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Agonistic-like responses from the torus semicircularis dorsalis elicited by $GABA_A$ blockade in the weakly electric fish *Gymnotus carapo*

T.T. Duarte[1], S.A.L. Corrêa[2], U.J. Santana[3], A.S.F. Pereira[1] and A. Hoffmann[1]

[1]Departamento de Fisiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil
[2]MRC Centre for Synaptic Plasticity, Department of Anatomy, School of Medical Sciences, University of Bristol, University Walk, Bristol, UK
[3]Departamento de Física, Universidade Católica de Goiás, Goiânia, GO, Brasil

Abstract

Findings by our group have shown that the dorsolateral telencephalon of *Gymnotus carapo* sends efferents to the mesencephalic torus semicircularis dorsalis (TSd) and that presumably this connection is involved in the changes in electric organ discharge (EOD) and in skeletomotor responses observed following microinjections of $GABA_A$ antagonist bicuculline into this telencephalic region. Other studies have implicated the TSd or its mammalian homologue, the inferior colliculus, in defensive responses. In the present study, we explore the possible involvement of the TSd and of the GABA-ergic system in the modulation of the electric and skeletomotor displays. For this purpose, different doses of bicuculline (0.98, 0.49, 0.245, and 0.015 mM) and muscimol (15.35 mM) were microinjected (0.1 µL) in the TSd of the awake *G. carapo*. Microinjection of bicuculline induced dose-dependent interruptions of EOD and increased skeletomotor activity resembling defense displays. The effects of the two highest doses showed maximum values at 5 min (4.3 ± 2.7 and 3.8 ± 2.0 Hz, $P < 0.05$) and persisted until 10 min (11 ± 5.7 and 8.7 ± 5.2 Hz, $P < 0.05$). Microinjections of muscimol were ineffective. During the interruptions of EOD, the novelty response (increased frequency in response to sensory novelties) induced by an electric stimulus delivered by a pair of electrodes placed in the water of the experimental cuvette was reduced or abolished. These data suggest that the GABA-ergic mechanisms of the TSd inhibit the neural substrate of the defense reaction at this midbrain level.

Correspondence
T.T. Duarte
Departamento de Fisiologia
FMRP, USP
Av. Bandeirantes, 3900
14049-900 Ribeirão Preto, SP
Brasil
Fax: +55-16-633-0017
E-mail: teduarte@rfi.fmrp.usp.br

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- Torus semicircularis dorsalis
- GABA-ergic system
- Electromotor modulation
- Agonistic behavior

Introduction

Gymnotiform weakly electric fishes modulate the frequency of their electric organ discharge (EOD) in different behavioral contexts, most robustly during courtship and aggressive encounters (1-3). The frequency of the EOD is controlled by inputs from the medullary pacemaker nucleus (PM), which contains three types of neurons: pacemaker, relay and parvocells. Pacemaker cells are electrotonically interconnected and make mixed electrotonic and chemical synaptic contacts with the relay cells. Relay cells are

output cells of the PM and their axonic terminals innervate spinal motoneurons that drive the electric organ (4-6). Parvocells receive electrotonic inputs from and produce chemical synapses back onto pacemaker and relay cells (7). The firing of PM can be modified by diencephalic inputs from the central-posterior/prepacemaker complex (CP/PPn), which contains separate cell clusters (8,9) or by mesencephalic inputs from the sublemniscal prepacemaker nucleus (SPPn) (3,10,11). Both CP/PPn and SPPn receive projections from the diencephalic nucleus electrosensorius (nE), which provides an interface between the electrosensory processing performed by the deep layers, mainly VIIIC and VIIID, of the torus semicircularis dorsalis (TSd) and the premotor control of PM (12-14).

The TSd, a large and laminated midbrain structure, is the terminal station of the lateral lemniscus and is homologous to the inferior colliculus of mammals (15). In the wave-type electric fish *Eigenmannia*, the torus has been related in particular to the jamming avoidance response, a frequency shift of the EOD that avoids jamming with neighboring conspecific EOD (16,17). The toral neurons are also involved in other types of behavior such as the pulse-types of *Gymnotus* and *Hypopomus*, in which jamming avoidance response is absent and the TSd is involved in novelty detection (18). *Gymnotus carapo* responds to sensory novelties with a transient frequency increase (novelty response, NR) (19), a behavior which shows a partial habituation when the stimulus is repeated, and is influenced by seasonal variation (19).

At the level of PM cells, the different patterns of EOD are mediated by gamma-aminobutyric acid (GABA) or glutamate receptors (2,3,6,20-22). GABA is a major neurotransmitter in the central nervous system, acting essentially as a hyperpolarizing or inhibitory transmitter. GABA neurons are widespread within the nervous system of vertebrates including teleosts. Kennedy and Heiligenberg (22) demonstrated GABA immunoreactivity in the PM of the weakly electric fish *Hypopomus*. A study from our laboratory showed that microinjection of the $GABA_A$ antagonist bicuculline into the dorsolateral telencephalon of *G. carapo* leads to changes in EOD frequency and in skeletomotor activity, supposedly by connections with the TSd (23).

In addition to the well-known involvement of the TSd and the optic tectum in sensory processing, some studies have implicated these structures or their mammalian homologues, the inferior and superior colliculus, respectively, in integrative functions. In all vertebrates studied, they are concerned with orienting (24) and defensive responses (25). Defense-like reactions can be obtained from these regions by a variety of stimulating agents, including GABA blockers and excitatory amino acids (26,27). Glutamate microinjected into the regions of the mesencephalic tegmentum near the TS of the toad *Bufo paracnemis* induces a flight response (28).

In mammals there is evidence that the neural substrates responsible for defensive behavior in the inferior colliculus (IC) are also regulated by GABA-ergic and glutamatergic mechanisms. Brandão et al. (26) showed that microinjections of bicuculline into the ventral aspects of the IC of rats produce a behavioral activation together with jumps and these results suggest that GABA exerts a tonic inhibitory action on these neurons implicated in the generation or elaboration of aversive responses. Studies by Cardoso et al. (27) also showed the involvement of excitatory amino acids in the IC in the expression of defensive reactions. The activation of glutamate receptors at ventral aspects of the IC elicits freezing behavior, one of the main features of the fear-like display in rats. Furthermore, Moreira et al. (29) showed that microinjection of nitric oxide donors into the central nucleus of the IC of rats induced flight reactions and that this

reaction could be prevented by pretreatment with the NMDA receptor antagonist amino-7-phosphonoheptanoic acid.

To our knowledge, no such studies have been undertaken in teleosts, specifically in weakly electric fishes, in which the role of TSd in electrosensory processing and its connections with other brain regions have been the subject of several studies (12-14,30).

The objective of the present study was to identify the local regulatory role of GABA receptors in the expression of the electro- and skeletomotor components of behavior to further explore the role of the TSd and to contribute to a better understanding of evolutionary processes. To achieve this, different amounts of bicuculline and an agonist (muscimol) of $GABA_A$ receptors were microinjected into the TSd of awake, unrestrained *G. carapo* fish and the effects on the EOD and on behavior were recorded.

Material and Methods

Thirty-five specimens of *G. carapo* without sex distinction, originating from the Tietê River in the State of São Paulo, were purchased on the local market and kept in individual aquaria (40 x 22 x 20 cm) containing continuously aerated water with resistivity ranging from 20 to 30 kΩ/cm, pH 6-7, at 25º to 29ºC. The animals were 22.5 ± 0.44 cm long and weighed 44.5 ± 2.5 g. They were fed small live fish every 2 days and maintained on a natural photoperiod. The experiments were carried out in compliance with the guidelines set up by Colégio Brasileiro de Experimentação Animal (Princípios Éticos na Experimentação Animal (1991), http://www.cobea.org.br/etica.htm#3) and approved by the Ethics Committee of our Institution (No. 047/2003).

Surgical procedure

The animals were submerged in a solution of MS222 (0.2 g/L tricaine methanesulfonate; Sigma, St. Louis, MO, USA) until postural loss and disappearance of opercular movements were observed before surgery. During surgery, anesthesia was maintained by perfusion of a 0.18 g/L MS222 aerated solution into the animal's mouth and across the gills. The skin was removed from the skull and a small hole was drilled above the midbrain to expose this region. A guide cannula prepared from a hypodermic needle (OD 0.7 mm, length 7 mm) was attached to the holder of the stereotaxic apparatus and placed over the exposed surface of the midbrain at the level of the TSd, according to references of the atlas of the telencephalon of *G. carapo* (31) and the brain atlas of *Apteronotus leptorhynchus* (32). Coordinates were adjusted as necessary according to histology. The orifice around the cannula was filled with a paste consisting of a mixture of equal parts of paraffin and glycerin, and the cannula was fixed to the skull with acrylic resin.

Experimental procedure

Following surgery, the animal was kept in an individual aquarium for 48 h and then placed in a cuvette (31 x 5 x 5 cm) containing aerated freshwater and left there for some minutes to familiarize itself with the experimental environment. It was then removed from the cuvette by means of a nylon net and placed on a table for a few seconds for microinjection of bicuculline into the TSd (0.98, 0.49, 0.245, and 0.015 mM; Sigma), muscimol (15.35 mM; Sigma) or saline solution, respectively. After injection, the fish was immediately returned to the cuvette, and the experiment was started. The highest dose of bicuculline and the dose of muscimol employed were the same as those used in the basolateral nucleus of the amygdaloid complex of Wistar rats (33). Drugs were diluted in fish Ringer's solution. Drugs or saline were microinjected centrally through a segment of a Mizzy needle (OD 0.3 mm) con-

nected to a 2-µL Hamilton microsyringe by PE10 polyethylene tubing. The injection needle was provided with a cursor made of a piece of polyethylene, placed with the aid of a caliper (Mitutoyo Industrial Ltd., Itatiba, SP, Brazil) 2.4 ± 0.24 mm above the end of the needle, and lowered through the guide cannula to reach the TSd. Microinjection of a constant volume of 0.1 µL took approximately 10 s. This microinjection of 0.1 µl of solution spread to a diameter of 240 µm (34) and since the TSd of *G. carapo* is 1.7 mm wide, 5 mm in its rostrocaudal extent and 1.4 mm deep, we considered that there was no diffusion away from this structure.

EOD acquisition and alerting stimulus

The EOD signal was recorded with a pair of electrodes coupled to the internal wall of the experimental cuvette, perpendicular to the rostrocaudal axis of the fish and monitored by a Tektronix model 5111 storage oscilloscope (Beaverton, OR, USA). For EOD acquisition a 10/26-type 10-bit digital analogue data acquisition board (Lynx Ltd., São Paulo, SP, Brazil) was used, installed in a PC-AT 3.86 DX 40 computer and having the capacity to record signals of up to 10 kHz. The EOD frequencies, reported as Hz, were recorded over a period of 3 s 1.5 min following microinjection of the drugs and subsequently every 5 min for 30 min.

The alerting stimulus used to evoke a novelty response was released into the water by a Nikon-Kohden (Long Branch, NJ, USA). voltage/current generator model SEN 1101 through electrodes coupled to the internal wall of the experimental cuvette. In view of the characteristics of the EOD in this species, the stimulus consisted of square wave pulses of 1-ms duration, 70-Hz frequency and a 100-µA current, yielding a 150 mV/cm field in the median region of the electrodes (35). The alerting stimulus was applied for 2 s before and 5 min after the microinjection of the drugs.

Behavioral responses

Skeletomotor activity was recorded using a written shorthand description. This method was found to be most efficient for simultaneously recording in an easy manner a wide variety of patterns of the skeletomotor repertoire. The skeletomotor patterns observed in the present study following intracerebral drug microinjections were classified according to the earlier study on *G. carapo* by Black-Cleworth (1). Patterns not described in the previous paper were also noted and included. The following responses were recognized:

Rolling. The fish rolls over on its side.

Tail curling. Pronounced lateral undulations of the body with little or no forward movement.

Retreat. Indicated by forward or backward rapid movements.

Postural change. The fish assumes dorsal or lateral decubitus and displays one of the above-described skeletomotor patterns.

Evaluation of agonistic skeletomotor patterns

The appearance and intensity of the skeletomotor patterns, recognized as agonistic responses (1), were evaluated. Each fish was scored according to an intensity scale (0-3) for each behavioral unit, where 0 = absence of the agonistic skeletomotor patterns; 1 = occasional occurrence; 2 = constant presence during the experiment, and 3 = constant presence with high intensity. The agonistic scores described were those observed for a period of 2 min, 4 min after the microinjections.

Histological procedures

At the end of the experiments, the animals were deeply anesthetized by immersion in an MS222 (0.2 g/L) solution, and a metallic microelectrode connected to the anode of a source of continuous current was

inserted through the guide cannula. This microelectrode was equipped with a cursor that permitted it to be inserted to the same depth as the injection needle. A reference electrode was placed on the animal's tail and a continuous current of 1 mA was passed for 5 s. The resulting electrolytic lesion permitted subsequent localization of the microinjection sites. After removal of the acrylic resin, the head of the fish was detached and immersed in 10% formalin for 4 days, after which the brain was removed from the skull and embedded in paraffin. The midbrain was cut serially with a microtome into 15-µm frontal sections and stained by the Nissl method for subsequent analysis under the light microscope. The location of the microinjection sites was recognized and plotted on a series of drawings of frontal sections of the mesencephalon.

Data and statistical analysis

Electric organ discharge frequencies (Hz) prior to and following microinjection of drugs or saline were counted. For the construction of the curves showing the effects of the drugs, individual EOD frequencies were calculated and plotted as a function of the experimental time. Mean value (± SEM) curves for each dose of bicuculline were constructed and compared with the curve for saline. Graphic presentations were performed using Origin 6 and Corel Draw 9.

Specific parametric statistical tests were used to assess the significance of each experimental treatment. The level of significance was set at $P < 0.05$ and the analyses were performed using the Sigma Stat software (version 2.03).

Experimental groups

Group 1 animals received a microinjection of bicuculline (0.98 mM, N = 6; 0.49 mM, N = 6; 0.245 mM, N = 6; 0.015 mM, N = 6) into the TSd; group 2 animals received a microinjection of muscimol (15.35 mM, N = 6) into the TSd, and group 3 animals received a microinjection of saline (N = 5) into the TSd. The animals were used only once.

Results

EOD frequency

The effects of different doses of the $GABA_A$ antagonist bicuculline, of muscimol and of saline injected into the deep layers of the TSd on individual EOD frequency are illustrated in Figure 1. At all doses except 0.015 mM, bicuculline induced interruptions of the EOD intermingled with resumed total or partial activity. The latency values as well as the duration of the interruptions varied with the dose of the drug. Episodes of EOD interruptions started 10-60 s after the microinjection of 0.98 mM bicuculline, showed maximum values at 5 min, a mean duration of 11.2 ± 1.5 min and in some cases persisted until the end of the experiments (30 min). In animals microinjected with 0.49 mM bicuculline, EOD interruptions had a mean duration of 7.1 ± 1.7 min, were established 1-2 min following microinjection, and persisted for 10 min, after which the frequencies gradually returned to control values. In the animals treated with 0.245 mM bicuculline, EOD interruptions appeared 1.5 min after the microinjection, with an approximate duration of 2 s, and lasted up to 5 min. After the dose of 0.015 mM bicuculline, no alteration in EOD frequency was observed. No effect was observed on EOD frequency after microinjection of muscimol (15.35 mM) or saline (Figure 1E and F). When the four curves describing the mean effects of the different doses tested were compared with the curve for saline (Figure 2), the frequency values for the two highest doses of bicuculline (0.98 and 0.49 mM) after 1.5 min (0 Hz for both), 5 min (4.3 ± 2.7 and 3.8 ± 2.0 Hz, respectively) and 10 min

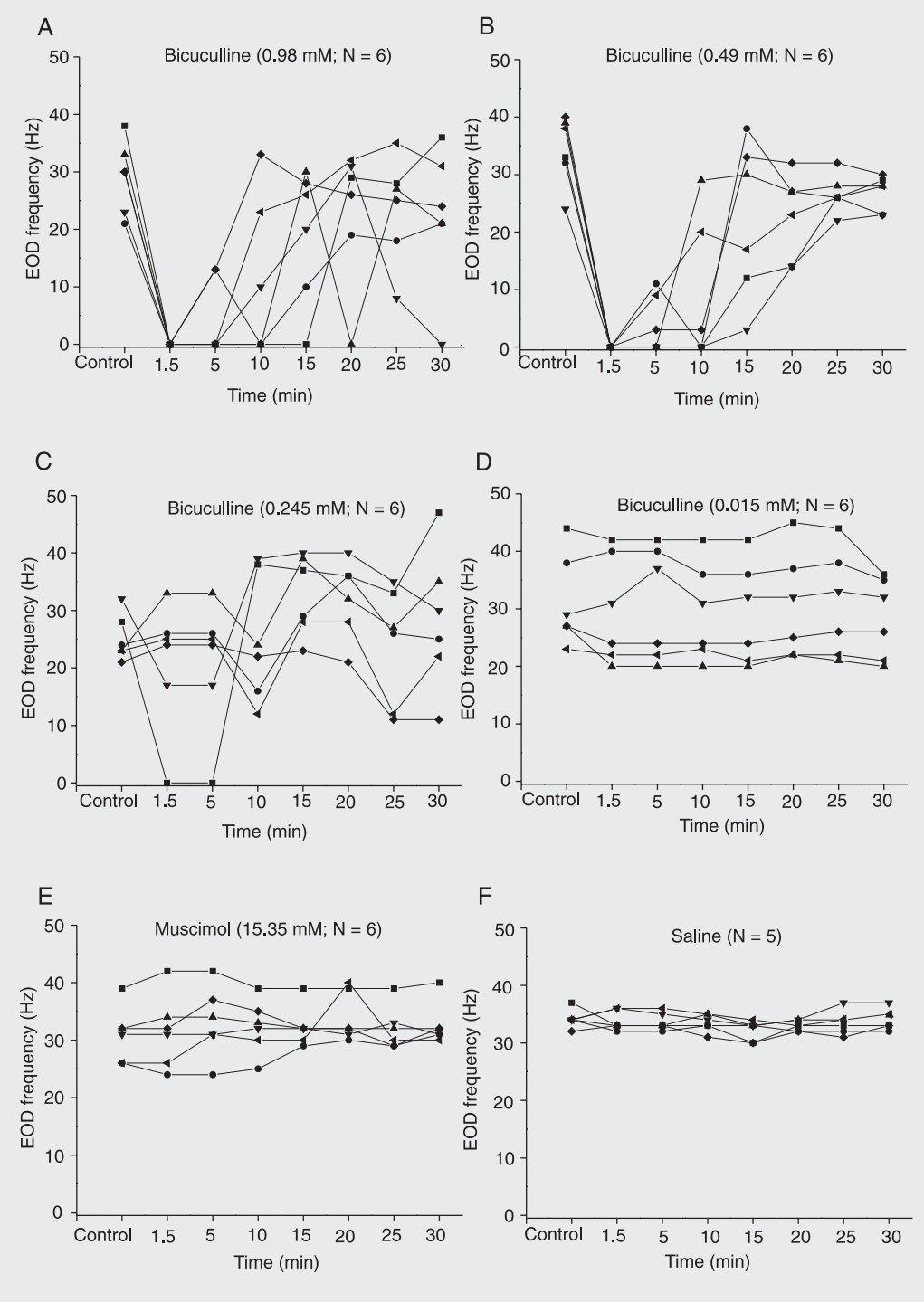

Figure 1. Time course of individual values of the electric organ discharge (EOD) frequencies (Hz) of awake *Gymnotus carapo* before (control) and after microinjections of different doses of bicuculline (A to D), muscimol (E) or saline (F) into the torus semicircularis dorsalis. With the higher doses of bicuculline the interruptions of the EOD persisted longer and were occasionally intermingled with partial or total resumption of activity. Muscimol and saline were ineffective.

(11 ± 5.7 and 8.7 ± 5.2 Hz, respectively) were significantly different from the value for saline (31.8 ± 1.51, 31.8 ± 1.7 and 32.1 ± 1.4 Hz, respectively), at the same time intervals ($P < 0.05$, one-way ANOVA on ranks followed by the Dunnett multiple comparisons test).

Novelty response

Animals of all groups responded with an increase in EOD frequency (novelty response) to the artificial electric stimulus presented prior to the microinjections of saline (Figure 3A) or drug. During the EOD interruptions elicited by the microinjection of the highest dose of bicuculline (0.98 mM), the NR did not occur (Figure 3B). After the other doses of 0.49 mM (Figure 3C) and 0.245 mM, the EOD interruptions were overcome by the NR.

Skeletomotor response

Microinjections of bicuculline into the TSd induced patterns of motor activity that resembled the components of agonistic behavior (Figure 4). The motor responses were established at the same time as the EOD interruptions. The highest dose of bicuculline (0.98 mM) elicited a strong behavioral activation expressed by a combination of different patterns: rolling, postural change, forward or backward (retreat) movements, and lateral undulations of the body (tail curling). The agonistic scores of all skeletomotor patterns were different from the values obtained with saline ($P < 0.05$, unpaired *t*-test). After lower doses (0.49 and 0.245 mM) a reduction of the number of patterns expressed by the animals, as well as of the intensity of the movements, was observed. With the dose of 0.49 mM, except for postural change, the agonistic scores of the skeletomotor patterns were different from saline ($P < 0.05$). Rolling was less expressed with the dose of 0.245 mM, but the agonistic scores for the other patterns differed from saline ($P < 0.05$). No detectable effect was seen following 0.015 mM bicuculline, muscimol or saline microinjection. In view of the relatively small dimensions of the experimental cuvette, the 0.98 mM dose of bicuculline was also microinjected into animals subsequently placed in a 44 x 22 x 26-cm aquarium. The results

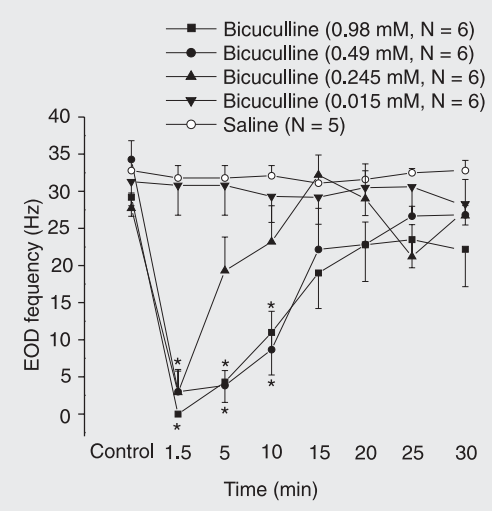

Figure 2. Time course of the frequencies (mean ± SEM) of the electric organ discharge (EOD) prior to (control) and following microinjections of bicuculline (0.98, 0.49, 0.245, and 0.015 mM) or saline into the torus semicircularis dorsalis of *Gymnotus carapo*. *P < 0.05 compared to the saline group (one-way analysis of variance followed by the Dunnett multiple comparisons test).

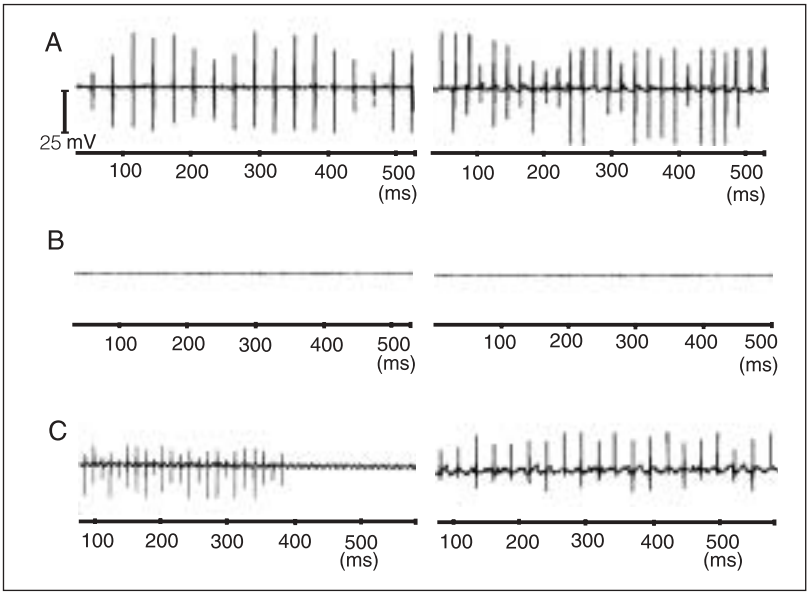

Figure 3. Examples of electric organ discharge in *Gymnotus carapo* before (left side) and during (right side) application of an alerting stimulus (square wave pulses of 5-ms duration, 70-Hz repetition frequency and a 100-µA current applied to the water of the experimental cuvette) in animals submitted to microinjections of saline (A), 0.98 mM bicuculline (B), and 0.49 mM bicuculline (C) into the torus semicircularis dorsalis. Vertical bar = 25 mV for A, B and C.

corroborated the observations obtained in the cuvette.

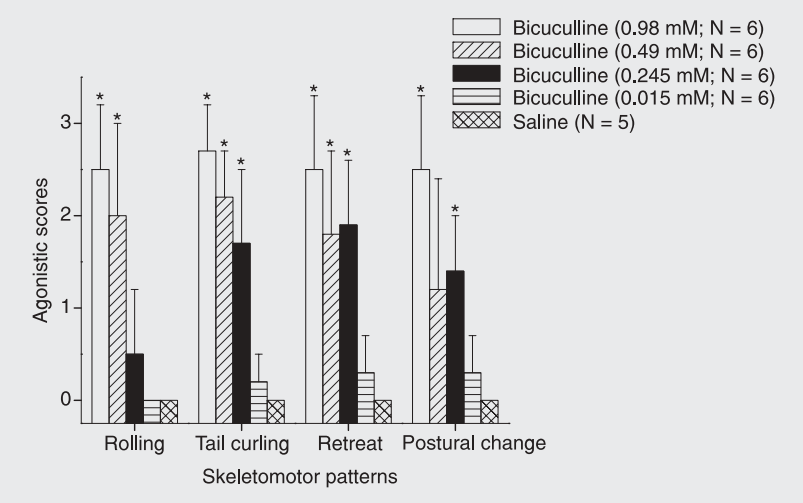

Figure 4. Intensity scores of skeletomotor patterns evaluated over a period of 2, 4 min after microinjection of different concentrations of bicuculline or saline into the torus semicircularis dorsalis of *Gymnotus carapo*. *P < 0.05 compared to the saline group (unpaired *t*-test)

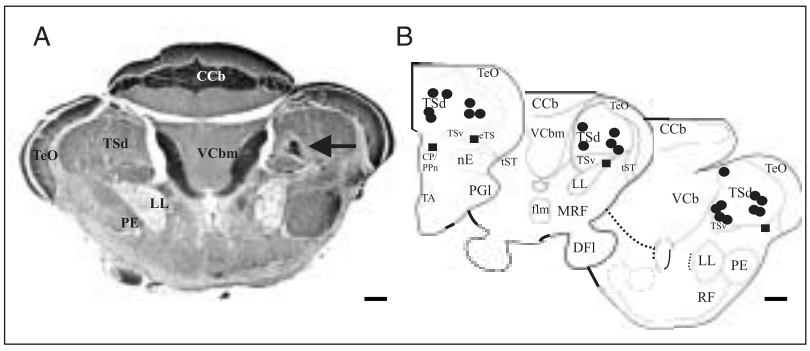

Figure 5. *A*, Photomicrograph of a frontal section of the mesencephalon of a representative fish showing the electrolytic lesion placed at the injection site into the torus semicircularis dorsalis (TSd) (arrow). Scale bar = 500 µm. *B*, Schematic drawings of frontal sections of the mesencephalon of *Gymnotus carapo*, showing the TSd and associated brain structures. Circles indicate the location of sites inside the TSd in which microinjections of bicuculline elicited motor and electromotor responses. The number of points in the figure is less than the total number of fish used because of several overlaps. Squares indicate the location of sites outside the TSd that were unresponsive to the drug. Scale bar for drawing = 650 µm. CCb = corpus cerebelli; CP/PPn = central-posterior/prepacemaker complex; DFl = nucleus diffusus lateralis of inferior lobe; eTS = torus semicircularis efferents; flm = fasciculus longitudinalis medialis; LL = lateral lemniscus; nE = nucleus electrosensorius; MRF = mesencephalic reticular formation; PE = periglomerular nucleus; PGl = preglomerular nucleus, lateral subdivision; RF = reticular formation (rhombencephalon); TA = nucleus tuberis anterior; TeO = optic tectum; TSd = torus semicircularis dorsalis; tST = subtectal tract; TSv = torus semicircularis ventralis; VCb = valvula cerebelli; VCbm = valvula cerebelli pars medialis.

Histology

Only fish showing histologically confirmed placement of the microinjections into the TSd were employed for data analysis. Figure 5A is a photomicrograph of a frontal section of the mesencephalon of *G. carapo* showing a typical microinjection site in the most medial aspect of the deep layers of the TSd. Figure 5B is a series of drawings of frontal sections of the mesencephalon of several fish showing the location of sites from which EOD interruptions were elicited by bicuculline as well as sites outside the torus (N = 4) which were unresponsive to the drug.

Discussion

Our data demonstrate for the first time that microinjection of the GABA-ergic antagonist bicuculline into the TSd of the awake unrestrained weakly electric fish *G. carapo* induces EOD alterations (variable duration of beat interruptions) that are dose-dependent, together with a strong skeletomotor response, by interfering with tonic toral GABA-ergic inhibition. The highest dose of bicuculline (0.98 mM) not only caused the most pronounced EOD effect (long-lasting complete interruption), but also abolished the NR when the evoking stimulus was presented during the EOD interruption.

Previous studies have shown that the decrease in EOD frequency involves different brain regions such as the TSd, ventral portion of the nE, SPPn and PM (10,11). In *Eigenmannia*, the pathway that controls EOD deceleration starts with neurons in the TSd that are selective for positive frequency differences, subsequently involves the ventral nE, which in turn lowers the tonic activity of the SPPn via its GABA-ergic input. The diminished activity of the SPPn finally reduces the glutamatergic NMDA-mediated input to the PM (10). Iontophoretic application of L-glutamate to the ventral nE or of

GABA into the SPPn results in a prompt reduction of the fish's EOD frequency (11). In *Hypopomus pinnicaudatus* the activation of the ventral nE provides glutamatergic activation of the SPPn, which in turn causes a long depolarization of relay cells due to selective activation of NMDA receptors (6). The sustained depolarized relay cells fail to transmit the pacemaker cell rhythm to the motoneurons that drive the electric organ, interrupting the regular EOD, while pacemaker cells keep firing regularly. So, when the relay cells repolarize, the EOD rhythm restarts at a frequency very close to that observed prior to the interruption (6).

The same pathway could be involved in the interruptions of the EOD described in this study in *G. carapo* since in this species tract tracing experiments showed the existence of a projection from the TSd to the nE (Duarte TT, Hoffmann A, Pereira ASF and Correa SAL, unpublished results). $GABA_A$ receptors of the TSd may play a role in modulating this pathway by controlling the intensity of the tonic inhibition of the projecting neurons. Since the inhibitory effect is a tonic one, muscimol microinjections had no additional effect.

We also observed in the present study that the artificial electric stimulus did not trigger the NR during the EOD interruption elicited by the microinjection of the highest dose of bicuculline into the TSd. Other doses of bicuculline, muscimol or saline did not cause this effect. In *Hypopomus*, the increase in EOD frequency that occurs in response to novelties can be elicited by excitatory inputs from a subnucleus of the CP/PPn complex, the CP/PPn-G, onto the NMDA receptors of the pacemaker cells of the PM (2). In contrast, the EOD interruption is elicited through excitatory inputs from the SPPn to the somata of the relay cells of the PM (2). The liberated glutamate binding to NMDA receptors triggers a sustained depolarization in relay cells and, while depolarized, the electric organ no longer fires coherently, so that the regular EOD is interrupted (6). We postulate that in our experiments, after microinjection of the highest dose of bicuculline into the TSd, the relay cells submitted to a sustained depolarization fail to transmit the rise of the pacemaker cell rhythm elicited by the alerting stimulus. With the lower doses of bicuculline this sustained depolarization may be overcome by inputs coming from a transient activation of the CP/PPn-G caused by the novelty stimulus.

The role of the electroreception system in the agonistic interaction of *G. carapo* is considered to be of great importance and the discharge frequencies of the animals are positively correlated with social status in dominance hierarchies (36). Two main signals are observed in this species during agonistic encounters: short-lived frequency increases or bursts that are used as threatening signals by dominant individuals and complete interruption of discharge which may be of variable duration and indicates submission. According to Black-Cleworth (1), the EOD interruptions result from a conflict between fleeing tendencies induced by an enemy producing electrical pulses and other tendencies, which vary in different situations, rendering the intensity of the fleeing responses proportional to the duration of the EOD interruption. Furthermore, the interruption of a discharge is a natural choice for an appeasement display.

In our experiments, microinjections of different doses of bicuculline into the deep layers of the TSd elicited different types of skeletomotor patterns. In nature, when two *G. carapo* individuals interact, their behavior is markedly affected by the dominance relationship between them and during their agonistic encounters the fishes adopt characteristic agonistic orientations with respect to each other (1). Among the responses evoked by bicuculline microinjection, some belong to the defense repertoire of the species. Tail curling would bring the fish's electric organ very close to the receptors of the

opponent, maximizing the effect of the dipole moment. Rolling may be a method of cushioning the shock of a butt from the side, causing the fish to rotate away from the attack. A fish in the rolled posture also presents less surface area to the opponent, so that butts may be less likely to occur (1). Responses like approach, parallel orientation of two fish, and nipping and thrusting, that are part of the aggressive behavior (36), were not observed.

Since $GABA_A$ receptor blockade of the TSd in *Gymnotus* resulted in interruptions of the EOD, together with strong skeletomotor activation expressed by a combination of different patterns of defensive behavior, we suggest that the GABA-ergic mechanisms of the TSd appear to inhibit the neural substrate of the defense reaction at this midbrain level of the fish. In mammals, it has been demonstrated that sensory collicular input from a given spatial location is not used only for orientation but also to generate a motor output critical for survival. In toads of the genus *Bufo*, chemical or electrical stimulation of the torus semicircularis induces species-specific defensive responses (28,37). We therefore postulate that collicular control of defense systems is a phylogenetically old, retained mechanism since it is common to representatives of all vertebrate groups studied. We conclude that the electrosensory information arriving at the TSd is converted into electromotor and skeletomotor commands leading to orientation and defense responses.

References

1. Black-Cleworth P. The role of electrical discharges in the nonreproductive social behavior of *Gymnotus carapo* (Gymnotidae, Pisces). *Anim Behav Monogr* 1970; 31: 1-17.
2. Kawasaki M, Heiligenberg W. Distinct mechanisms of modulation in a neuronal oscillator generate different social signals in the electric fish *Hypopomus*. *J Comp Physiol [A]* 1989; 165: 731-741.
3. Keller CH, Kawasaki M, Heiligenberg W. The control of pacemaker modulations for social communication in the weakly electric fish *Sternopygus*. *J Comp Physiol [A]* 1991; 169: 441-450.
4. Bennett MV. Electrolocation in fish. *Ann N Y Acad Sci* 1971; 188: 242-269.
5. Dye JC, Meyer JH. Electroreception. In: Bullock TH, Heiligenberg W (Editors), *Electroreception*. New York: Wiley; 1986. p 71-102.
6. Spiro JE. Differential activation of glutamate receptor subtypes on a single class of cells enables a neural oscillator to produce distinct behaviors. *J Neurophysiol* 1997; 78: 835-847.
7. Smith GT, Lu Y, Zakon HH. Parvocells: a novel interneuron type in the pacemaker nucleus of a weakly electric fish. *J Comp Neurol* 2000; 423: 427-439.
8. Heiligenberg W, Finger T, Matsubara J, Carr C. Input to the medullary pacemaker nucleus in the weakly electric fish, *Eigenmannia* (Sternopygidae, Gymnotiformes). *Brain Res* 1981; 211: 418-423.
9. Kawasaki M, Heiligenberg W. Individual prepacemaker neurons can modulate the pacemaker cycle of the gymnotiform electric fish, *Eigenmannia*. *J Comp Physiol [A]* 1988; 162: 13-21.
10. Metzner W. The jamming avoidance response in *Eigenmannia* is controlled by two separate motor pathways. *J Neurosci* 1993; 13: 1862-1878.
11. Heiligenberg W, Metzner W, Wong CJ, Keller CH. Motor control of the jamming avoidance response of *Apteronotus leptorhynchus*: evolutionary changes of a behavior and its neuronal substrates. *J Comp Physiol [A]* 1996; 179: 653-674.
12. Carr CE, Maler L, Heiligenberg W, Sas E. Laminar organization of the afferent and efferent systems of the torus semicircularis of gymnotiform fish: morphological substrates for parallel processing in the electrosensory system. *J Comp Neurol* 1981; 203: 649-670.
13. Bastian J, Yuthas J. The jamming avoidance response of *Eigenmannia*: Properties of a diencephalic link between sensory processing and motor output. *J Comp Physiol [A]* 1984; 154: 895-908.
14. Keller CH. Stimulus discrimination in the diencephalon of *Eigenmannia*: the emergence and sharpening of a sensory filter. *J Comp Physiol [A]* 1988; 162: 747-757.
15. Meek JR, Nieuwenhuys R. Holosteans and teleosts. In: Nieuwenhuys R, ten Donkelaar HJ, Nicholson C (Editors), *The central nervous system of vertebrates*. Berlin, Heidelberg, New York: Springer-Verlag; 1998. p 855.
16. Heiligenberg W, Rose G. Phase and amplitude computations in the midbrain of an electric fish: intracellular studies of neurons participating in the jamming avoidance response of *Eigenmannia*. *J Neurosci* 1985; 5: 515-531.
17. Grau HJ, Bastian J. Neural correlates of novelty detection in pulse-type weakly electric fish. *J Comp Physiol [A]* 1986; 159: 191-200.
18. Lissmann HW. On the function and evolution of electric organs in fish. *J Exp Biol* 1958; 35: 156-191.
19. Corrêa SAL, Hoffmann A. Novelty response in the weakly electric fish *Gymnotus carapo*: seasonal differences and the participation of the telencephalon in its modulation. *Comp Biochem Physiol* 1998; 119: 255-262.
20. Kawasaki M, Heiligenberg W. Different classes of glutamate receptors and GABA mediate distinct modulations of a neuronal oscillator, the medullary pacemaker of a gymnotiform electric fish. *J Neurosci* 1990; 10: 3896-3904.
21. Dye JC, Heiligenberg W, Keller CH, Kawasaki M. Different classes of glutamate receptors mediate distinct behaviors in a single brain-

stem nucleus. *Proc Natl Acad Sci U S A* 1989; 86: 8993-8997.
22. Kennedy G, Heiligenberg W. Ultrastructural evidence of GABA-ergic inhibition and glutamatergic excitation in the pacemaker nucleus of the gymnotiform electric fish, *Hypopomus*. *J Comp Physiol [A]* 1994; 174: 267-280.
23. Santana UJ, Roque-da-Silva AC, Duarte TT, Correa SA. Interference with the GABAergic system in the dorsolateral telencephalon and modulation of the electric organ discharge frequency in the weakly electric fish *Gymnotus carapo*. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2001; 187: 925-933.
24. Sparks DL. Translation of sensory signals into commands for control of saccadic eye movements: role of primate superior colliculus. *Physiol Rev* 1986; 66: 118-171.
25. Dean P, Redgrave P, Westby GW. Event or emergency? Two response systems in the mammalian superior colliculus. *Trends Neurosci* 1989; 12: 137-147.
26. Brandão ML, Tomaz C, Borges PC, Coimbra NC, Bagri A. Defense reaction induced by microinjections of bicuculline into the inferior colliculus. *Physiol Behav* 1988; 44: 361-365.
27. Cardoso SH, Coimbra NC, Brandão ML. Defensive reactions evoked by activation of NMDA receptors in distinct sites of the inferior colliculus. *Behav Brain Res* 1994; 63: 17-24.
28. Hoffmann A, Brazil Romero SM, de Oliveira LM. Agonistic behavior and its cardiovascular components elicited by microinjection of L-glutamic acid into the basal midbrain of the toad *Bufo paracnemis*. *Brain Behav Evol* 1993; 41: 316-325.
29. Moreira FA, Molchanov ML, Guimarães FS. Flight reactions to nitric oxide in the inferior colliculus of rats depend on NMDA receptor activation. *Pharmacol Biochem Behav* 2003; 76: 35-41.
30. Carr CE, Maler L. A Golgi study of the cell types of the dorsal torus semicircularis of the electric fish *Eigenmannia*: functional and morphological diversity in the midbrain. *J Comp Neurol* 1985; 235: 207-240.
31. Correa SA, Correa FM, Hoffmann A. Stereotaxic atlas of the telencephalon of the weakly electric fish *Gymnotus carapo*. *J Neurosci Methods* 1998; 84: 93-100.
32. Maler L, Sas E, Johnston S, Ellis W. An atlas of the brain of the electric fish *Apteronotus leptorhynchus*. *J Chem Neuroanat* 1991; 4: 1-38.
33. Albuquerque-Araujo WIC, Brentegani MR, Hoffmann A. $GABA_A$ receptors of amigdala are involved in the modulation of dorsal immobility. *Abstr Soc Neurosci* 1996; 178.16 (Abstract).
34. Nicholson C. Diffusion from an injected volume of a substance in brain tissue with arbitrary volume fraction and tortuosity. *Brain Res* 1985; 333: 325-329.
35. Baffa O, Correa SL. Magnetic and electric characteristics of the electric fish *Gymnotus carapo*. *Biophys J* 1992; 63: 591-593.
36. Westby GWM. Further analysis of the individual discharge characteristics predicting social dominance in the electric fish *Gymnotus carapo*. *Anim Behav* 1975; 23: 249-260.
37. Ewert JP. Neural mechanisms of prey-catching and avoidance behavior in the toad (*Bufo bufo* L.). *Brain Behav Evol* 1970; 3: 36-56.