A proposed neural pathway for vocalization in South African clawed frogs, *Xenopus laevis*

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Summary. 1. Vocalizations of South African clawed frogs are produced by contractions of laryngeal muscles innervated by motor neurons of the caudal medulla (within cranial nerve nucleus IX–X). We have traced afferents to laryngeal motor neurons in male and female frogs using retrograde axonal transport of horseradish peroxidase conjugated to wheat germ agglutinin (HRP-WGA).

2. After iontophoretic injection of HRP-WGA into n. IX–X, retrogradely labelled neurons were seen in the contralateral n. IX–X, in rhombencephalic reticular nuclei, and in the pre-trigeminal nucleus of the dorsal tegmental area (DTAM) of both males and females.

3. Injection of HRP-WGA into DTAM resulted in labelled cells in the striatum, preoptic area and thalamus. Posterior to DTAM, labelled cells were found in the rhombencephalic reticular nuclei as well as n. IX–X of males. Results in females were similar with the exception that n. IX–X labelled cells were only seen after very large injections of unconjugated HRP into DTAM and surrounding tegmentum. Thus, the projection of n. IX–X neurons to DTAM is not as robust in females as males.

4. These anatomical studies revealed candidate brain nuclei contributing to the generation of vocal behaviors and confirmed some features of a model for anuran vocal behavior proposed by Schmidt (1976).

5. Comparison of calling candidate brain nuclei to the location of steroid accumulating neurons (Kelley 1981) reveals that most calling nuclei contain hormone concentrating cells. Androgens may act to promote calling by influencing neural activity at multiple sites within the vocalization pathway.

Introduction

Male South African clawed frogs, *Xenopus laevis*, emit a distinctive vocalization, the mate call, during the breeding season. Female *X. laevis* also vocalize; the female-typical vocalization, ticking, is produced by sexually unreceptive frogs in response to a clasp attempt by a male (Russell 1954; Kelley 1982). Vocalizations are produced by contractions of the laryngeal bipinnate muscles (Yaeger 1982) innervated by motor neurons in cranial nerve nucleus IX–X of the caudal medulla (Kelley 1980).
The goal of this study was to identify sources of afferent input to laryngeal neurons in order to establish neural candidates for participation in the generation of calling. Our results suggest that laryngeal afferent brain nuclei are connected in a manner with many similarities to the vocal model of Schmidt (1971, 1976). We thus suggest that these brain nuclei control the production of vocal behaviors in *X. laevis*.

In male *Xenopus laevis*, vocal behaviors are modulated by the androgens, testosterone and dihydrotestosterone (Wetzel and Kelley 1983). Androgen target neurons are located in a restricted set of diencephalic, mesencephalic and medullary brain nuclei (Kelley et al. 1975) which includes the laryngeal motor neurons (Kelley 1980). These androgen target brain nuclei appear homologous to those identified by Schmidt (1971, 1976) in other frog species as participating in the motor control of calling. Thus in this study we also compared the locations of laryngeal afferents with those of androgen-concentrating neurons and with Schmidt’s (1971) neural model for anuran vocal behavior.

Female *X. laevis* do not mate call (Hannigan and Kelley, submitted). We wished to determine whether sex differences in calling reflect differences in connectivity of the brain nuclei involved in vocalization. We compared the connections of laryngeal afferent brain nuclei in male and female brains. The projection from laryngeal motor neurons to their major tegmental afferent nucleus (DTAM) is less robust in female than in male brains. This difference in connectivity could contribute to sex differences in *X. laevis* vocal behaviors.

**Materials and methods**

*Experimental approach.* Retrograde transport of horseradish peroxidase (HRP) coupled to the lectin, wheat germ agglutinin (WGA) was used to anatomically identify afferents to laryngeal motor neurons. Laryngeal motor neurons (n. IX-X) occupy a slender column approximately 200 μm wide, 300 μm in diameter and 2,500 μm long in the caudal medulla. Use of the HRP-peroxidase (HRP) coupled to the lectin, wheat germ agglutinin (WGA) was used to anatomically identify afferents to laryngeal motor neurons (Kelley 1980). These androgen target brain nuclei appear homologous to those identified by Schmidt (1971, 1976) in other frog species as participating in the motor control of calling. Thus in this study we also compared the locations of laryngeal afferents with those of androgen-concentrating neurons and with Schmidt’s (1971) neural model for anuran vocal behavior.

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