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Sex-specific role of a glutamate receptor subtype in a pacemaker nucleus controlling electric behavior

Laura Quintana [a,*], Erik Harvey-Girard [b], Carolina Lescano [a], Omar Macadar [a], Daniel Lorenzo [a]

[a] Unidad Bases Neurales de la Conducta, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
[b] Department of Cell and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

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ABSTRACT

Electric communication signals, produced by South American electric fish, vary across sexes and species and present an ideal opportunity to examine the bases of signal diversity, and in particular, the mechanisms underlying sexually dimorphic behavior. Gymnotiforms produce electric organ discharges (EOD) controlled by a hindbrain pacemaker nucleus (PN). Background studies have identified the general cellular mechanisms that underlie the production of communication signals, EOD chirps and interruptions, typically displayed in courtship and agonistic contexts. *Brachyhypopomus gauderio* emit sexually dimorphic signals, and recent studies have shown that the PN acquires the capability of generating chirps seasonally, only in breeding males, by modifying its glutamatergic system. We hypothesized that sexual dimorphism was caused by sexual differences in the roles of glutamate receptors. To test this hypothesis, we analyzed NMDA and AMPA mediated responses in PN slice preparations by field potential recordings, and quantified one AMPA subunit mRNA, in the PNs of males and females during the breeding season. In situ hybridization of GluR2B showed no sexual differences in quantities between the male and female PN. Functional responses of the PN to glutamate and AMPA, on the other hand, showed a clear cut sexual dimorphism. In breeding males, but not females, the PN responded to glutamate and AMPA with bursting activity, with a temporal pattern that resembled the pattern of EOD chirps. In this study, we have been successful in identifying cellular mechanisms of sexual dimorphic communication signals. The involvement of AMPA receptors in PN activity is part of the tightly regulated changes that account for the increase in signal diversity during breeding in this species, necessary for a successful reproduction.

1. Introduction

Animal signaling is a rich field of study, since social communication is widespread and occurs over an extensive range of modalities. Many animal communication systems are uniquely suited to identifying cellular mechanisms that account for a diverse behavioral repertoire, and they may have signature features in common. Firstly, the communication signal, be it a birdsong, a fish vocalization or an electric organ discharge, is stereotyped and quantifiable. The organs that produce the signal are muscle or muscle-derived, and therefore have similar control as motor systems. Furthermore, when the signal is sexually dimorphic, these organs are sensitive to steroid hormones (reviewed by Bass, 1989). It has been known for several decades that the nervous system has sexually dimorphic features, and some dimorphisms occur in brain regions with known links to social behavior, as reviewed in Atkins-Regan (2005). A challenging problem is to understand how anatomical and/or functional sexual dimorphism of brain structures relates causally to sex-specific behavior.

Electric communication signals, produced by South American electric fish, vary across sexes and species and are generated by a well-known control system. This presents an ideal opportunity to examine the bases of signal diversity, and in particular, the mechanisms underlying sexually dimorphic behavior. These fish have the ability to produce and perceive electric organ discharges (EOD) which they use to serve two critical functions: to sense

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; EOD, electric organ discharge; GluR2B, glutamate AMPAR subunit type 2B; NMDA, N-methyl-D-aspartate; PN, pacemaker nucleus; PPN, diencephalic pre-pacemaker; PPNc, diencephalic pre-pacemaker chirp region; PPNg, diencephalic pre-pacemaker gradual rises region; sPPN, sublemnical pre-pacemaker.

* Corresponding author. Address: Unidad Bases Neurales de la Conducta, Instituto de Investigaciones Biológicas Clemente Estable, Avda Italia 3318, 11600 Montevideo, Uruguay. Tel.: +598 24875532; fax: +598 2 487 546.

E-mail addresses: lquintana@iibce.edu.uy (L. Quintana), erikhg@uottawa.ca (E. Harvey-Girard).

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objects in the environment, as the EOD is a self-generated carrier signal (active electrolocation) and to interact socially (electrocommunication). Gymnotiformes have well characterized electric behavioral displays that play a fundamental role in, among others, reproductive and aggressive interactions (Batista et al., 2012; Hagedorn, 1988; Hagedorn and Heiligenberg, 1985; Hopkins, 1972, 1974b, 1988; Hupe and Lewis, 2008; Kawasaki and Heiligenberg, 1989; Perrone et al., 2009; Zubizarreta et al., 2012). It is one of the few vertebrate groups in which the control of specific behavioral displays can be reliably traced to a single conspicuous hindbrain nucleus (Bennett et al., 1967a; Bennett, 1971; Ellis and Szabo, 1980; Szabo and Enger, 1964). This pacemaker nucleus (PN) fires spontaneously and controls, in a one to one fashion, the timing of each EOD in all weakly electric fish. It is composed of electrotonically coupled intrinsic pacemaker cells, which contact projecting relay cells (Bennett, 1971; Dye and Meyer, 1986; Elekes and Szabo, 1981, 1985). The PN has a pivotal role in the electromotor system: it receives and integrates superior inputs into its own oscillating activity and conveys the resulting command to the system that ultimately generates the EOD. This is performed within the constraints imposed by the PŃs dual, overlapping key roles in environment exploration and social communication.

The PN can change the timing of its own discharge by the input it receives from various pre-pacemaker structures, onto its pacemaker or relay neurons, thus commanding different EOD rate modulations. This work will focus on modulations that are typically displayed in social interactions of most species of gymnotiforms. Brief rate rises, chirps (abrupt and transient EOD frequency increments with amplitude decay and waveform distortion) and EOD interruptions are the most common modulations, and the information they encode in each behavioral context is species-specific (Hagedorn, 1988; Hagedorn and Heiligenberg, 1985; Hopkins, 1972, 1974a, 1974b, 1988; Hupé and Lewis, 2008; Kawasaki and Heiligenberg, 1989; Perrone et al., 2009; Zupanc et al., 2006).

Gymnotiformes appear to have a common neural design in which the diencephalic pre-pacemaker nucleus (PPN) projects from its different subregions to certain target sites within the PN, provoking changes in its regular activity (Heiligenberg et al., 1981; Kawasaki and Heiligenberg, 1989, 1990; Kawasaki et al., 1988). Subregion PPNg projects to pacemaker cells producing gradual EOD frequency rises mediated by NMDA receptors, and subregion PPNc projects to relay cells, possibly on dendritic sites, generating chirps via AMPA receptors (Dye et al., 1989; Juranek and Metzner, 1997, 1998; Kawasaki and Heiligenberg, 1989, 1990; Kawasaki et al., 1988; Keller et al., 1991; Metzner, 1993; Spiro, 1997; Spiro et al., 1994). On the other hand, the sublemniscal pre-pacemaker (sPPN) triggers EOD interruptions, by projecting to relay neuron somas and blocking them by depolarization via NMDA receptors (Dye et al., 1989; Juranek and Metzner, 1997, 1998; Kawasaki and Heiligenberg, 1989, 1990; Kawasaki et al., 1988; Keller et al., 1991; Spiro, 1997; Spiro et al., 1994). The studies aforementioned on the cellular and circuital mechanisms underlying EOD rate modulations provide ample background for understanding how these mechanisms relate to naturally occurring behavior.

Pulse-type fish of the family Hypopomidae are, among gymnotiforms, champions in signal diversity. *Brachyhypopomus gauderio* (Giora and Malabarba, 2009) displays various social electric signals during its breeding season, in courtship and aggressive behavior, extensively studied both in the field and laboratory settings (Perrone et al., 2009; Quintana et al., 2011b; Silva et al., 2007, 2008; Zubizarreta et al., 2012). This species synchronizes spawning by "dialoguing" in a back and forth volley of three different kinds of chirps, emitted exclusively by males, and EOD interruptions, produced only by females (Perrone et al., 2009). Agonistic encounters between males include EOD interruptions and a fourth type of chirp (Perrone et al., 2009). Recent studies have yielded fundamental insight into the seasonal and sex-specificity basis of these signals, which lie in the command nucleus (Fig. 1). The PN, in spite of not showing morphological differences across sexes nor seasons (Quintana et al., 2011a), presents a remarkable seasonal plasticity which accounts for the functional sexual dimorphism underlying communication signals (Quintana et al., 2011b). The PN is able to command EOD interruptions in both breeding and nonbreeding adults, but its capability of generating chirps is exhibited in males during the breeding season. This sexual dimorphism is brought about by modifications in its glutamatergic system, evinced in a spatially restricted portion of the PN (Silva et al., 2008).

Previous studies in different species of gymnotiformes have shown that glutamate receptor subtypes mediate distinct rhythms in the PN (Dye et al., 1989; Juranek and Metzner, 1997, 1998; Kawasaki and Heiligenberg, 1989, 1990; Kawasaki et al., 1988; Keller et al., 1991; Spiro, 1997; Spiro et al., 1994). We are interested in understanding the neural underpinnings of signal diversity in the framework of seasonal plasticity in naturally occurring behavior in *B. gauderio*, a species in which temporary sexual dimorphism in communication signals has been pinpointed to changes in the glutamatergic system in a single nucleus. We hypothesized that this dimorphism is caused by sexual differences in the roles of glutamate receptors. To test this hypothesis, we analyzed NMDA and AMPA mediated responses in PN slice preparations, and quantified one AMPA subunit mRNA, in breeding males and females.

2. Materials and methods

2.1. Animals

Thirty five adult *B. gauderio* were used in this study (16 males ranging from 15 to 23 cm long and 17 females ranging from 12.5 to 18 cm long). During breeding, the morphological sexual dimorphism exhibited by this species allowed us to easily distinguish males from females. Males are larger than females and display a long broad caudal filament, whereas females have a shorter caudal filament and exhibit protruding ovaries (Hopkins, 1991; Hopkins et al., 1990).

2.2. Fish collection and housing

Fish were detected and collected using a "fish detector", an electronic audio amplifier connected to a pair of electrodes, as described elsewhere (Quintana et al., 2004; Silva et al., 2003) from

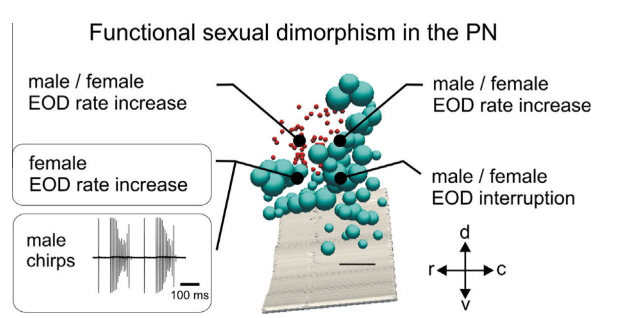

Fig. 1. Functional sexual dimorphism in the PN as evinced in an in vivo preparation. Glutamate injected in the PN provokes sexually dimorphic effects on EOD discharge, exclusively during the breeding season. Only in breeding males, an injection of glutamate in a ventral–rostral site of the PN triggers chirps. In sagittal 3D reconstruction of PN blue spheres: relay neurons, red spheres: pacemaker neurons. Anatomical directions. r: rostral, d: dorsal, c: caudal, v: ventral. Calibration bar: 100 μm. Modified from Quintana et al. (2011b).