay cells, whereas kainate/quisqualate receptors mediate the brief and rapid modulations, or chirping, of relay cell firing. This observation is in agreement with studies in other systems, such as the spinal cords of lampreys (Grillner et al., 1987) and larval *Xenopus* (Roberts et al., 1986) or the X cells in the mammalian lateral geniculate (Heggelund and Hartveit, 1989), demonstrating that, as a likely consequence of their differences in kinetics, NMDA receptors mediate slow and sustained processes, while kainate/quisqualate receptors mediate fast and transient processes.

Figure 9 represents our current hypothesis of the prepacemaker–pacemaker organization in *Hypopomus* and *Eigenmannia.* In both genera, gradual rises in pacemaker frequency appear to be generated through effects on the pacemaker cells, whereas chirping is brought about by selective recruitment of the relay cells (Dye, 1988; Dye et al., 1989; Kawasaki and Heiligenberg, 1989). In *Eigenmannia,* we assume that different input fibers from the PPN induce frequency rises and chirps, respectively, by terminating onto NMDA- and kainate/quisqualate-sensitive postsynaptic membranes of different cell types. An additional complexity emerges in *Hypopomus,* however, which has the behavioral option of selectively silencing its relay cells through sustained depolarization (Kawasaki and Heiligenberg, 1989). Because this form of EOD interruption is blocked by APV, this behavior appears to be mediated by NMDA receptors. The relay cells of *Hypopomus* thus are subject to the dual control by NMDA and kainate/quisqualate receptors. It remains to be determined whether the NMDA receptors mediating sudden interruptions reside at locations different from those of the kainate/quisqualate receptors mediating chirping. The inhibitory control over the PN demonstrated in *Hypopomus* represents an additional complexity of the prepacemaker–pacemaker organization in this genus.

By separate modulatory innervations of pacemaker cells and relay cells, gymnotiform fish are able to alter the firing of their PN in different ways and to generate a variety of distinct social signals. The independence of the control of pacemaker cells and relay cells in *Hypopomus* becomes most obvious when a stimulation site in the PPN triggers both silencing of the pacemaker cells and chirping of the relay cells. In this instance, chirps appear in the absence of normal EOD activity. Although, in the normally behaving animal, chirping occurs in conjunction with a gradual rise in EOD frequency, this observation demonstrates that such slow rises are not required for chirping.

While chirping, the relay cells of *Hypopomus* fire in synchrony, at a rhythm much faster than that of the pacemaker cells (Kawasaki and Heiligenberg, 1989). Because chirping can be elicited directly by glutamate injection to the PN, the synchronization of the relay cells does not require the control of the PPN and therefore must be based upon local, yet unknown network properties.

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