Integrative and Comparative Biology, volume 50, number 6, pp. 1081–1090
doi:10.1093/icb/icq147

SYMPOSIUM

A Male Poecillid's Sexually Dimorphic Body Plan, Behavior, and Nervous System

Nydia L. Rivera-Rivera,* Noraida Martinez-Rivera,* Irma Torres-Vazquez,* Jose L. Serrano-Velez,* George V. Lauder† and Eduardo Rosa-Molinar[1,*]

*Biological Imaging Group, University of Puerto Rico-Rio Piedras, San Juan, Puerto Rico, USA; †Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA

From the symposium "Contemporary Approaches to the Study of the Evolution of Fish Body Plan and Fin Shape" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2010, at Seattle, Washington.

[1]E-mail: ed@hpcf.upr.edu

Synopsis Here we review the literature of a male poecillid's sexually dimorphic body plan, behavior, and nervous system, including work dating from the mid 1800s to the mid 1990s as well as work in press or in preparation for publication. Rosa-Molinar described the remodeling of the sexually dimorphic anal fin appendicular support, confirmed earlier claims about the development of the male and female secondary sex characteristics in the Western Mosquitofish, *Gambusia affinis* and provided for the first time direct embryonic evidence suggesting that remodeling of the sexually dimorphic anal fin appendicular support is biphasic. The first process begins in embryos and proceeds similarly in immature males and females; the second process occurs only in males and results in the anterior transposition of the anal fin and its appendicular support to the level of vertebra 11 [Rosa-Molinar E, Hendricks SE, Rodriguez-Sierra JF, Fritzsch B. 1994. Development of the anal fin appendicular support in the western mosquitofish, *Gambusia affinis* (Baird and Girard, 1854): a reinvestigation and reinterpretation. Acta Anat 151:20–35.] and the formation of a gonopodium used for internal fertilization. Studies using high-speed video cameras confirmed and extended Peden's and others' observations of copulatory behavior. The cameras showed that circumduction is a complex movement combining in a very fast sequence abduction, extension and pronation, S-start-type fast-start (defined as torque–thrust), and adduction movements. Recent work on the nervous system demonstrated dye-coupling between motor neurons and interneurons via gap junctions, suggesting an attractive substrate for the rapid motions involved in poecillid copulatory reflexes.

Revisiting the poecillid body plan

This review of work dating from the mid 1800s to the mid 1990s as well as work in press or in preparation promotes the idea that the radical changes in the body plan of the male Western Mosquitofish, *Gambusia affinis* (Baird and Girard 1854; Rauchenberger 1989; *Gambusia* hereafter) are reflected in, and related to, sexually dimorphic behavior and nervous system organization and connectivity. It is worth keeping in mind that some aspects of the spinal neural circuit controlling *Gambusia*'s motor reflexes may be difficult to delineate. Further, how much spinal neural circuits in *Gambusia* will teach us about neural circuits of other poecillid fishes, whether or not *Gambusia*'s neural circuit can be further elucidated in a way described in this review, and whether or not these *Gambusia* spinal neural circuits bear a fundamental resemblance to other poecillid spinal neural circuits—only time will tell.

The primary ichthyological literature and textbooks as well as the primary comparative vertebrate anatomical literature and textbooks describe the proto-typical teleost axial skeleton vertebral formulae as being composed of two vertebral regions [i.e., anterior trunk (rib containing vertebrae) and posterior caudal] body plan (Rosen and Bailey 1963; Webster and Webster 1974; Kluge 1977; Lagler et al. 1977; Wake 1979; Parenti 1981; Romer and

Advanced Access publication October 12, 2010

Parson 1986; Rosa-Molinar et al. 1994; Kardong and Zalisko 1998; Helfman et al. 2009). However, the body plan of internal-fertilizing male poeciliid fish in the family Poeciliidae, specifically males of the genus *Gambusia*, have a three-part body plan consisting of anterior trunk, posterior caudal, and a third region known as the ano-urogenital region (Rosa-Molinar et al. 1994). In 1994 Rosa-Molinar and colleagues described the remodeling of the sexually dimorphic anal fin appendicular support, confirmed earlier claims about the development of the male and female secondary sex characteristics in this species, and provided for the first time direct embryonic evidence suggesting that remodeling of the sexually dimorphic anal fin appendicular support is biphasic, involving one) anteriorization of the most anterior caudal segments and two) growth and elongation of hemal spines of vertebrae 14–16 [note that in this review the 14th and 16th hemal spines previously termed "gonapophyses" will be referred to as hemal spines] (Rosa-Molinar et al. 1994). The first process, anteriorization, involves a sequential homeotic-like transformation of the hemal spines of vertebrae 11–13 through resorption of mineralized connective tissue, thus forming parapophyses that bear pleural ribs (Rosa-Molinar et al. 1994). This process begins in embryos and proceeds similarly in immature males and females (Rosa-Molinar et al. 1994).

During this same period, the second process, occurring only in males and probably mediated by male gonadal hormones, causes the addition of mineralized connective tissue at the hemal spines of vertebrae 14–16 (Rosa-Molinar et al. 1994). This second process elongates and bends the hemal spines of vertebrae 14–16 anteriorly (Rosa-Molinar et al. 1994) and results in the anterior transposition of the anal fin and its appendicular support to the level of vertebra 11 (Rosa-Molinar et al. 1994).

It appears that the developmental programs of female and male *Gambusia* lead to a third region of six vertebrae that are markedly different from any vertebrae anterior to 11 (anterior trunk region) or posterior to 16 (posterior caudal region) (Rosa-Molinar et al. 1994). The anterior transposition of the anal fin and its axial and appendicular support in *Gambusia* represents a significant reorganization of the teleost axial formulae (Rosa-Molinar et al. 1994). Although data led to the proposition that the axial formulae of *Gambusia* is differentiated into three regions (i.e., anterior trunk, ano-urogenital, and posterior caudal) (Rosa-Molinar et al. 1994), it did not explain how, provide the mechanism for, or determine the critical size required for the vertebral column of *Gambusia* to differentiate into three regions, issues of some speculation since 1926 (Rosa-Molinar et al. 1994). Additional work by Rosa-Molinar et al. (1998) addressed these issues.

The male Mosquitofish accomplishes internal fertilization via a sexually dimorphic intromittent organ, the gonopodium, that results from the above described anterior transposition and is a modified portion of the median unpaired anal fin (Collier 1936; Rosen and Gordon 1953; Rosen and Tucker 1961; Breder and Rosen 1966; Peden 1970, 1972a, 1972b, 1973, 1975; Lagler et al. 1977; Liley 1983; Constantz 1989; Farr 1989; Rosa-Molinar et al. 1994, 1996, 1998, 2005). The anterior transposition of the anal fin and its appendicular support along the anteroposterior (AP) axis aligns the anal fin with the fish's axial skeleton center of gravity or center of mass, and these radical changes are essential for the male *Gambusia* to generate the complex motor reflexes needed to transfer spermatozeugmata (Collier 1936; Rosen and Gordon 1953; Rosen and Tucker 1961; Breder and Rosen 1966; Peden 1970, 1972a, 1972b, 1973, 1975; Lagler et al. 1977; Liley 1983; Constantz 1984, 1989; Farr 1989; Rosa-Molinar et al. 1994, 1996, 1998, 2005).

Rethinking poecillid copulatory behavior

Research on *Gambusia's* motor reflexes has been hampered by the speed and frequency of the male's movements as well as by the limitations of photographic technology, including inadequate speed, inadequate depth-of-field and field-of-view, and poor or inappropriate lenses. Nevertheless, and remarkably, given the available technology, Peden (1970, 1972a, 1972b, 1975) provided one of the most detailed analyses of the courtship behavior of male and female Eastern Mosquitofish, *Gambusia holbrooki* (Girard 1859). Peden (1970, 1972a, 1972b, 1975) reported that male *G. holbrooki* approach females from behind and slightly below them and engage in "swing and thrust" behavior; the male swings the gonopodium forward, then thrusts it as he moves upwards toward the urogenital sinus of the female. Peden's observations and those of others led to the conclusion that the pectoral fin supports the gonopodium during copulation; explanations of the placement of the gonopodium on the pectoral fin varied somewhat (Hubbs and Reynolds 1957; Warburton et al. 1957; Rosen and Tucker 1961; Peden 1970, 1972a, 1972b, 1973, 1975; Rosa-Molinar et al. 1996).

Three synchronized high-speed video cameras (1024 by 1024 pixel resolution) operated at

1000•frames•s–1 (1/1000•s shutter speed) provided clear, crisp, ventral, lateral, and frontal views of the gonopodium during circumduction (Fig. 1 and Table 1 for description of the movements) and during attempted copulations. In this review we use "circumduction of the gonopodium" versus "swing and thrust" to describe in an anatomically correct manner the movement of the gonopodium towards the female's urogenital sinus (see Table 1 for description of the movements). The cameras clearly confirmed that male *Gambusia* circumduct the gonopodium without ever displaying precopulatory behaviors. The camera images made it possible to calculate the speed of the circumduction as well as that of the torque–thrust motion and to determine that circumduction occurs in ~1300 ms; the extremely rapid male torque–thrust motion takes 20–50 ms. The short duration of the torque–thrust motion of the circumduction of the gonopodium is one of the most rapid behaviors known in fishes, and it is similar in time-course to the C-type fast-start response.

As Peden observed, males approach females from behind and below them. High-speed images show that when males are directly below females, they circumduct the gonopodium. The images show that circumduction in *G. affinis* (and in *G. holbrooki*) is a complex movement in which abduction, extension and pronation, S-start-type fast-start, and adduction movements are combined in a very rapid sequence (see Fig. 1 and Table 1 for description of the movements). After extension and pronation of the gonopodium during circumduction, males' bodies bend in an S-shape (the S-start-like portion of the circumduction of the gonopodium takes ~20 ms) that is similar to the rapid-acceleration S-start type of fast-starts, used by teleost fish during predator–prey interactions (Domenici and Blake 1997; Hale 2002;

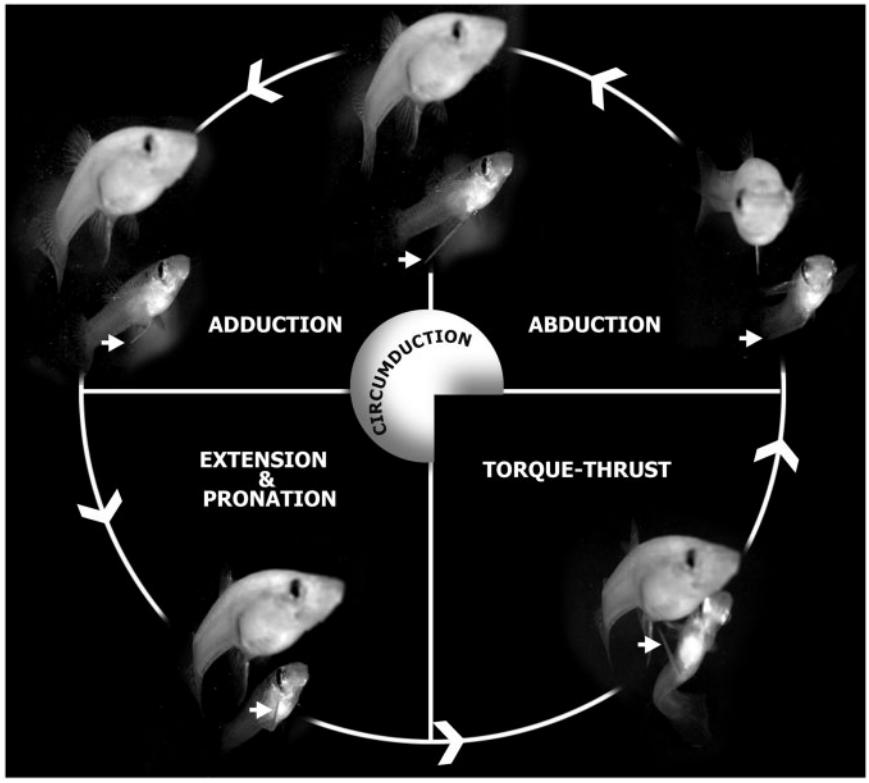

Fig. 1 Five lateral-view frames from high-speed (1000 Hz) video of a male *G. affinis* showing circumduction of the gonopodium (for details see "Filming"). With the gonopodium abducted, the male approaches the female from behind and directly underneath her where he adducts the gonopodium. Just prior to circumducting, the gonopodium is extended and pronated to a point that is nearly parallel with the body. The male *G. affinis* bends his body into an S-shape-type fast-start behavior during the torque–thrust motion of the circumduction of the gonopodium to transfer spermatozeugmata. The sequence ends with the gonopodium adducted. Filming: *Gambusia* were placed in a glass container on a stand with a three Photron APX FastCam camera (1024 by 1024 pixel resolution, Photron, Inc., San Diego, CA, USA) mounted to provide dorsal, ventral, and lateral views. The stand did not have a solid plate for a dish base. *Gambusia* were illuminated with fiber optic lights from both above and below the container and were allowed to swim freely in water ~2 cm deep at room temperature (24.5°C). We analyzed the speed of the circumduction of the gonopodium. All sequences were filmed at 1000·frames·s–1.

Table 1 Definition of Movements

General Movements:		
Adjusting an angle between two parts	Extension: a straightening movement that increases the angle between body parts	
Adjusting a structure's or structures' relationship to the midline of the body	Abduction: A motion that pulls a structure or part away from the midline of the body (or, in the case of anal fin, spreading the fin rays apart)	Adduction: a motion that pulls a structure or part towards the midline of the body, or towards the midline of the anal fin. Bringing the anal fin rays together is an example of adduction
Special Movements:		
Circumduction: the circular (or, more precisely, conical) movement of a body part, such as that of a ball-and-socket joint. It consists of a combination of extension, adduction, and abduction		
Pronation: a rotational movement		

Hale et al. 2002; Fig. 1). Finally, contrary to previous reports of courtship behavior (Hubbs and Reynolds 1957; Warburton et al. 1957; Rosen and Tucker 1961; Peden 1970, 1972a, 1972b, 1973, 1975; Rosa-Molinar et al. 1996), analyses of high-speed camera video did not confirm that the sexually dimorphic pectoral fin supports the gonopodium.

During circumduction of the gonopodium, *G. holbrooki* as well as *G. affinis* males pronate the gonopodium prior to making contact with the female's urogenital sinus. This pronation results in the gonopodium's achieving its functional position (erect), with the dorsal groove now facing upwards. Note (Figs. 1 and 2) that the dorsal groove runs from the base of the gonopodium, ending rostrally at the base of the hooks at the distal end of the gonopodium and that the groove is permanent, not temporary (Collier 1936; Peden 1970, 1972a, 1972b, 1975). How spermatozeugmata travel down the dorsal groove is unknown. Similarly, the function of the distal tips in facilitating transfer of spermatozeugmata has not been elucidated.

Reevaluating gap junctions and considering a spinal neural circuit linked to fast copulatory behavior

The development of neural circuits requires the establishment of millions of synaptic connections, and numerous studies have focused on the development of chemical synapses (Peinado et al. 1993; Bennett 1997, 2000; Condorelli et al. 1998; Cohen-Cory 2002; Pereda et al. 2003; Scheiffele 2003; Connors and Long 2004; Szabo et al. 2004; Waites et al. 2005; Marin-Burgin et al. 2005, 2006, 2008; Szabo and Zoran 2007). Fewer studies have focused on the formation or roles of electrical synapses, with the exception of studies of their role in the retina and other systems in which the role of electrical coupling between cells has long been accepted (O'Brien et al. 1996, 2004; Söhl 1998). Recently, however, new interest has been shown in the sequential development of electrical (hereafter we use the term "gap junction") and chemical synapses as well as in the role that gap junctions might play in helping to guide the formation of synaptic connections in neural circuits (Harris and Landis 1986; Peinado et al. 1993; Mills and Massey 2000; Szabo et al. 2004; Harris 2007; Szabo and Zoran 2007).

In invertebrates, a "switch" to chemical neurotransmission following an initial transient expression of gap junctional synaptic coupling has been observed (Szabo et al. 2004). In vertebrates, transient gap junctions have been proposed as being essential in the formation of neuronal networks (Gibson et al. 1999; Marin-Burgin et al. 2005; Szabo and Zoran 2007). Now, a growing body of ultrastructural and immunocytochemical evidence shows the presence and persistence of gap junctions at "mixed" (i.e., chemical and electrical combined) synapses in the adult CNS (Sotelo and Korn 1978; Vaney 1991; Kalb 1994; Rash et al. 1996, 1997, 1998, 1999, 2000, 2001, 2005; Kamasawa et al. 2006). This persistence has been taken to suggest that gap junctions provide a previously unrecognized means of communication between vertebrate CNS neurons (Rash et al. 2007). Recent advances in neuroanatomical tract tracing and imaging have made it possible to begin detailed analyses of neuronal cells and connectivity in the CNS of vertebrates.

Retrograde labeling using 3000 MW Texas Red® dextran amine (3 kDa TDA), previously presumed to be gap junction-impermeant because of its size and anionic charge, revealed extensive dye-coupling between motor neurons (MNs) and commissural primary ascending interneurons (CoPA INs; Hale et al. 2001; Fig. 3). In this review, "extensive dye-coupling" refers to the condition in which fluorescence permeates throughout the entire labeled neuron(s) but the relative fluorescence intensity is always higher in the parent neuron(s), in this case, MNs, than it is in the dye-coupled neuron(s), in this case CoPA INs (Fig. 3). Some of the dye-coupled neuronal somata are >105 μm away from the somata of the nearest labeled parent MNs. In CoPA INs, 3 kDa TDA revealed extensive dendritic

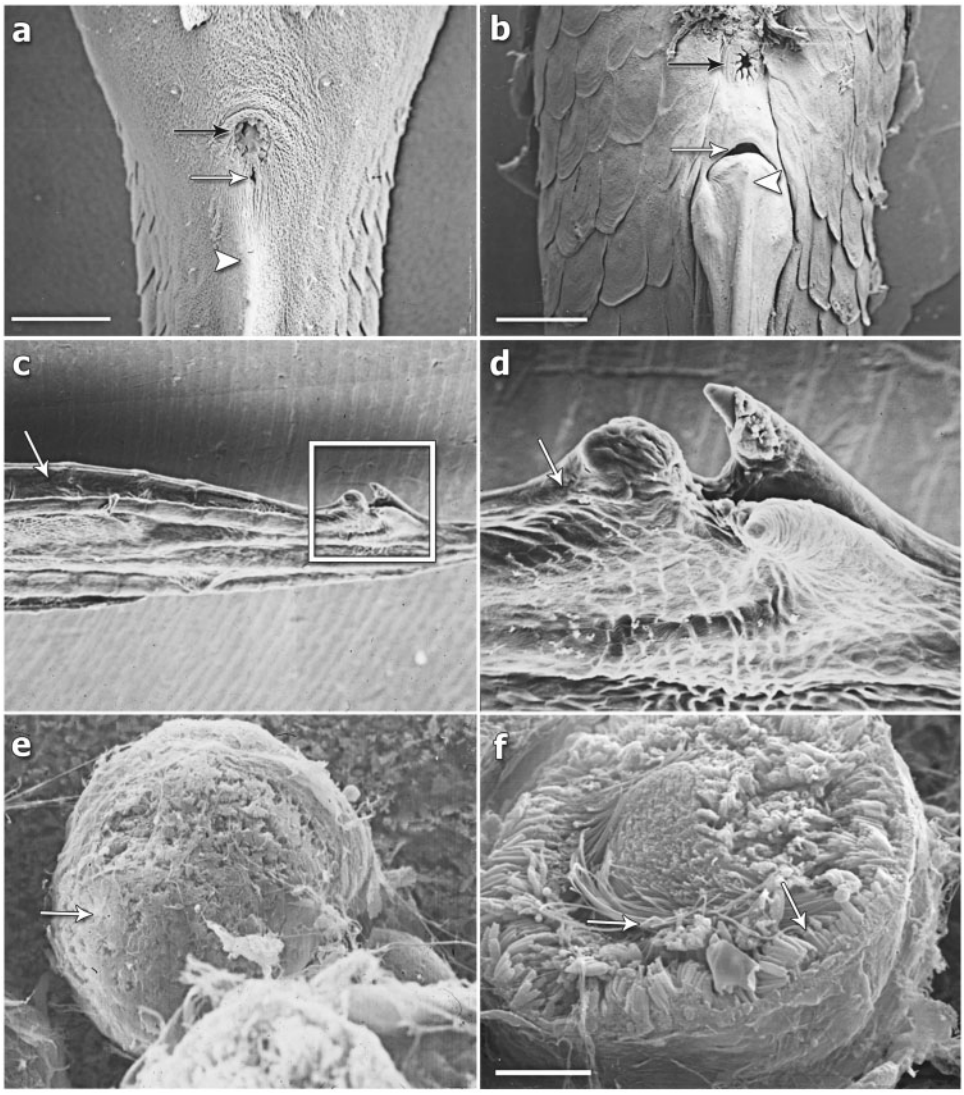

Fig. 2 Scanning electron microscopy (SEM) of the gonopodium of a male *G. affinis* [for details see "Scanning Electron Microscopy (SEM) Specimen Procedure"]. Ventral view of the ano-urogenital region of an immature (**a**) immature and (**b**) mature *G. affinis* male (scale bar = 250 μm in both a and b). Note that the mechanism of anterior transposition aligns the base of the terminally differentiated gonopodium with the ano-urogenital sinus, thus facilitating the transfer of the spermatozeugmata down the permanent dorsal groove. (**c**) Lateral view showing the permanent dorsal groove (arrows) extending rostrally to the distal tip of the gonopodium (see box). (**d**) Dorsal view showing that the permanent dorsal groove ends at the base of the hooks on the distal tip of the gonopodium (see arrow). (**e**) Dorsal view of an intact spermatozeugmata following release from the ano-urogenital pore (**e**). Note the glycocalyx-like fuzzy outer layer (arrow). (**f**) Dorsal view of the same spermatozeugmata in which we etched away the glycocalyx-like fuzzy layer to reveal sperm (arrow scale bar = 250 μm). SEM specimen procedure: Specimens were fixed in 2.5% glutaraldehyde, dehydrated through a graded ethanol series, immersed in hexamethyldisilazane (HMDS) for 5 min three times, and air dried. The samples were then mounted on an aluminium stub and sputter coated with ~150 Å thickness of gold-palladium and viewed with a JEOL JSM-6610LV SEM.

arbors and branches associated with the bipolar "T-shaped" dorsal dendrites that extend rostrally and caudally; it also revealed a long axon of the CoPA INs (Fig. 3). Because of researchers' skepticism of dye-coupling studies, the following should be noted: the dye-coupling technique used does not disturb distant regions of the spinal cord because the tracers were retrogradely introduced from a site ~5 mm away, representing ca. a quarter of the fish's body length. Likewise, absence of tracer in the extracellular space around commissural primary ascending interneurons disallows extracellular trans-ynaptic transport and large-scale endocytosis into INs. Artifactual labeling of commissural primary ascending interneurons by dye diffusion was thus unlikely.

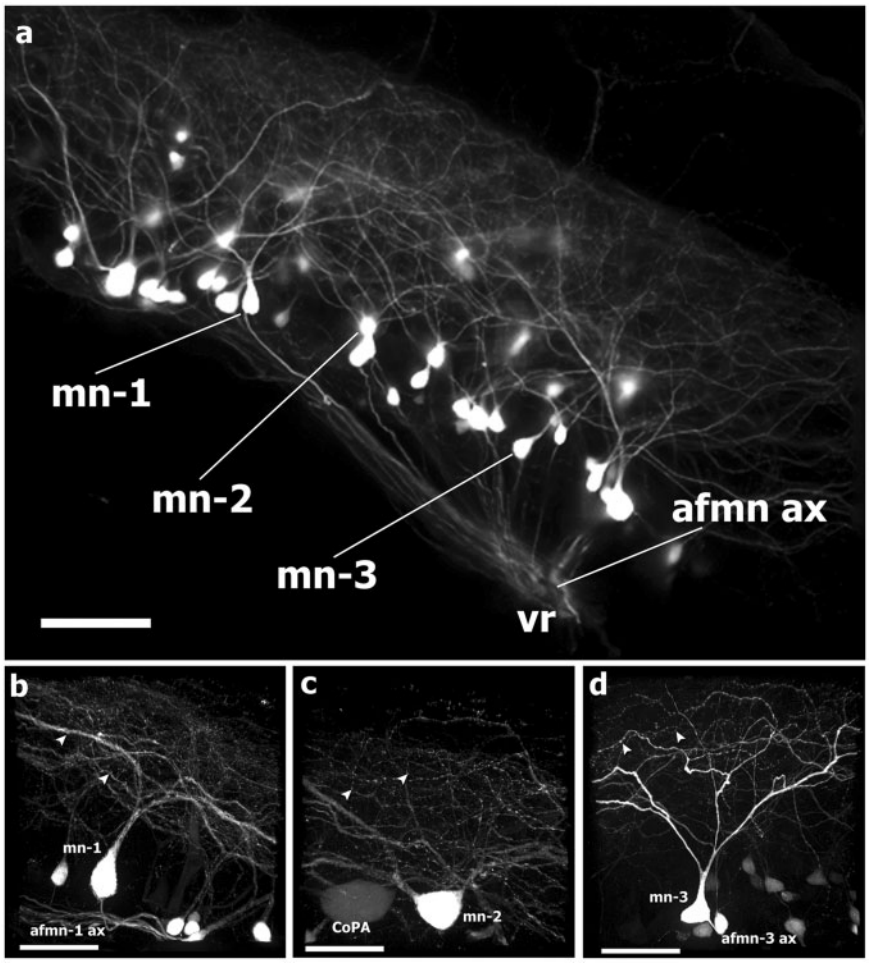

Fig. 3 (**a**) Introduction of 3000 MW Texas Red® dextran amine [3 kDA TDA] into the superficial and deep muscles of the female *G. affinis* anal fin and male *G. affinis* gonopodium results in Golgi-like retrograde labeling of mn's (mn-1, mn-2, and m-3) via their axons (afmn ax) associated with the 14th ventral root (vr). (**b**) mn-1′s (average number labeled was 16 for three ventral roots) have a unipolar primary dendritic branch from which there are extensive dendritic branches and dendritic spines (arrows) and a ventrally projecting axon (afmn-1 ax). (**c**) mn-2′s (average number labeled was 32 for three ventral roots) have bipolar primary dendritic branches from which there are extensive dendritic branches and dendritic spines (see arrows; for details see "Labeling and Imaging" below). 3 kDA TDA also labeled interneurons of the commissural primary ascending (CoPA) class. (**d**) mn-3's (average number labeled was 12 for three ventral roots) have bipolar primary dendritic branches from which there are extensive dendritic branches and dendritic spines (see arrow) and a laterally projecting axon. Scale bar: 50 um. Retrograde labeling and imaging: *Gambusia* were anesthetized by immersion with benzocaine (1:2000). Filter paper fibers saturated with fluorescent and/or non-fluorescent gap junction-permeant and gap junction-impermeant tracers were surgically implanted directly into pinched nerves innervating the deep muscles ca. 2 mm from the base of the adult male gonopodium and 2 mm from the base of the adult female anal fin of *Gambusia*; *Gambusia* were revived and the tracer was allowed to transport 8.0 h. For fixation, *Gambusia* were euthanized by immersion in benzocaine (1:4000) and intracardially perfused with teleost buffer pH 7.4 followed with 4.0% formaldehyde (PFA) in teleost buffer pH 7.4. The spinal cord associated with vertebral segments 7-17 was removed, dissected free and post-fixed overnight with 4% PFA in teleost buffer pH 7.4. Spinal cords were covered with mounting medium and cover-slipped. Spinal cord whole-mount preparations were viewed and digitally photographed using a Nikon CFI Super Fluor 20× objective (N.A. 0.50; W.D. 2.10) and a Nikon CFI Plan Fluor 60× 0.11–0.23 correction-collar spring-load objective (N.A.0.85; W.D. 0.30) in a Nikon Eclipse 800 epifluorescence microscope. High-resolution images were taken using a Qimaging Retiga Exi 12-bit CCD camera with a HRF50L1 High Resolution 0.5× coupler. Spinal cord whole-mount preparations were also viewed with a Nikon C1 Laser Scanning Confocal Microscope (LSCM) using a Nikon CFI Super Fluor 20× objective (N.A. 0.50; W.D. 2.10) and a Nikon CFI-PLAN APO 60× objective with correction collar and spring load (N.A. 0.85; W.D. 0.30).

Combining neural-tract tracing with confocal microscopy and freeze-fracture replica immunogold labeling (FRIL; Fujimoto 1995) confirmed spinal motor neuron-to-interneuron coupling and showed the presence of mixed synapses (Fig. 4). Given that mixed synapses offer a powerful means of synchronizing fast motor behavior (Galarreta and Hestrin 1999, 2001a, 2001b; Saint-Amant and Drapeau

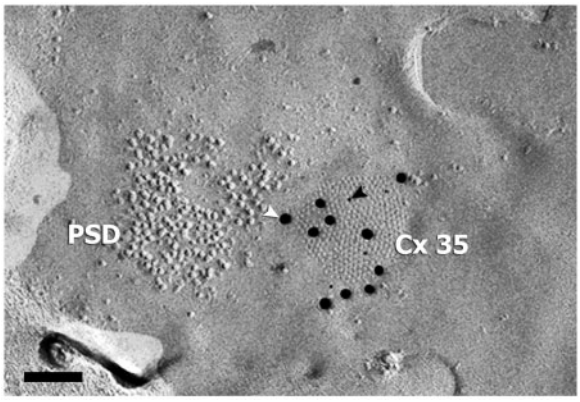

Fig. 4 Freeze-fracture replica immunogold labeling (FRIL) revealed gap junctions between dye-coupled motor neurons and commissural primary ascending interneurons in the ventral spinal cord of a male *G. affinis*. The Cx 35 gap junctions were labeled using a Cx35 antibody that recognizes the perch Cx 35. The Cx35 antibody does not cross-react with Cx 34.7, a very closely related Cx protein (O'Brien et al. 1998). The Cx35 labeling was visualized using immuno-colloidal gold [colloidal gold particles range from 6 nm (black arrows) to 18 nm (arrows)]. FRIL labeling and imaging: The freeze-fracture replica and immunogold labeling (FRIL) protocol used for this paper has been described in detail (Pereda et al. 2003; Rash and Yasumura 1999). The labeled spinal cord region associated with vertebral segments 7–17 (see the labeling section above for details) was first placed in a 3% low melting agarose, then transferred and embedded in 6% agarose, followed by refrigeration until fully gelled. Coronal sections were cut (100 μm) using a Lancer Vibrotome 3000 that maintained the tissue sections at 4°C. Spinal cord sections were infiltrated with 30% glycerol, mounted on solid aluminum stubs, and frozen by contact with a liquid nitrogen-cooled metal mirror. Frozen samples were fractured and replicated in a JOEL/RMC 9010 freeze-fracture device, then bonded to gold "index grids by using 2.0% Lexan dissolved in dichloroethane. After solvent evaporation at −35°C, the Lexan-stabilized samples were thawed; samples were viewed and digitally photographed using a 20× (0.50 N.A.; 2.10 W.D.) objective in a Zeiss Meta Laser Scanning Confocal Microscope. Replicas were washed in 2.5% SDS detergent in 0.16% Tris–HCl buffer (pH 8.9) for 29 h at 48.5°C. After the initial wash in 2.5% SDS (4.0 h), the samples were digested 1.25 h in 4.0% collagenase D in 0.15 M Sorensen's phosphate buffer (pH 7.4), followed by an additional 18-24 h in SDS solution. The replicas were rinsed in "labeling-blocking buffer" (1 mg/mL LBB), then incubated for 1–1.5 h at 22–24°C in 1:100 primary antibody solution in LLB (LBB consists of 1.5% fish gelatin plus 10% heat-inactivated goat serum in Sorensen's phosphate buffer). The replicas were labeled for 12–16 h with species-specific secondary antibodies (goat anti-rabbit) coupled to 12- and 30-nm gold beads, or to 6-nm aλnd 18-nm gold beads. All FRIL replicas were viewed with a JEOL 2000 EX-II transmission electron microscope operated at 100 kV. Stereoscopic images (8° included angle) allowed assessment of the "sidedness" of the gold beads and the level of background immunogold labeling. Cell-specific ultrastructural markers were used to confirm cell identifications. FRIL images were correlated with confocal microscopic images obtained before SDS washing.

2000, 2001; Tresch and Kiehn 2000; Bonnot et al. 2002; Drapeau et al. 2002; Kiehn and Tresch 2002), the results reported above offer an attractive substrate for the rapid motions involved in poecillid copulatory reflexes.

Conclusion

The work reviewed here is a small part in a continuum of studies of poeciliid fishes. Previous and ground-breaking studies provided fundamental knowledge of the anatomy, physiology, and reproductive behavior of poeciliids. Data reviewed here show the following: *Gambusia* have a three-part, not a two-part, body plan. Male *Gambusia* engage in extraordinarily fast copulatory behavior that appears to be linked to a remodeling of the CNS that accompanies the radical sexually dimorphic remodeling of the male body plan. Gap junctions link four different neural cell types (i.e., three distinctive and identifiable motor neuron types and one distinctive and identifiable interneuron type) in the *Gambusia* spinal cord. These neural cells work together and may have a role in a distinct neural circuit that controls male *Gambusia's* rapid copulatory behavior. Building on the foundational work that began in the mid 1800s and using modern tools and technologies will continue to increase understanding of how sex-specific behaviors are encoded by neural circuits.

Acknowledgments

The use and care of animals and the procedures described in this article were conducted under guidelines established by the National Institutes of Health and the University of Puerto Rico-Rio Piedras Institutional Animal Care and Use Committee. All fish were collected and introduced under state permits issued by the Puerto Rico Department of Natural Resources. The authors thank Jo Taylor for helpful discussions and editorial comments on this article and John Rash, Kim Davidson, and Tom Yasamura at Colorado State University for their assistance with the FRIL aspects. The authors acknowledge the contributions of all current and past members of the Biological Imaging Group who are too numerous to mention.

Funding

The research described in the paper was supported by a grant from the National Institute of Neurological Disorders and Stroke (NS30405). Additional funds were provided by Brad Weiner,

Ph.D., Dean, College of Natural Sciences, University of Puerto Rico-Rio Piedras.

References

Baird SF, Girard G. 1854. Descriptions of new species of fishes collected by Mr. J. H. Clark on the U. S. and Mexican boundary survey under Lt. Col. J. D. Graham. Proc Acad Nat Sci Phila 6:390.

Bennett MV. 1997. Gap junctions as electrical synapses. J Neurocytol 26:349–66.

Bennett MV. 2000. Electrical synapses, a personal perspective (or history). Brain Res Brain Res Rev 32:16–28.

Bonnot A, Whelan PJ, Mentis GZ, O'Donovan MJ. 2002. Spatiotemporal pattern of motoneuron activation in the rostral lumbar and the sacral segments during locomotor-like activity in the neonatal mouse spinal cord. J Neurosci 22:RC203.

Breder CM Jr, Rosen DE. 1966. Modes of Reproduction in Fishes. Garden City: Natural History Press. p. 941.

Cohen-Cory S. 2002. The developing synapse: construction and modulation of synaptic structures and circuits. Science 298:770–6.

Collier A. 1936. The mechanism of internal fertilization in *Gambusia*. Copeia 1936:45.

Condorelli DF, Parenti R, Spinella F, Trovato-Salinaro A, Belluardo N, Cardile V, Cicirata F. 1998. Cloning of a new gap junction gene (Cx36) highly expressed in mammalian brain neurons. Eur J Neurosci 10:1202–8.

Connors BW, Long MA. 2004. Electrical synapses in the mammalian brain. Annu Rev Neurosci 27:393–418.

Constantz GD. 1984. Sperm competition in poeciliid fishes. In: Smith R, editor. Sperm Competition and the Evolution of Animal Mating Systems. Orlando: Academic Press. p. 465–85.

Constantz GD. 1989. Reproductive biology of poeciliid fishes. In: Meffe GK, Snelson FF, editors. Ecology and Evolutionary of Live Bearing Fishes (Poeciliidae). New Jersey: Prentice Hall, Englewood Cliffs. p. 33–50.

Domenici P, Blake RW. 1997. The kinematics and performance of fish fast-start swimming. J Exp Biol 200:1165–78.

Drapeau P, Saint-Amant L, Buss RR, Chong M, McDearmid JR, Brustein E. 2002. Development of the locomotor network in zebrafish. Prog Neurobiol 68:85–111.

Dulka JG, Demski LS. 1986. Sperm duct contractions mediate centrally evoked sperm release in goldfish. J Exp Zool 237:271–9.

Farr JA. 1989. Sexual selection and secondary sexual differentiation in Poeciliids: determinant of male mating success and the evolution of female choice. In: Meffe GK, Snelson FF, editors. Ecology and Evolution of Live Bearing Fishes (Poeciliidae). New Jersey: Prentice Hall, Englewood Cliffs. p. 91–123.

Fujimoto K. 1995. Freeze-fracture replica electron microscopy combined with SDS digestion for cytochemical labeling of integral membrane proteins. Application to the immunogold labeling of intercellular junctional complexes. J Cell Sci 108:3443–9.

Galarreta M, Hestrin S. 1999. A network of fast-spiking cells in the neocortex connected by electrical synapses. Nature 402:72–5.

Galarreta M, Hestrin S. 2001a. Spike transmission and synchrony detection in networks of GABAergic interneurons. Science 292:2295–9.

Galarreta M, Hestrin S. 2001b. Electrical synapses between GABA-releasing interneurons. Nat Rev Neurosci 2:425–33.

Gibson JR, Beierlein M, Connors BW. 1999. Two networks of electrically coupled inhibitory neurons in neocortex. Nature 402:75–9.

Girard C. 1859. Ichthyological notices. Proc Acad Nat Sci Philadelphia 11:113–22.

Hale ME, Ritter DA, Fetcho JR. 2001. A confocal study of spinal interneurons in living larval zebrafish. J Comp Neurol 437:1–16.

Hale ME. 2002. Alternative neuromotor mechanisms of the fast-start: Kinematics and EMGs of S- and C-start escape responses. J Exp Biol 205:2005–16.

Hale ME, Long JH, McHenry MJ, Westneat MW. 2002. Evolution of behavior and neural control of the fast-start escape response. Evolution 56:993–1007.

Harris KM, Landis DM. 1986. Membrane structure at synaptic junctions in area CA1 of the rat hippocampus. Neuroscience 19: 857–72.

Harris AL. 2007. Connexin channel permeability to cytoplasmic molecules. Prog Biophys Mol Biol 94:120–43.

Helfman G, Collette BB, Facey DE, Bowen BW. 2009. The Diversity of Fishes: Biology, Evolution, and Ecology. 2nd Edition. Boston: Wiley-Blackwell. p. 736.

Hubbs C, Reynolds RA. 1957. Copulatory function of the modified pectoral fin of gambusiin fishes. Am Nat 91:333–5.

Kalb RG. 1994. Regulation of motor neuron dendrite growth by NMDA receptor activation. Development 120:3063–71.

Kamasawa N, et al. 2006. Abundance and ultrastructural diversity of neuronal gap junctions in the OFF and ON sublaminae of the inner plexiform layer of rat and mouse retina. Neuroscience 142:1093–117.

Kardong K. 2008. Vertebrates: Comparative Anatomy, Function, Evolution. 5th Edition. Boston: McGraw-Hill. p. 800.

Kiehn O, Tresch MC. 2002. Gap junctions and motor behavior. TINS 25:108–15.

Kluge AG, editor. 1977. Chordate Structure and Function. 2nd Edition. New York: MacMillan. p. 628.

Lagler KF, Bardach JE, Miller RR, Passino DRM. 1977. Ichthyology. New York: John Wiley and Sons. p. 506.

Liley NR. 1983. Hormones, pheromones, and reproductive behavior in fishes. In: Hoar WS, Randall DJ, editors. Fish Physiology. Vol. 3. New York: Academic Press. p. 73–116.

Marin-Burgin A, Eisenhart FJ, Baca SM, Kristan WB Jr, French KA. 2005. Sequential development of electrical and chemical synaptic connections generates a specific behavioral circuit in the leech. J Neurosci 25:2478–89.

Marin-Burgin A, Eisenhart FJ, Kristan WB Jr, French KA. 2006. Embryonic electrical connections appear to pre-figure a behavioral circuit in the leech CNS. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 192:123–33.

Marin-Burgin A, Kristan WB Jr, French KA. 2008. From synapses to behavior: development of a sensory-motor circuit in the leech. Dev Neurobiol 68:779–87.

Mills SL, Massey SC. 2000. A series of biotinylated tracers distinguishes three types of gap junction in retina. J Neurosci 20:8629–36.

O'Brien J, Al-Ubaidi MR, Ripps H. 1996. Connexin 35: a gap-junctional protein expressed preferentially in the skate retina. Mol Biol Cell 7:233–43.

O'Brien J, Bruzzone R, White TW, Al-Ubaidi MR, Ripps H. 1998. Cloning and expression of two related connexins from the perch retina define a distinct subgroup of the connexin family. J Neurosci 18:7625–37.

O'Brien J, Nguyen HB, Mills SL. 2004. Cone photoreceptors in bass retina use two connexins to mediate electrical coupling. J Neurosci 24:5632–42.

Parenti LR. 1981. A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). Bull Am Mus Nat Hist 168:335–557.

Peden AE. 1970. Courtship behavior of *Gambusia* (Poeciliidae) with emphasis on isolation mechanism. Unpublished PhD Dissertation, University of Texas at Austin, Austin, Texas.

Peden AE. 1972a. Differences in the external genitalia of female gambusiin fishes (Poeciliidae). Southwest Nat 17:265–72.

Peden AE. 1972b. The function of gonopodial parts and behavioral pattern during copulation by *Gambusia* (Poeciliidae). Can J Zool 50:955–68.

Peden AE. 1973. Variation in anal spot expression of gambusiin females and its effect on male courtship. Copeia 1973:250–63.

Peden AE. 1975. Differences in copulatory behavior as partial isolating mechanisms in the poeciliid fish *Gambusia*. Can J Zool 53:1290–6.

Peinado A, Yuste R, Katz LC. 1993. Extensive dye coupling between rat neocortical neurons during the period of circuit formation. Neuron 10:103–14.

Pereda A, O'Brien J, Nagy JI, Bukauskas F, Davidson KG, Kamasawa N, Yasumura T, Rash JE. 2003. Connexin35 mediates electrical transmission at mixed synapses on Mauthner cells. J Neurosci 23:7489–503.

Rash JE, Dillman RK, Bilhartz BL, Duffy HS, Whalen LR, Yasumura T. 1996. Mixed synapses discovered and mapped throughout mammalian spinal cord. Proc Natl Acad Sci USA 93:4235–9.

Rash JE, Duffy HS, Dudek FE, Bilhartz BL, Whalen LR, Yasumura T. 1997. Grid-mapped freeze-fracture analysis of gap junctions in gray and white matter of adult rat central nervous system, with evidence for a ''panglial syncytium'' that is not coupled to neurons. J Comp Neurol 388:265–92.

Rash JE, Olson CO, Davidson KG, Yasumura T, Kamasawa N, Nagy JI. 2007. Identification of connexin36 in gap junctions between neurons in rodent locus coeruleus. Neuroscience 147:938–56.

Rash JE, Staines WA, Yasumura T, Patel D, Furman CS, Stelmack GL, Nagy JI. 2000. Immunogold evidence that neuronal gap junctions in adult rat brain and spinal cord contain connexin-36 but not connexin-32 or connexin-43. Proc Natl Acad Sci USA 97:7573–8.

Rash JE, Yasumura T. 1999. Direct immunogold labeling of connexins and aquaporin-4 in freeze-fracture replicas of liver, brain, and spinal cord: factors limiting quantitative analysis. Cell Tissue Res 296:307–21.

Rash JE, Yasumura T, Davidson KG, Furman CS, Dudek FE, Nagy JI. 2001. Identification of cells expressing Cx43, Cx30, Cx26, Cx32 and Cx36 in gap junctions of rat brain and spinal cord. Cell Commun Adhes 8:315–20.

Rash JE, Yasumura T, Dudek FE. 1998. Ultrastructure, histological distribution, and freeze-fracture immunocytochemistry of gap junctions in rat brain and spinal cord. Cell Biol Int 22:731–49.

Rash JE, et al. 2005. Ultrastructural localization of connexins (Cx36, Cx43, Cx45), glutamate receptors and aquaporin-4 in rodent olfactory mucosa, olfactory nerve and olfactory bulb. J Neurocytol 34:307–41.

Rauchenberger M. 1989. Systematics and biogeography of the genus *Gambusia* (Cyprinodontoformes: Poecilidae). Am Mus Novitates 2951:1–74.

Romer AS, Parsons TS. 1986. The Vertebrate Body. 6th Edition. Philadelphia: Saunders. p. 679.

Rosa-Molinar E. 2005. Edwin S. Goodrich's theory of transposition revisited: the shift to a sexually dimorphic axial formulae and nervous system in a poeciliine Fish. In: Uribe MC, Grier HJ, editors. Viviparous Fishes. Homestead, Florida: New Life Publications Inc. p. 60–70.

Rosa-Molinar E, Fritzsch B, Hendricks SE. 1996. Organizational-activational concept revisited: sexual differentiation in an atherinomorph teleost. Horm Behav 30:563–75.

Rosa-Molinar E, Hendricks SE, Rodriguez-Sierra JF, Fritzsch B. 1994. Development of the anal fin appendicular support in the western mosquitofish, *Gambusia affinis affinis* (Baird and Girard, 1854): a reinvestigation and reinterpretation. Acta Anat 151:20–35.

Rosa-Molinar E, Proskocil BJ, Hendricks SE, Fritzsch B. 1998. A mechanism for anterior transposition of the anal fin and its appendicular support in the western mosquitofish, *Gambusia affinis affinis*. Acta Anat 163:75–91.

Rosen DE, Gordon M. 1953. Functional anatomy and evolution of male genitalia in poeciliid fishes. Zoologica 38:1–47.

Rosen DE, Bailey RM. 1963. The poeciliid fishes (Cyprinodontoformes), their structure, zoogeography and systematics. Bull Am Mus Nat Hist 126:1–176.

Rosen DE, Tucker A. 1961. Evolution of secondary sexual characters and sexual behavior patterns in a family of viviparous fishes (Cyprinodontiformes: Poeciliidae). Copeia 1961:201–12.

Saint-Amant L, Drapeau P. 2000. Motoneuron activity patterns related to the earliest behavior of the zebrafish embryo. J Neurosci 20:3964–72.

Saint-Amant L, Drapeau P. 2001. Synchronization of an embryonic network of identified spinal interneurons solely by electrical coupling. Neuron 31:1035–46.

Scheiffele P. 2003. Cell-cell signaling during synapse formation in the CNS. Annu Rev Neurosci 26:485–508.

Söhl G, Degen J, Teubner B, Willecke K. 1998. The murine gap junction gene connexin 36 is highly expressed in mouse retina and regulated during brain development. FEBS Lett 428:27–31.

Sotelo C, Korn H. 1978. Morphological correlates of electrical and other interactions through low-resistance pathways between neurons of the vertebrate central nervous system. Int Rev Cytol 55:67–107.

Szabo TM, Faber DS, Zoran MJ. 2004. Transient electrical coupling delays the onset of chemical neurotransmission at developing synapses. J Neurosci 24:112–20.

Szabo TM, Zoran MJ. 2007. Transient electrical coupling regulates formation of neuronal networks. Brain Res 1129:63–71.

Tresch MC, Kiehn O. 2000. Motor coordination without action potentials in the mammalian spinal cord. Nat Neurosci 3:593–9.

Vaney DI. 1991. Many diverse types of retinal neurons show tracer coupling when injected with biocytin or neurobiotin. Neurosci Lett 125:187–90.

Waites CL, Craig AM, Garner CC. 2005. Mechanisms of vertebrate synaptogenesis. Annu Rev Neurosci 28:251–74.

Wake MH, editor. 1979. Hyman's Comparative Vertebrate Anatomy. 3rd Edition. Chicago: University of Chicago Press. p. 788.

Warburton B, Hubbs G, Hagen DW. 1957. Reproductive behavior of *Gambusia heterochir*. Copeia 1957:299–300.

Webster D, Webster M. 1974. Comparative Vertebrate Morphology. New York: Academic Press. p. 517.