Characterization of Projections from a Sexually Dimorphic Motor Nucleus in the Spinal Cord of Adult Green Anoles

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ABSTRACT

Male green anoles possess two copulatory organs (hemipenes), which are independently controlled by bilateral muscles: the transversus penis (TPN) and retractor penis magnus (RPM). Adult females do not possess hemipenes or either of the two related muscles. Motoneurons projecting to the TPN lie in spinal segments trunk 17 and sacral 1 (T17–S1). Overall, motoneurons in this region are larger and more numerous in males than females. The present studies were designed to determine 1) whether motoneurons projecting to the RPM are located in the same sexually dimorphic nucleus, 2) other targets of T17–S1 motoneurons, and 3) the approximate proportion of motoneurons projecting to each muscle. In Study 1, unilateral injection of the retrograde tracer Fast Blue (FB) into RPMs and simultaneous unilateral injection of either Cholera Toxin-fluorescein (CT-FITC) or Diamidino Yellow into TPNs revealed that RPM and TPN motoneurons are indeed interdigitated in T17–S1. In Study 2, FB was used to characterize other targets of this nucleus in both males and females. In adult males, projections to four muscles accounted for 96% of the T17–S1 motoneurons: the TPN, RPM, caudifemoralis (CF), and cloacal sphincter (SC). In adult females, projections to the CF and SC comprised 70% of this nucleus. These data demonstrate that the T17–S1 nucleus is a mixed spinal nucleus that has projections to muscles present in both sexes, as well as those present only in males and specialized for male copulatory behavior. J. Comp. Neurol. 471:180–187, 2004.

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Neuromuscular systems are exceptionally well suited to investigations of structure/function relationships in the central nervous system. The direct link between neural activity and muscle contraction, as well as the often obvious behavioral function of the target structure, make it easier to identify the adaptive significance of dimorphisms in these compared to more complicated neural systems (Breedlove et al., 2002). The function of structural differences is particularly evident in sexually dimorphic neuromuscular systems involved in reproductive behaviors, and examples of such systems exist in all vertebrate classes. In species that exhibit male vocal courtship behavior (including frogs, songbirds, and some fish), the neuromuscular system mediating production of vocalizations contains motoneurons and/or muscles that are larger in males than females (e.g., Sassoon and Kelley, 1986; Simpson et al., 1986; Bass and Marchaterre, 1989a,b; Wade and Buhlman, 2000). The system underlying mammalian copulation shows striking dimorphisms in motoneuron and muscle fiber number as well as size, again favoring males (reviewed in Breedlove et al., 2002).

The green anole lizard (Anolis carolinensis) has similar sexually dimorphic neuromuscular systems, one involved in courtship and the other in copulation. Adult males...
court females by performing head bobbing displays coupled with extension of a red throat fan, called a dewlap. Females have much smaller dewlaps than males and extend them far less frequently, only in agonistic encounters (Greenberg and Noble, 1944; Nunez et al., 1997; Jenssen et al., 2000). The motoneurons controlling dewlap extension are in the caudal brainstem and innervate the paired ceratohyoid muscles, located in the throat (Font and Rome, 1990; Wade, 1998); contraction of these muscles causes the second ceratobranchial cartilage to bow away from the ventral surface of the throat, extending the dewlap (Bels, 1990). Similar to the systems outlined above, many components, including motoneuron somata and ceratohyoid muscle fibers, are larger in males than females (Wade, 1998; O’Bryant and Wade, 1999, 2002).

More recently, the neuromuscular system underlying copulation in green anoles has been investigated. Male squamate reptiles (lizards and snakes) each possess two penises called hemipenes. These hemipenes are located bilaterally in the tail and only one is extended through the cloaca during each copulation. Two muscles mediate extension (transversus penis, TPN) and retraction (retractor penis magnus, RPM) of each hemipene (Ruiz, 1984). Adult female anoles do not have hemipenes or either of these muscles (Ruiz and Wade, 2002). Injection of the retrograde tracer biocytin into the TPN revealed that its corresponding motoneurons are located in segments trunk 17 and sacral 1 (T17–S1) in the caudal spinal cord. These cells are larger and more numerous in males than females (Ruiz and Wade, 2002).

Before one can appreciate the relationships between structure and function in a given neuromuscular system, one needs to understand how the motoneurons, nerve(s), and muscle(s) that comprise it are organized. While this work has been largely completed for other sexually dimorphic neuromuscular systems (e.g., Nottebohm et al., 1976; Schroder, 1980; Bass, 1985; McKenna and Nadelhaft, 1986; Simpson et al., 1986; Wade, 1998), organization of the anole copulatory system has not yet been fully characterized. Importantly, the motoneurons projecting to the RPM must be located. The first goal of the present investigation was to find these cells in males (Study 1). Additional goals of this investigation were to identify other muscle targets for T17–S1 motoneurons and to estimate the relative proportions of T17–S1 motoneurons that project to each in both males (Study 2a) and females (Study 2b). In addition to the TPN, the caudifemoralis (CF) receives projections from T17–S1 motoneurons (Ruiz and Wade, 2002). The cloacal sphincter (SC) was also considered a likely target, as SC motoneurons are located in a similar pelvic region of the spinal cord in birds (Ohmori and Watanabe, 1989; Seiwert and Adkins-Regan, 1998). Furthermore, external sphincter motoneurons are interdigitated with copulatory motoneurons and share the pudendal nerve in rats (Schroder, 1980; McKenna and Nadelhaft, 1986), and cloacal muscles receive projections via the pudendal nerve in iguanas (Akita, 1992).

**MATERIALS AND METHODS**

**Animals and housing**

Adult (>50 mm snout–vent length) male and female green anoles were purchased from Charles Sullivan Co (Nashville, TN). Prior to the commencement of each experiment, animals were group housed (1 male with 3–6 females) in 110-L glass aquaria (76 × 30 × 48 cm). Lizards were housed in a 14:10 hour light:dark cycle with fluorescent and full-spectrum lights, as well as a heat lamp placed on one end of each aquarium. Temperature ranged from 28°C (ambient) to 38°C (directly under heat lamp) during the day to 18°C at night. These conditions approximate those the animals experience in the spring in the field and stimulate breeding in the lab. Aquaria were sprayed daily to help maintain 70% relative humidity, and water was provided ad libitum. Animals were fed crickets or mealworms three times a week. Following injection of tract tracers (see below), all animals were housed individually in 21-L glass aquaria (42 × 30 × 24 cm) and cared for as above. All procedures were approved by the Michigan State University All University Committee on Animal Use and Care and conform to NIH guidelines.

**Treatment, tissue collection, and analyses**

**Study 1.** Seven adult male lizards were anesthetized with isofluurane, placed on ice, and incisions were made in the ventral surface of the tail. The retrograde tracer Fast Blue (FB; Illing Plastics, Bergfeld, Germany; 0.5 μl at 2.5–5% in 0.9% saline) was bilaterally injected into the RPMs. Additionally, the retrograde tracer Cholera Toxin-B subunit conjugated to fluorescein (CT-FITC; List Biological Laboratories; 0.5 μl at 2% in 0.9% saline) was bilaterally injected into the TPNs of the same individuals. To determine whether the projections were ipsilateral and to confirm that there were no double-labeled motoneurons, which would indicate tracers leaking into a muscle not intentionally injected or an unusual situation of a motoneuron projecting to more than one muscle, an additional six males were injected as above with FB into one RPM and Diamidino Yellow (DY; 0.5 μl at 3% in 0.9% saline; Sigma, St. Louis, MO) into the ipsilateral TPN. CT-FITC and FB both label the cytoplasm of the cell (Kuypers et al., 1980; Dederen et al., 1994), whereas DY primarily labels the nucleus (Keizer et al., 1983). As such, we were better able to identify possible double-labeled motoneurons when FB was used with DY. To decrease possible contamination, excess tracer was removed with a cotton swab and gel foam prior to suturing the incision with silk.

Three days following injection, lizards were overdosed with Brevital Sodium (0.03 ml) and perfused with 0.1M phosphate-buffered saline (PBS; pH 7.4) and 4% paraformaldehyde in PBS. Spinal segments were marked with India ink to permit identification of individual segments. Cords were extracted (segments T15–S2), embedded in gelatin, postfixed in 4% paraformaldehyde for 2.5 hours, then transferred to 20% sucrose in PBS overnight. The hemipenes, TPNs, and RPMs were also extracted from the rostral tail in one block (which also included the CFs) and processed as above. Cross-sections of all tissue were cut frozen at 30 μm into PBS. Two series of alternate sections of spinal cord were mounted onto gelatin-coated slides from 9:1 dH2O:PBS. Two series of every fourth section of muscle/hemipene tissue was used for fluorescent analyses (see below). They were soaked in 0.1% sodium borohydride in PBS for 10 minutes to decrease autofluorescence (Clancy and Cauller, 1998) and rinsed twice with PBS and once with dH2O prior to dehydration. Alternate spinal cord
sections were stained with thionin. In some cases, series of tail tissue were stained using the trichrome method to facilitate localization of specific muscles. However, leakage of fluorescent dyes into muscles neighboring those injected was obvious even in unstained tail sections. All tissue was dehydrated, cleared in xylene, and overslipped using either DPX (for fluorescent tissue) or Permorn (for thionin- and trichrome-stained tissue).

Spinal cord and muscle tissue was analyzed using a microscope (Olympus BX60) capable of simultaneous visualization of the multiple fluorochromes. Spinal segments T15 through S2 were analyzed for the presence of FB+, CT-FITC+, and DY+ motoneurons; sections containing labeled cells were compared to thionin-stained tissue in which the ink marking the segments was obvious. In addition, it was noted whether the positively labeled motoneurons were ipsilateral or contralateral to the injection, and whether any double-labeled motoneurons were detected. In all cases, muscle tissue was analyzed to confirm appropriate and accurate injection sites.

**Study 2a.** To identify other muscle targets for T17–S1 motoneurons, and to determine the proportions of cells projecting to each muscle, adult male lizards received a single tracer injection in one of four muscles: TPN, RPM, CF, or SC. Injections were unilateral in the TPN, RPM, and CF. The SC wraps around the cloaca (Arnold, 1984) and was injected at the midline in both rostral and caudal fibers. To control for possible differences in transport efficacy between tracers, all males were injected with FB (as above). For estimation of percentages of motoneurons projecting to each muscle (see below), three males were used per muscle. While more were injected, a few were not analyzed either because analysis of the tail tissue indicated the tracer had leaked outside the intended muscle or too much autofluorescence existed in the spinal cord tissue. All tissue (spinal cord and muscle) was collected and processed as described for Study 1 for animals with TPN, RPM, or CF injections. When SCs were injected, the muscle dissection included the entire cloacal region.

**Study 2b.** To characterize T17–S1 motoneuron projections in females, five adult female anoles received a single injection of FB into either the CF (unilateral; n = 3) or the CS (n = 2). Tissue processing and analyses were performed as outlined for Study 2a.

For all animals in Study 2, FB+ neurons were counted in spinal segments T15–S2. All cells labeled following TPN, RPM, and CF injections were unilateral, so total counts from the alternate sections were multiplied by two to provide an estimate for one side of the spinal cord (see Table 1). With SC injections, labeled cells were detected on both sides of the cord, so they were compared to the total number of thionin-stained motoneurons found bilaterally; SC counts represent an average of the two sides of the spinal cord. All data points come from separate individuals (see Materials and Methods).

### RESULTS

#### Study 1

Injecting FB into the RPM and either CT-FITC or DY into the TPN revealed that TPN and RPM motoneurons are interdigitated in the T17–S1 motoneuron nucleus (Fig. 1). Positively labeled motoneurons (FB, CT-FITC, and DY) were located throughout the rostral-caudal extent of T17

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TABLE 1. Counts of Fast Blue-Labeled Motoneurons in T17–S1 Following Injection into Either One Transversus Penis (TPN), One Retractor Penis Magnus (RPM), One Caudifemorals (CF), or the Cloacal Sphincter (SC)

Percentages of labeled motoneurons, based on counts from thionin-stained alternate sections, are also indicated. Means for each muscle are outlined in grey. All cells labeled following TPN, RPM, and CF injections were unilateral, so the percent labeled cells was based on thionin counts from the same side of the spinal cord. With SC injections, labeled cells were detected on both sides of the cord, so they were compared to the total number of thionin-stained motoneurons found bilaterally; the left and right SC counts were then averaged to provide an estimate for one side of the spinal cord. To avoid double-counting of cells across sections, motoneurons were assessed using the physical dissector technique (as in Ruiz and Wade, 2002; Gunderson, 1986), whereby cells were counted if the nuclei come into focus and disappear within a given section. This technique was not used for counting of fluorescent motoneurons, as their nuclei are not always identifiable due to the homogenus nature of the FB label. However, double-counting of FB+ motoneurons was unlikely, given that cells were counted 1) in alternate 30-μm sections (and diameter of these motoneurons is ~20-μm), and 2) only if they were large and bright (i.e., not cell fragments or split nuclei). As with Study 1, muscle tissue was analyzed to confirm appropriate and accurate injection sites. For each animal the FB+ total was divided by the total motoneuron count to provide an estimated percentage of the number of T17–S1 motoneurons that project to that muscle. In order to confirm that T17–S1 motoneurons were sexually dimorphic in number (Ruiz and Wade, 2002) in the present sample, total motoneuron counts (from thionin-stained tissue) were compared between males (n = 12) and females (n = 5) using an unpaired t-test.

Fluorescent photomicrographs were obtained with an Olympus 55mm camera and Kodak Elite Chrome 400 film for color slides. Images were then digitally scanned (Polaroid Sprint Scan 35 Plus). Nissl photomicrographs were obtained using a Q Imaging MicroPublisher 5.0 digital camera. All images were sized, compiled, and labeled in Adobe Photoshop 7.0 (San Jose, CA). Brightness and/or contrast were adjusted slightly as necessary to produce images matching those visible through the microscope. No other types of modifications were made.

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M.M. HOLMES AND J. WADE

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*injection of FB into either the CF (unilateral; n = 3) or the CS (n = 2). Tissue processing and analyses were performed as outlined for Study 2a.*
and S1 but were not found in any other spinal segment (T15, T16, or S2); no labeled cells were found outside of the ventral horn. Unilateral injection of FB into the RPM with DY into the ipsilateral TPN 1) did not reveal any double-labeled motoneurons (Fig. 1F), and 2) demonstrated that both the TPN and RPM receive only ipsilateral motoneuron projections.

Study 2a

In adult males, unilateral injection of FB into either the TPN, RPM, or CF revealed only ipsilateral labeling of motoneurons, whereas SC injections resulted in bilateral labeling of motoneurons. As with Study 1, labeled motoneurons projecting to each muscle were lo-
cated throughout spinal segments T17–S1 with roughly equivalent distributions. Approximately one-third of T17–S1 motoneurons each projected to the TPN and CF, whereas the remaining third projected to either the RPM or the SC. These estimates account for 96% of the motoneurons in the T17–S1 nucleus in males (Fig. 2, Table 1). The TPN counts with FB in the present study are comparable to those obtained with biocytin, as reported in Ruiz and Wade (2002).

Study 2b

In adult females, unilateral injection of FB in the CF resulted in ipsilateral labeling and SC injections resulted in bilateral labeling of motoneurons. Almost half of the T17–S1 motoneurons projected to the CF, while approximately one-third projected to the SC. These estimates account for 70% of the motoneurons in the T17–S1 nucleus in females (Table 1).
Comparison of the number of T17–S1 motoneurons (on one side of the spinal cord) between males (Study 2a) and females (Study 2b) demonstrates that adult males have more motoneurons in this region than adult females (t = 5.77; P < 0.001; Fig. 3).

**DISCUSSION**

**Summary**

In adult male anoles, the sexually dimorphic T17–S1 nucleus is comprised of motoneurons projecting to muscles directly involved in copulation (TPN and RPM) as well as two other muscles (CF and SC, present data; Ruiz and Wade, 2002). More than half of this motoneuron population projects to the copulatory muscles (Table 1). Adult female anoles do not possess either TPN or RPM muscles, but like males, their T17–S1 nucleus projects to the CF and SC. Unilateral injection of a retrograde tracer into the TPN, RPM, and SC all resulted in ipsilateral labeling of motoneurons. Injection of the SC, which wraps around the cloaca, results in bilateral labeling of motoneurons. For all muscles (TPN, RPM, CF, and SC), labeled motoneurons were located in the lateral ventral horn and were interdigitated throughout spinal segments T17–S1; specific subpopulations were not obviously segregated by medial-lateral or rostral-caudal position.

**Organization of T17–S1 motoneuron projections**

Retrograde tracer injections into the TPN, RPM, CF, and SC resulted in labeling of the vast majority of T17–S1 motoneurons in males (Table 1), suggesting that these four muscles comprise all major muscle targets for this motoneuron population. However, in adult females, while FB injections into the CF and SC labeled approximately two-thirds of the T17–S1 motoneurons, not all were accounted for (Table 1). This discrepancy between males and females may be attributed to variability between the sexes in either the degree to which tracer filled the respective muscles or in transport efficacy. However, analysis of the injection sites revealed no obvious sex difference in the extent to which each muscle (CF and SC) was filled with FB. Also, a sex difference in transport efficacy may exist, but if so, it is likely due to a factor other than circulating testosterone levels; gonadal androgens do not affect axonal transport of another retrograde tracer, cholera toxin-conjugated horseradish peroxidase (Leslie et al., 1991).

Another possibility is that females have motoneurons in the T17–S1 nucleus that project to an additional muscle(s), as yet unidentified. Other leg and tail muscles are located near the CF and SC, including the retractor medialis, the retractor lateralis, and the protractor commissurale (Arnold, 1984). Perhaps the best candidate is the iliocaudalis tail muscle (ILC; also called the flexor caudae externus; Akita, 1992). This muscle is present in both sexes and lies adjacent to the CF (in both sexes) and TPN (in males), and the ILC and TPN are both supplied by the same nerve in male iguanas (Akita, 1992). Additional projections in T17–S1 in females would be particularly interesting. As virtually all (96%) of these motoneurons have been identified in males with TPN, RPM, CF, and SC injections, it would mean that females have T17–S1 projections that are minimal or nonexistent in males.

Alternatively, female anoles may have residual motoneurons in the T17–S1 nucleus that do not actually project to muscles. Female lizards do develop hemipenes and the corresponding musculature embryonically; however, in some species, including anoles, these structures regress by hatching by sexing (Dufaure and Hubert, 1961; Raynaud and Pieg, 1985; Holmes and Wade, unpubl. obs.). It would be exciting if a subset of the corresponding motoneurons were maintained into adulthood without target musculature (perhaps via glial support), which may be consistent with previous reports in mammals (Glicksman et al., 1998). In at least one other reptile species, leopard geckos (Eublepharis macularius), adult females can develop fully evertable hemipenes when treated with testosterone (Rhen et al., 1999). The degree to which this hemipene plasticity is accompanied by corresponding development of TPN and RPM musculature and motoneurons is currently under investigation. One possibility is that female leopard geckos maintain copulatory motoneurons into adulthood, which then support function of the remainder of the system when it develops (as opposed to having adult motoneuron genesis, which would be exciting but quite surprising).

Finally, given that both females and males have motoneurons projecting to the CF and SC, it appears that the sex difference in T17–S1 motoneuron number (present data; Ruiz and Wade, 2002) is largely due to females not having TPN or RPM motoneurons (but see above). One prediction that arises from this fact is that females should have higher percentages of cells projecting to CF and SC given their lower number of total T17–S1 motoneurons, and indeed they do, although the effect is certainly more striking for SC (27% in females vs. 9% in males) than CF (43% in females vs. 32% in males) projections (Table 1).

**Implications for structure/function relationships within this system**

The ipsilateral projections to the TPN and RPM are consistent with the organization of copulatory behavior in this species. Male anoles use only one hemipene per copulation (Crews, 1978), suggesting that each is independently controlled by its own neuromuscular system. Furthermore, over relatively short periods of time male anoles alternate...
hemipene use in order to maximize sperm transfer (Tokarz, 1988; Tokarz and Slowinski, 1990; Tokarz and Kirkpatrick, 1991); green anoles in particular rarely use individual hemipenes more than two times in succession (Crews, 1978). While there is evidence to suggest that sensory feedback from the hemipenes and/or testes mediates this effect in part (Crews, 1978), it may be that muscular fatigue also participates in this behavioral pattern.

The bilateral labeling of motoneurons observed following injection of the SC is consistent with at least two avian species (Japanese quail and brown leghorn fowl), in which, similar to the present data, motoneurons projecting to the SC are located in the pelvic region of the spinal cord (Ohmori and Watanabe, 1989; Seiwert and Adkins-Regan, 1998). This bilateral labeling may be because the SC receives bilateral projections with axons crossing the midline. Alternatively, the SC may receive only ipsilateral projections, but they could not be detected because the muscle wraps around the cloaca and all injections filled fibers on both sides.

Finally, interdigitation of T17–S1 motoneurons projecting to different muscles is consistent with nerve organization in other reptilian and avian species. In male iguanas, RPM, CF, and SC muscles all receive projections via the same nerve trunk, which branches into the pudendal nerve (serving SC and RPM) and the nerve serving the CF (Akita, 1992). Furthermore, SC and CF motoneurons are interdigitated and may share the connexus caudalis nerve in domestic fowl (Ohmori and Watanabe, 1989). This organization of the nerve is not surprising given the close proximity of these muscles within the lizard tail as well as the fact that, arguably, all four muscles are used (either directly or indirectly) in copulation. While the TPN and RPM are directly involved in the control of the hemipene during intromission, the CF is likely involved in postural positioning during mounting and the SC may also be involved in facilitating intromission, either by passively or actively permitting the hemipene to extend through the cloaca.

Comparison to organization of other dimorphic neuromuscular systems

In male anoles, more than half of T17–S1 motoneurons project to the TPN and RPM, both muscles critical for male copulatory behavior. The remaining motoneurons project to muscles that are not directly involved in copulation: the CF (a leg muscle) and SC. This composition is reminiscent of spinal nuclei in laboratory rats in which the dorsolateral nucleus (DLN) and spinal nucleus of the bulbocavernous (SNB), both sexually dimorphic, have motoneurons projecting to both copulatory and noncopulatory muscles (e.g., Schroeder, 1980; McKenna and Nadelhaft, 1986), including external sphincters involved in waste elimination. Interestingly, in the neuromuscular systems underlying vocalization in fish, frogs, and songbirds, while populations of motoneurons within brain nuclei can project to different muscle targets, those projecting to sexually dimorphic muscles are usually clustered, rather than interdigitated, populations (Nottageholz et al., 1976; Bass, 1985; Simpson et al., 1986).

Some differences in neuromuscular systems are often not limited to motoneuron somata and muscle size. For example, they can include dimorphisms in dendritic arborization, neuromuscular junction size, neurotransmitter release, and synaptic efficacy (reviewed in Breedlove et al., 2002). Indeed, in the neuromuscular system that extends the anole dewlap during courtship, not only do adult males have larger motoneurons and muscle fibers, they also have longer cartilage, more muscle fibers, larger nerve cross-sectional area, and larger neuromuscular junctions than adult females (O’Bryant and Wade, 1999, 2002). Now that organization of the anole copulatory neuromuscular system is better understood, we are primed to uncover the full extent of dimorphisms in this set of structures and the mechanisms regulating them. This work can then be related to that from copulatory systems of mammalian species. Importantly, it will also allow direct comparisons between copulatory neuromuscular systems and those regulating courtship, traditionally studied in non-mammalian vertebrates. In anoles, both types of dimorphic neuromuscular systems are required for the full suite of masculine reproductive behaviors.

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LITERATURE CITED


Jenssen TA, Orrell KS, Lovern MB. 2000. Sexual dimorphisms in aggres-
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