To the graduate Council:

I am submitting herewith a dissertation written by Enrique Font entitled "Functional Morphology and Central Control of Dewlap Extension in the Displays of Anolis equestris (Sauria, Iguanidae)." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Life Sciences.

Neil Greenberg, Major Professor

We have read this dissertation and recommend its acceptance:

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

FUNCTIONAL MORPHOLOGY AND CENTRAL CONTROL OF DEWLAP EXTENSION IN THE DISPLAYS OF *ANOLIS EQUESTRIS* (SAURIA, IGUANIDAE)

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Enrique Font

December 1988

DEDICATION

To Maria Jose, my wife

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ABSTRACT

Dewlap extension is an important component of inter- and intraspecific displays of Anolis lizards. The dewlap, an extendible flap of skin ordinarily folded under the throat, is supported by the hyoid apparatus. The mechanism and neural pathway for dewlap extension were investigated in Anolis equestris (Sauria, Iguanidae), a large arboreal lizard with a prominent dewlap.

Specializations of the hyoid skeleton for dewlap extension include elongated second ceratobranchials and highly movable joints between the ceratohyals and the hypohyals and between the first ceratobranchials and the body of the hyoid. A well developed M. ceratohyoideus extends between the ceratohyals and the first ceratobranchials. The mechanism of dewlap extension was studied using X-rays, electrical stimulation, and denervation and electromyography of key hyoid muscles. During dewlap extension, the hyoid apparatus acts as a first order lever. Contraction of M. ceratohyoideus pulls the ceratohyals posterio-laterally causing the hypohyals and the body of the hyoid to rotate dorsally around the first ceratobranchial/body joints. This movement results in the second ceratobranchials swinging forward and down, unfolding the dewlap. A comparison is made of dewlap extension and other hyoid displays.

The brain stem origins of efferent fibers to M. ceratohyoideus were investigated by retrograde transport of horseradish peroxidase (HRP). Following application of HRP to

M. ceratohyoideus or to its nerve supply on one side of the throat, large polygonal motoneurons were found ipsilaterally in the vagal (Amb X) and glossopharyngeal (Amb IX) parts of the medullary nucleus ambiguus. Labelled neurons were more abundant and more heavily labelled in Amb X than in Amb IX. In addition, small spindle-shaped cells were labelled ipsilaterally in three parasympathetic nuclei that innervate glandular structures in the pharynx and the larynx.

HRP injections of the larynx labelled cells in many of the same locations, including Amb X and Amb IX. Thus, nucleus ambiguus in A. equestris contains motoneurons for supply of striated muscles in the hyoid (i.e., M. ceratohyoideus) and the larynx. The distribution of these motoneuronal pools shows no evidence of topographical organization.

Afferent sources to Amb X were tentatively identified by injection of HRP or wheat germ agglutinin-HRP conjugate (WGA-HRP) into the caudal medulla. These injections labelled cells in several nuclei in the diencephalon, mesencephalon and rhombencephalon. Comparison of locations of HRP labelled cells and results of previous experiments using electrical stimulation of the brain suggests candidate nuclei subserving dewlap extension. In particular, it is proposed that nuclei in the reticular formation and the mesencephalic laminar nucleus of the torus semicircularis (Torl) are part of the neural network for control of dewlap extension. The implications of these findings for the evolutionary origins of dewlap displays are discussed.

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CHAPTER I

INTRODUCTION AND OVERVIEW

The dewlap of Anolis (Sauria, Iguanidae) lizards is a specialized display organ used to communicate with conspecifics, other lizards or potential predators. With well over 200 extant species, Anolis is one of the most speciose vertebrate genera. It has undergone extensive radiation, particularly in the Caribbean islands, resulting in a remarkable diversity of morphology, ecology and behavior (Williams, '69, '83). Anolis dewlaps differ greatly in relative size, shape and coloration. This diversity of form has stimulated many studies of dewlap function (see below), but few have examined the muscles and neural mechanisms effecting its display.

A neuroeffector pathway consists of a peripheral effector organ (a muscle or set of muscles) and the motoneurons associated with it (Bass, '86). Abundant descriptive information has accumulated on the display behavior of all major groups of vertebrates (see review in Sebeok, '77). Much less is known about neuroeffector pathways used to produce those displays, particularly visual displays. Yet, most agree that questions of immediate causation are pertinent to the study of animal behavior (e.g., Dawkins, '83). The present study is concerned with the neuroeffector pathway for dewlap extension in the lizard Anolis equestris. My goal is to proceed from a description of movement and muscle activity to the underlying

neural structures controlling dewlap extension. This chapter presents a brief review of studies of dewlap function and introduces material to be discussed in subsequent chapters.

Comments on Dewlap Function

Crests, fins and bold body patterns are common social signals among iguanid lizards (Carpenter and Ferguson, '77). The benefits of conspicuousness and enhanced visibility that they accord their bearers entail a cost in the form of increased predation risk, particularly because they are continuously displayed. *Anolis* lizards have many natural predators (Crews, '80). However, dewlaps are barely visible when retracted, thus reducing the risk of predation.

Traditionally, analyses of dewlap function have focused on the role of dewlap coloration in species recognition. Color and pattern of the dewlap are often strikingly different among sympatric species in multi-species *Anolis* faunas, suggesting that differences in dewlap coloration may serve as a reproductive isolation mechanism (Rand and Williams, '70; Webster and Burns, '73; Williams and Rand, '77). Experimental demonstration of the species recognition role of dewlap coloration has been provided by Losos ('85). He has shown that dewlap coloration affects levels of interspecific aggression in the sibling species *A. marcanoi* and *A. cybotes*.

In addition to species recognition, Williams and Rand ('77) list the following putative uses of the dewlap:

1. to identify the sex of the displaying animal;

2. to distinguish between juveniles and adults;

3. to indicate the size of the displaying animal;

4. to discriminate territory holders from wandering or subordinate animals;

5. to signal the mood of an animal;

6. to call attention to an animal by increasing its conspicuousness;

7. to increase the apparent size of an animal in close agonistic contacts between males.

It seems unlikely that all the proposed uses of the dewlap are present in a single species. In general, different species will emphasize different uses of the dewlap depending on their social system, life history strategies, ecology, and the presence or absence of syntopic congeners. For instance, evidence from over 40 species of mainland anoles suggests a correlation between dewlap morphology and habitat seasonality. In seasonally dry climates that limit reproduction to a short breeding season, dewlaps are relatively large and brightly colored. Conversely, dewlaps tend to be small and dull colored in the relatively aseasonal climates of tropical rainforests and cloud forests (Fitch and Hillis, '84). Adoption of a non-territorial social system has led to extreme reduction of the dewlap in Anolis agassizi, the Malpelo island anole (Rand et al., '75; Williams and Rand, '77). The rudimentary dewlaps of the hendersoni group of Hispaniola, on the other hand, are

probably the result of intense predation pressure from two larger congeners, A. coelestinus and A. cybotes (Fitch and Henderson, '87).

Sexual selection is usually invoked to account for the evolution of male traits that are used in male-male combat (intrasexual selection) or to attract females (intersexual selection) (Trivers, '85). In many species of Anolis, males have dewlaps that are larger and of brighter coloration than those of females, suggesting a role for sexual selection in the evolution of the dewlap. Intermale competition is intense in most anoles. Since large lizards tend to dominate smaller ones in aggressive interactions (e.g., Tokarz, '85), the significance of dewlap extension may lie in increasing the apparent size of the displaying lizard (Williams and Rand, '77). Theoretical models of intersexual selection often revolve around the evolutionary origins of female choice. Active female choice is recognized as an important selective force in the evolution of male secondary sexual characters, perhaps including the dewlap. Sigmund's ('83) experiments suggest that dewlap coloration in A. carolinensis may be important for passive female attraction due to increased male detectability, but do not demonstrate female choice. Other investigations of the role of male dewlap coloration in female choice have produced inconclusive results (Greenberg and Noble, '44; Crews, '75).

Attempts to trace the evolutionary history of the dewlap are, at present, likely to fail due to insufficient information

concerning its occurrence and functions in different taxa. Carpenter and Ferguson ('77) argue that dewlaps are primitive for the class Reptilia, since they occur in all lizard families and in other reptiles, including Sphenodon. Their conclusion, however, fails to discriminate two superficially similar behaviors: throat and dewlap extension. Both are common elements in the displays of many lizards but can be distinguished functionally as well as by reference to their underlying anatomy (see Chapter II). Yet, too many behavioral descriptions lack the detail necessary to discriminate among them. In fact, throat extension occurs in most reptiles, while dewlap extension is probably restricted to the lacertilian infraorder Iguania, which includes the families Agamidae, Chamaeleontidae and Iguanidae (Carpenter and Ferguson, '77).

Choice of Subject

The lizard Anolis equestris (Merrem, 1820) was chosen for this study because its large size facilitates surgical and other experimental manipulations. The superspecies Anolis equestris comprises five species (A. equestris, A. luteogularis, A. noblei, A. smallwoodi and A. baracoae), all endemic to Cuba (Schwartz and Garrido, '72). The species A. equestris was included in Etheridge's ('59) Alpha section of the genus based on the structure of the caudal vertebrae, and in Williams' ('76) carolinensis series (a division of the Alpha section) based on morphological and karyological characters. Ecologically, A.

equestris has been characterized as a giant crown anole (Williams, '69), a highly arboreal species usually found at heights above 5 m on orchards, palm groves and trees along roads (Collette, '61; Ruibal, '64). In 1952 the subspecies A. equestris equestris was introduced into suburban Miami (King and Krakauer, '66), where it continues to expand its range (Dalrymple, '80).

Anolis equestris is an insectivorous lizard with a tendency toward omnivory (Dalrymple, '80). Its diet in the wild consists mostly of insects and spiders, but includes occasional gastropods, tree frogs, nestling birds, smaller anoles and fruit (Pseudophoenix and Ficus berries) (Collette, '61; Ruibal, '64; Brach, '76; Dalrymple, '80). In the laboratory, lizards were fed crickets, cockroaches, katydids, mealworms, baby mice and rats, and adult A. carolinensis. Fruit (bananas, peaches) and Madagascan hissing cockroaches (Gromphadorhina sp) were offered but not taken by the lizards.

Information on the behavior of Anolis equestris is scant (Ruibal, '64; Brach, '76). Observations in the wild are hindered by this species' arboreal habits (personal observation). In laboratory enclosures, lizards exhibit dramatic defensive displays when approached by a human (see Chapter II). Headbobbing displays are more difficult to elicit. A study using multivariate clustering techniques identified five different display types from a sample of 40 displays recorded in the laboratory (Font and Kramer, submitted).

Although Anolis equestris are robust and recover well from surgery, a fraction of the lizards used in this study failed to adapt to captive conditions and over the course of several months dehydrated, lost weight and died. Post-mortem examination revealed a severe parasitic infection (Freitaxia sp) that may have been responsible for their demise. Several nematodes have been reported from wild populations of A. equestris (Barus and Otero, '68). Information on this species is insufficient to assess whether the heavy parasite loads of captive lizards are a cause or a consequence of their maladaptation to captive conditions.

Overview

Following this introduction, Chapter II examines the mechanism of dewlap extension in Anolis equestris. The dewlap is supported by the hyoid apparatus, which forms the skeletal framework of the buccal floor. Because of its value as a systematic character (e.g., Cope, 1892; Camp, '23), the hyoid of lizards has generated an extensive literature. However, available descriptions are contradictory and use inconsistent terminology. In many cases, this is complicated by the use of inappropriate illustrations or no illustrations at all. For these reasons, a study of muscles and skeletal elements in the buccal floor of A. equestris was undertaken prior to investigating the mechanism of dewlap extension.

Previous studies of the mechanism of dewlap extension (e.g.,

von Geldern, '19) had suggested that dewlap extension is accomplished by contraction of a single pair of hyoid muscles. Results of electromyography, electrical stimulation and denervation experiments confirmed this hypothesis and provided the basis for a study of the central control of dewlap extension.

Mapping of neural pathways has classically been done using lesion, stimulation and recording techniques. More recently, other anatomical procedures have been used to characterize functional pathways involved in the generation of specific behaviors. In particular, retrograde tracers such as horseradish peroxidase (HRP) have been used with success to map the motor output to a variety of effector organs. Because the dewlap is extended by a single pair of hyoid muscles, it lends itself well to this experimental approach. Chapter III describes experiments using HRP to identify brain stem motoneurons subserving the muscles that extend the dewlap. A second set of experiments uses similar techniques to identify afferent sources to those motoneurons. Results of these experiments suggest candidate brain nuclei contributing to the generation of the dewlap display.

Chapter IV summarizes the main findings of this study and presents ideas regarding the evolution of the dewlap. A final section is devoted to a comparison of the proposed neural pathway for dewlap extension with central networks for vocal control in vertebrates.

CHAPTER II

FUNCTIONAL MORPHOLOGY OF DEWLAP EXTENSION

Introduction

The dewlap or gular fan, an extendible flap of skin under the throat, is a conspicuous "morphological adjunct" (Greene, '87) in the displays of many lizards, especially agamids and iguanids of the genus Anolis (Sauria, Iguanidae). Because of its potential for inter- and intraspecific signalling, the dewlap of Anolis has long been a topic of interest (Greenberg and Noble, '44; Rand and Williams, '70; Crews, '75; Williams and Rand, '77; Echelle et al., '78; Sigmund, '83; Fitch and Hillis, '84; Losos, '85). However, few studies have examined the mechanism of dewlap extension in Anolis (Bell, 1826; von Geldern, '19; Gnanamuthu, '30, '37).

The dewlap is supported by the hyoid apparatus, a derivative of the hyoid arch and posterior branchial arches of fishes. From a primitive role as a support for the gills, the hyoid apparatus has undergone pronounced evolutionary change in all vertebrate taxa (Corsy, '33; Romer and Parsons, '77). The hyoid apparatus of modern lizards is used in feeding, drinking, respiration, chemoreception, and possibly sound reception (Gnanamuthu, '37; Arechaga, '77; Smith, '84, '86). In addition, the hyoid supports "morphological adjuncts" displayed during interactions with conspecifics, other lizards and potential

predators, such as the dewlap of Anolis and other lizards, and the frills of the agamids Chlamydosaurus kingii and Amphibolurus barbatus (= Pogona barbata) (Kent, 1895; Beddard, '05; Throckmorton et al., '85).

The morphology of the lacertilian hyoid has been examined by many authors (reviewed in Avery and Tanner, '71; Tanner and Avery, '82). However, available descriptions are not always in agreement and many lack appropriate illustrations. Functional studies of the hyoid of lizards are more infrequent. Smith ('84, '86) investigated the role of the hyoid in feeding, drinking and chemoreception but did not examine dewlap displays.

In this paper, I first describe the morphology of the hyoid apparatus and associated musculature in Anolis equestris, then propose a mechanism for dewlap extension based on results of morphological and experimental techniques. Last, dewlap extension is compared to other hyoid displays.

Materials and Methods

Experimental Animals and Maintenance

The knight anole, Anolis equestris (Merrem, 1820), was chosen because of its large size facilitating surgery and other experimental manipulations. Also, preliminary information was available on its captive care and display behavior (Brach, '76; Dalrymple, '80; Font and Kramer, submitted). This species is a recent introduction to the Miami area from Cuba (Dalrymple, '80).

Nineteen adult lizards (49-106 gm; 132-166 mm snout-vent length) were purchased through commercial animal dealers (Pet Farm Inc., Miami) and kept in the laboratory for up to two years prior to experimentation. Both male (N=15) and female (N=4) lizards were used in these experiments since Anolis equestris shows little sexual dimorphism in size or dewlap morphology (Collette, '61; Ruibal, '64).

The lizards were individually housed in 110 liter glass vivaria with sphagnum moss as ground litter and tree branches for perching. The vivaria were kept in a temperature controlled room (ca. 26° C). Ultraviolet supplemented lights (Vitalite) above the vivaria provided a 14 h daily photoperiod. The vivaria were sprinkled with water three times weekly and water was continuously available in shallow dishes. Food consisted of crickets, cockroaches, katydids, mealworms, baby mice and rats, and adult Anolis carolinensis. Occasionally, the largest food items were injected with a vitamin supplement (Avitron) before being offered to the lizards.

Morphological Techniques

Terminology

With few exceptions, terminology used in this study follows the work of Throckmorton et al. ('85). Their terminology emphasizes anatomical relations rather than presumed homologies of muscles and skeletal elements. Tables 1 and 2 present synonyms used in previous descriptions of the lacertilian hyoid.

Table 1. Comparisons of Terms Used for the Hyoid Apparatus of Lizards in this Study and in the Literature[1].

This Study	Literature
Body	(2,4,6,7,8,11,13,15,16,17) basihyal (1-4,11,12,14); basihyoid (5,9,13,15,16); corpus hyoideum (8); copula (8,10)
Entoglossal process	(4,5,8,9,11-17) glossohyal (1,3,12); entoglossal stylet (2); anterior extension (4); pars entoglossus (6); hypohyal (7,16); processus lingualis (8,10,15)
Hypohyal	(1,3,6,10,11,12,15,17) anterior horn, proximal portion (2,9); anterior horn (= ceratohyal), first part (4); anterior cornu (5); ceratohyal (7); hyoid cornu (8,15); body (= basihyoid) (13); anterior process (16)
Ceratohyal	(1,3,7,10,11,12,14-17) anterior horn, distal portion (2,9); anterior horn (= ceratohyal), second part (4); anterior cornu (5,13); stylohyal (6); epihyal (8,15)
Ceratobranchial I	(1,3,7,8,10,11,12,14-17) posterior horn (2,4); ceratobranchial (4); thyrohyal (4,6); posterior cornu (5,13); posterior cornu, proximal piece (9)
Ceratobranchial II	(1,3,7,8,10,11,12,14-17) accessory horn (2); processus retrobasalis (4); basibranchial (5); urohyal (6); basibranchial process (13)
Epihyal	(10,16,17)
Epibranchial I	(8,10,15,17) epibranchial (1,3,16); posterior cornu, distal piece (9); posterior cornu (13)

[1]Initial set of numbers indicates authors using terminology used in this study. Key to numbers: 1) Cope (1892); 2) Chemin (1899); 3) Willard ('15); 4) von Geldern ('19); 5) Gnanamuthu ('30, '37); 6) Kesteven ('44); 7) Oelrich ('56); 8) Romer ('56); 9) Sondhi ('58); 10) Jollie ('60); 11) Langebartel ('68); 12) Avery and Tanner ('71); 13) John ('72); 14) Rieppel ('78); 15) Tanner and Avery ('82); 16) Smith ('84, '86); 17) Throckmorton et al. ('85).

Table 2. Comparisons of Terms Used for the Buccal Floor Musculature of Lizards in this Study and in the Literature.

This Study	Literature
M. intermandibularis anterior	(2,6,8,9,10,13,14) mylohyoideus (1,3); mylohyoideus anterior (2,4,10,12,13); platysma myoides (3); intermandibularis + submentalis (5); mentalis (7); intermandibularis (11)
M. intermandibularis posterior	(2,6,8,9,10,13,14) mylohyoideus (1,3); mylohyoideus posterior (2,4,10,12,13); platysma myoides (3); intermandibularis (5,11); mylohyoideus anterior (7)
M. constrictor coli	(4,6,8-14) sphincter colli (2,6); superficial constrictor (5); mylohyoideus posterior (7)
M. ceratomandibularis externus	(14) cerato-maxillaire (1); ceratomandibularis 1 (2); mandibulohyoid (3); geniohyoideus + adductor inferior labioris (4); thyromandibularis (5); mandibulohyoideus I (6,9,12); geniohyoideus (7,10,13); ceratomandibularis (8); geniohyoideus II (11); mandibulohyoideus II (13)
M. ceratomandibularis internus	(14) cerato-genien (1); ceratomandibularis 2 (2); geniohyoideus (3,4,5,7,10,13); mandibulohyoideus II (6,9,12); ceratomandibularis (8); geniohyoideus I (11); mandibulohyoideus I (13)
M. hyomandibularis	(5,14) ceratomandibularis 3 (2); myloceratoid (3,4,10); ceratomandibularis (4); geniocerato ideus (4); mandibulohyoideus III (6,9,12,13); geniocerato ideus + mandibulohyoideus (7); geniohyoideus (8); mylohyoideus III (12); geniohyoideus + geniocerato ideus (13)
M. omohyoideus	(1-4,6,9-13) sternothyroideus (5); omohyoideus + sternohyoideus (7); omohyoideus + sternohyoideus superficialis (8); sternohyoideus (14)

This Study	Literature
M. sternohyoideus	(2,3,6,11,13) sterno-cleido-hyoideus (1); sternohyoideus + sternothyroideus (4,9,10,12); sternothyroideus (5,7); sternohyoideus profundus (8); omohyoideus (14)
M. ceratohyoideus	(2,3,4,8,10-14) cera-ceratoideus (1); hyoglossus (2); thyrohyoideus (5); branchiohyoideus (6,9,13); ceratohyoideus + interportialis + cornuohyoideus (7)
M. hyoglossus	(1,3-14) genioglossus (2)
M. genioglossus	(1,4-14) lng. lg. (2); myloglossus (3); geniohyoideus (12)

[1]Initial set of numbers indicates authors using the terminology used in this study. Key to numbers: 1) Chemin (1899); 2) Willard ('15); 3) von Geldern ('19); 4) Gnanamuthu ('30, '37); 5) Kesteven ('44); 6) Oelrich ('56); 7) Sondhi ('58); 8) Langebartel ('68); 9) Avery and Tanner ('71); 10) John ('72); 11) Rieppel ('78); 12) Tanner and Avery ('82); 13) Smith ('84, '86); 14) Throckmorton et al. ('85).

Such descriptions encompass a wide range of species. For Anolis (mainly A. carolinensis) information on the hyoid or its muscles can be found in Cope (1892), Willard ('15), von Geldern ('19), Gnanamuthu ('37), Kesteven ('44), Crews ('80), and Tanner and Avery ('82).

Dissection

The musculoskeletal components of the hyoid apparatus were examined by gross dissection in 12 Anolis equestris freshly killed or preserved in 10% formalin. Specimens included experimental animals and those drawn from the collection of N. Greenberg (University of Tennessee). Drawings of dissected material were done through a Wild binocular microscope using an iodine solution to increase contrast between muscles and surrounding tissue (Bock and Shear, '72). Comparative dissections were made of several specimens of A. carolinensis and A. cybotes, two related but much smaller species.

Histology

The composition of the hyoid apparatus was examined in two Anolis equestris by sectioning and histological staining. The hyoids and adjacent tissue were embedded in paraffin and serially sectioned in the transverse plane at 10 μm using a microtome. Alternate sections were stained either with Milligan trichrome stain, which distinguishes muscle and connective tissue, or Alizarine red S, specific for calcium deposits (Humason, '79). Sections were studied and drawn using an

Olympus microscope. Similarly stained sections from the hyoids of three A. carolinensis were available for comparison.

Radiography

X-rays of two live A. equestris with the hyoid at rest and during dewlap extension were made using a Siemens radiographic unit set at 40 kVp, 5 mAs. Dewlap extension was elicited by teasing or holding the lizard in the experimenter's hand, prompting a defensive display which included gaping and long bouts of dewlap extension. During defensive displays, the position of the hyoid in relation to other structures in the buccal floor could be studied by looking into the lizards' open mouth.

Experimental Techniques

Electrical Stimulation

The actions of muscles in the buccal floor were investigated by electrical stimulation in three Anolis equestris. Muscles and nerves in the buccal floor were exposed by dissection under deep ketamine anesthesia and stimulated through tungsten needles attached to a Grass S44 stimulator.

Nerve transection

Previous studies suggested that dewlap extension is effected by contraction of paired M. ceratohyoideus (e.g., von Geldern, '19). In lizards, M. ceratohyoideus is innervated by a branch of the ramus pharyngo-laryngeus (RPL), a combined nerve carrying

fibers from several cranial nerves (Willard, '15; Oelrich, '56). No other hyoid muscles are innervated by this branch of RPL[1]. The effects of interrupting the nerve supply to M. ceratohyoideus were studied in six Anolis equestris. In these animals, the RPL on one side was surgically transected at the posterior end of M. ceratohyoideus. Dewlap extension was elicited as described above.

Electromyography

Two large male Anolis equestris were used in electromyography (EMG) experiments. The animals were anesthetized by intramuscular injection of ketamine hydrochloride at dosages of 180-200 mg/kg (Font and Schwartz, '89). Incisions were made in the intermandibular region to expose the musculature of the buccal floor. Bipolar hook electrodes constructed with 60 μm insulated electromagnet copper wire were inserted into M. ceratohyoideus and into the lateral portion of M. omohyoideus using 22-gauge hypodermic needles (Loeb and Gans, '86). Electrode wires were run subcutaneously to connectors in the animal's back. Proper electrode placement was confirmed by electrical stimulation at the time of surgery and by post-mortem dissection.

After recovery from surgery the animals were presented in their home vivaria with a conspecific intruder or a mirror to

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[1]A complete list of abbreviations used in this Chapter may be found in Appendix A, page 98.

elicit headbobbing and dewlap displays. Their behavior was observed using a closed-circuit TV system attached to a video recorder. During recording, a light-weight cable with a swivel mechanism joined the connectors in the animal's back to the recording apparatus. The wires did not appear to impair the lizards' behavior. EMG signals were amplified by a Phipps and Bird amplifier-oscilloscope assembly set at a frequency range of 10-30,000 Hz, and recorded on a Teac A-3440 reel-to-reel tape recorder. One of the signals was simultaneously recorded on the audio track of the video tape to allow synchronization of video and EMG recordings.

Electromyograms were analyzed by playing back the tape recordings through a chart recorder at the same speed used for recording (15 in./sec). Video films were analyzed frame-by-frame using a TV projection system (Miami Flock Equipment Co.). For each display, the position of the lizard's snout and the extent of dewlap extension every 1/30 sec were plotted onto graph paper (Jenssen, '78; Jenssen and Gladson, '84). In order to correlate behavior and muscle activity patterns, headbobbing and dewlap displays were graphed on the same time scale as the EMG recordings.

Results

Behavioral Observations

Dewlap extension in Anolis equestris was observed in two contexts. When confronted by a human intruder, lizards

exhibited defensive (i.e., antipredator) displays. Similar displays in the wild are addressed to potential predators (Ruibal, '64). Defensive displays consist of lateral presentation, sagittal trunk compression, nuchal crest erection, gaping with the tongue partially extruded, throat and dewlap extension. The dewlap remains extended for long periods, even when the lizard is held by the experimenter (a "static" display; Barlow, '77). Headbobbing displays were addressed to conspecifics or mirrors placed inside the animals' vivaria. Headbobbing displays consist of stereotyped up-and-down movements of the head. Five different display types were identified (Font and Kramer, submitted), four of which incorporate a single dewlap pulse of relative short duration (<10 sec). Most headbobbing displays end in a side-to-side movement of the head, usually coinciding with the folding of the dewlap. "Dewlapping" (repeated dewlap extension and withdrawal) independent of headbobbing movements was occasionally observed. This dewlapping may represent a weakly stereotyped display and is present in other anoles (Jenssen, '77).

A presumed benefit of dewlap extension is the increased conspicuousness and apparent body size it accords a displaying lizard (Williams and Rand, '77; Chiszar, '78; Greene, '87). Planimetric measurements taken from Super-8 films indicate that dewlap extension increases the lateral profile of a displaying Anolis equestris by as much as 20% (Fig. 1). Further increases

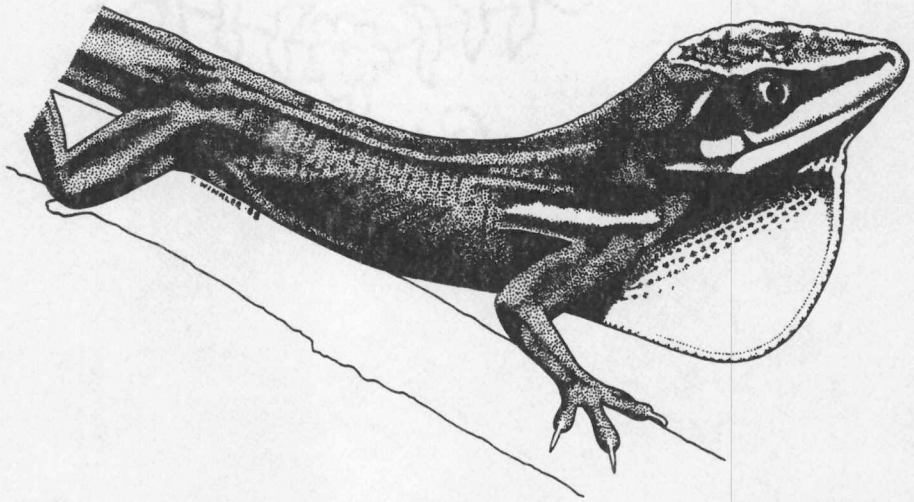

Figure 1. Dewlap Extension in Anolis equestris. Drawn from Super-8 film sequence of headbobbing lizard.

can be accomplished by sagittal trunk compression, nuchal crest erection and throat extension.

In addition to displays, dewlap extension in other iguanid lizards occurs during basking (Rand, '68), shedding, and after eating (Jenssen, '77) and drinking (Distel and Veazey, '82).

External Morphology of the Dewlap

The dewlap of Anolis lizards is a longitudinal fold of elastic skin located along the midventral line of the throat. The extended dewlap has a characteristic semicircular outline due to the flexibility of cartilaginous second ceratobranchials that unfold it (Fig. 1).

In Anolis equestris, most of the dewlap skin is devoid of scales. Only 4-6 horizontal rows of scattered gorgetals and sternals (terminology of Fitch and Hillis, '84) are visible across the dorsal third of the dewlap. This arrangement results in large portions of bare skin being exposed when the dewlap is extended. In other anoles (e.g., A. carolinensis), gorgetal-sternal rows almost cover the entire dewlap. In the Miami population of A. equestris the bare skin between dewlap scales is pale pink (2.5-5R 9/2; Munsell book of color), probably due to the presence of pteridine pigments (Ortiz et al., '63), and contrasts with the bright green-blue of the head, dorsum, limbs and tail.

The Hyoid Skeleton

The hyoid apparatus of Anolis equestris is embedded in the

tissue of the buccal floor, of which it forms the skeletal framework (Fig. 2). The hyoid and its musculature are separated from the oropharyngeal cavity by a sheet of mucosa containing abundant melanocytes. The hyoid apparatus is cartilaginous except for the first ceratobranchials, and is surrounded by a dense tendinous sheath which provides a surface for insertion of hyoid muscles (perichondrium; p in Fig. 3).

Directly beneath the larynx is the central portion, or body of the hyoid (Fig. 2). Anteriorly, the body is continuous with the unpaired entoglossal process. The entoglossal is short and stylet-like, separated from the musculature of the tongue by a fluid filled sac. Paired hypohyals project anterio-dorsally from the body and run for a short distance parallel to the entoglossal process. Slender ceratohyals and first ceratobranchials articulate with the distal ends of the hypohyals and with the body of the hyoid by movable synovial joints (s in Figs. 3B and 3D). Ceratohyals and first ceratobranchials extend posterio-laterally from their respective attachments, reaching around the sides of the head. Proximally, the first ceratobranchials are located ventral to the ceratohyals. Distally, they are dorsal to them due to the sharp curvature of the ceratohyals around M. pterygoideus. Short, cartilaginous epihyals and first epibranchials attach by fibrous joints to the distal ends of ceratohyals and first ceratobranchials, respectively. Posteriorly, the body of the hyoid is continuous with paired second ceratobranchials. The

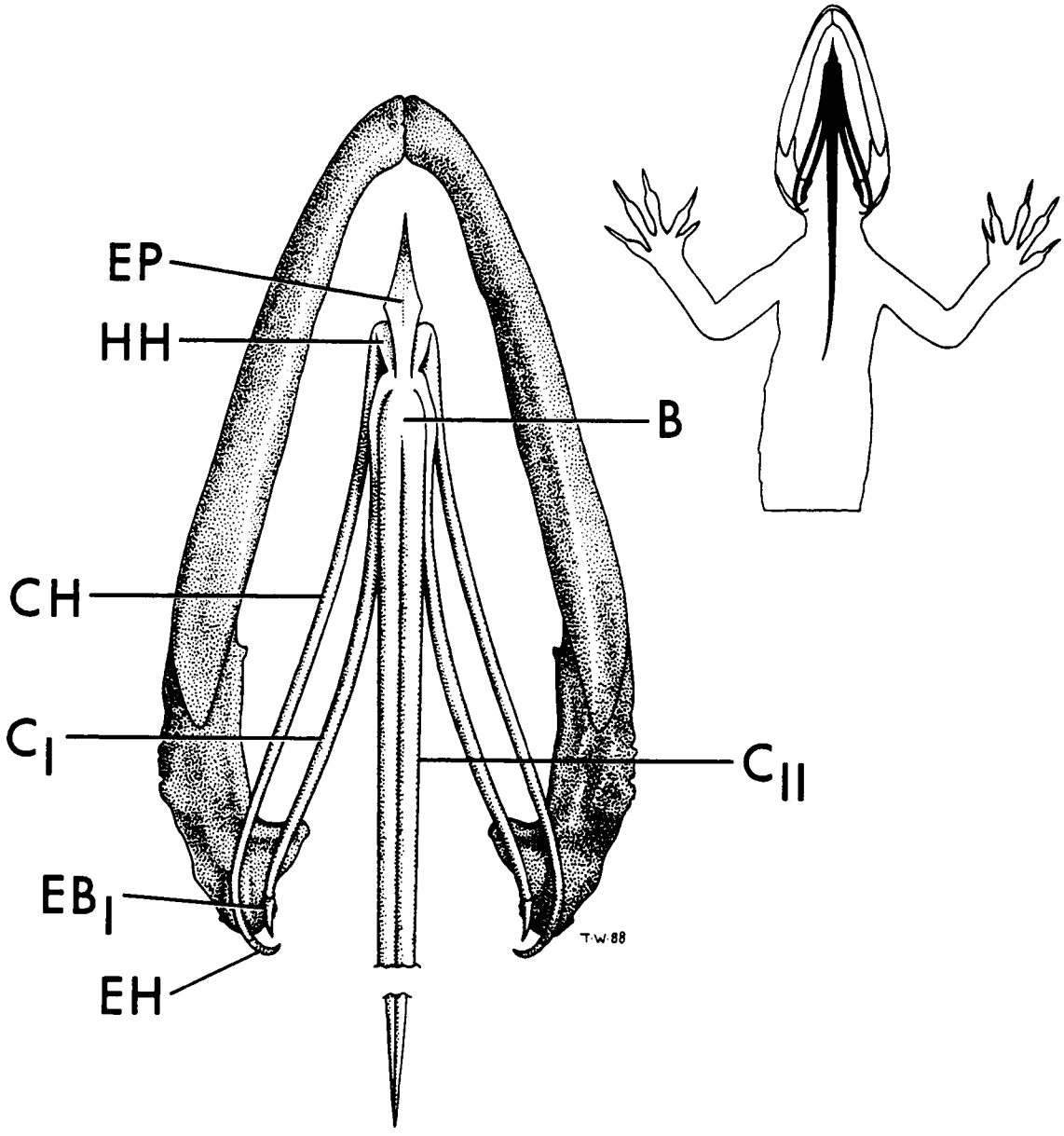

Figure 2. Hyoid Apparatus of Anolis equestris, Ventral View. Inset shows hyoid apparatus in situ. For abbreviations used in this Figure see Appendix A, page 98.

Figure 3. Histologic Composition of the Hyoid Apparatus. Drawn from frontal sections stained with Milligan trichrome stain or Alizarine red S. The level of the cross sections is indicated at the top. Note the presence of calcium deposits in most hyoid elements (crosshatching). Scale bar, 1 mm. For abbreviations used in this Figure see Appendix A, page 98.

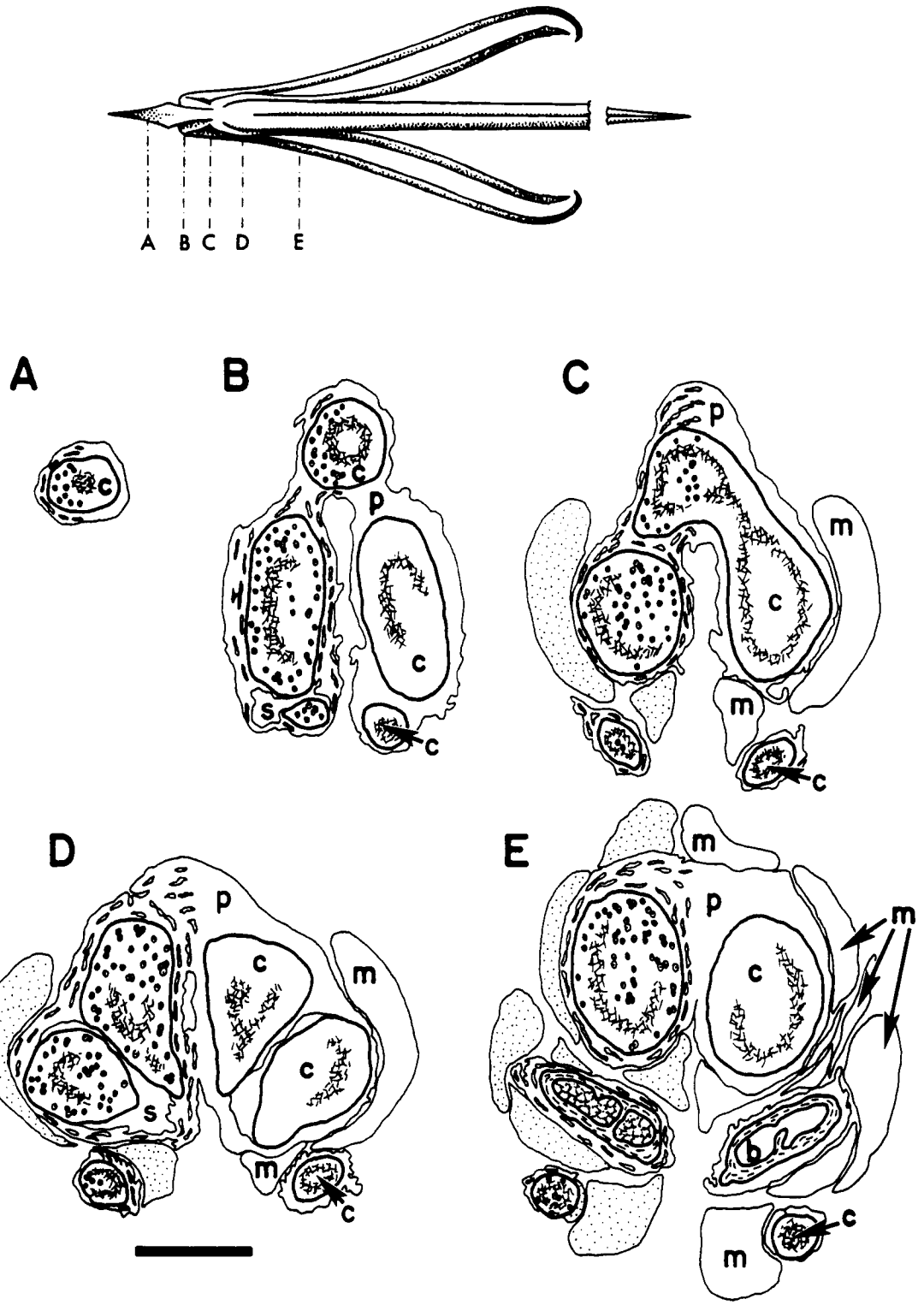

Figure 3

two lay in close apposition and share a common perichondrial sheath throughout most of their length, tapering considerably as they extend away from the body of the hyoid (Fig. 2). The second ceratobranchials support the dewlap during dewlap extension; they are the longest elements of the hyoid (40% of the lizards' snout-vent length) and reach as far back as the middle of the sternum. In three animals examined, old fractures were present in one of the second ceratobranchials.

With the hyoid in a resting position, the epihyals curve around the retroarticular process of the mandible. During dewlap extension they push against the overlying skin and form small ridges on either side of the neck.

The hyoid skeleton of Anolis carolinensis resembles that of A. equestris in its major components. In A. carolinensis the entoglossal process is longer relative to the size of the hyoid. This results in the body of the hyoid being located further back along the intermandibular space. Only a narrow constriction marks the transition between hypohyals and ceratohyals. As in A. equestris, the first ceratobranchials articulate with the body of the hyoid by movable synovial joints. No epihyals are present in the hyoid of A. carolinensis.

The hyoid skeleton of two male Anolis cybotes was also examined. As in A. carolinensis, the entoglossal is long and slender. The second ceratobranchials are remarkably long (67% of the lizards' snout-vent length), reaching as far caudally as the pelvic girdle. The ceratohyals consist of a proximal

cartilaginous portion and a distal ossified portion. The proximal portion is dorsoventrally flattened and blade-like. Long epihyals attach to the distal ends of the ceratohyals.

Histologic Composition of the Hyoid Apparatus

The first ceratobranchials are the only ossified elements of the hyoid apparatus in Anolis equestris. They provide insertions for seven different pairs of hyoid muscles: Mm. ceratomandibularis externus and internus, M. genioglossus, M. hyoglossus, M. ceratohyoideus, M. omohyoideus and M. sternohyoideus. In cross section, the first ceratobranchials appear dorsoventrally flattened (Fig. 3). They are hollow, with a prominent marrow cavity which in A. equestris, but not in A. carolinensis, is traversed by numerous transverse septa. The remaining portions of the hyoid are made of hyaline cartilage. The cellular elements or chondrocytes in this cartilage are arranged in clusters of four (isogenic groups), presumably arising from mitotic divisions of a single cell (Junqueira et al., '77). The acellular matrix contains heavy calcium deposits, occupying the core of most cartilaginous elements of the hyoid (except epihyals and epibranchials). Calcium deposits in the second ceratobranchials are arranged in a semicircle open ventrally (Fig. 3E). The effect of such calcification upon the structural properties of the hyoid remains to be studied.

Musculature of the Buccal Floor

The muscles of the buccal floor are classified into six

groups according to their origins and insertions (von Geldern, '19; Smith, '84). The superficial throat musculature consists of thin muscles with no connection to the hyoid skeleton. Supra- and infrahyoid musculature refers to those muscles with insertions on the hyoid and origins in the mandible or the shoulder girdle. The intrinsic hyoid musculature consists in Anolis equestris of a single paired muscle, M. ceratohyoideus, extending between two arms of the hyoid. Of the tongue musculature, only the extrinsic muscles bear connections with the hyoid and might be involved in the mechanism of dewlap extension (see below). Descriptions of the intrinsic tongue muscles in lizards can be found in McDowell ('72), Smith ('84, '86, '88) and Schwenk ('86).

Dissections of the buccal floor revealed an additional muscle, M. pterygoideus, which inserts on the retroarticular process of the mandible. This massive muscle is part of the jaw adducting musculature and lacks connections to the hyoid (Smith, '82).

Superficial Throat Musculature

The superficial throat muscles in Anolis equestris are the Mm. intermandibularis anterior and posterior, and the M. constrictor coli (Fig. 4). The Mm. intermandibularis are thin, sheet-like muscles; they are the most superficial muscles on the ventral aspect of the rostral two thirds of the head. Both Mm. intermandibularis insert along a midline connective tissue raphe except for two distinct bundles of M. intermandibularis anterior

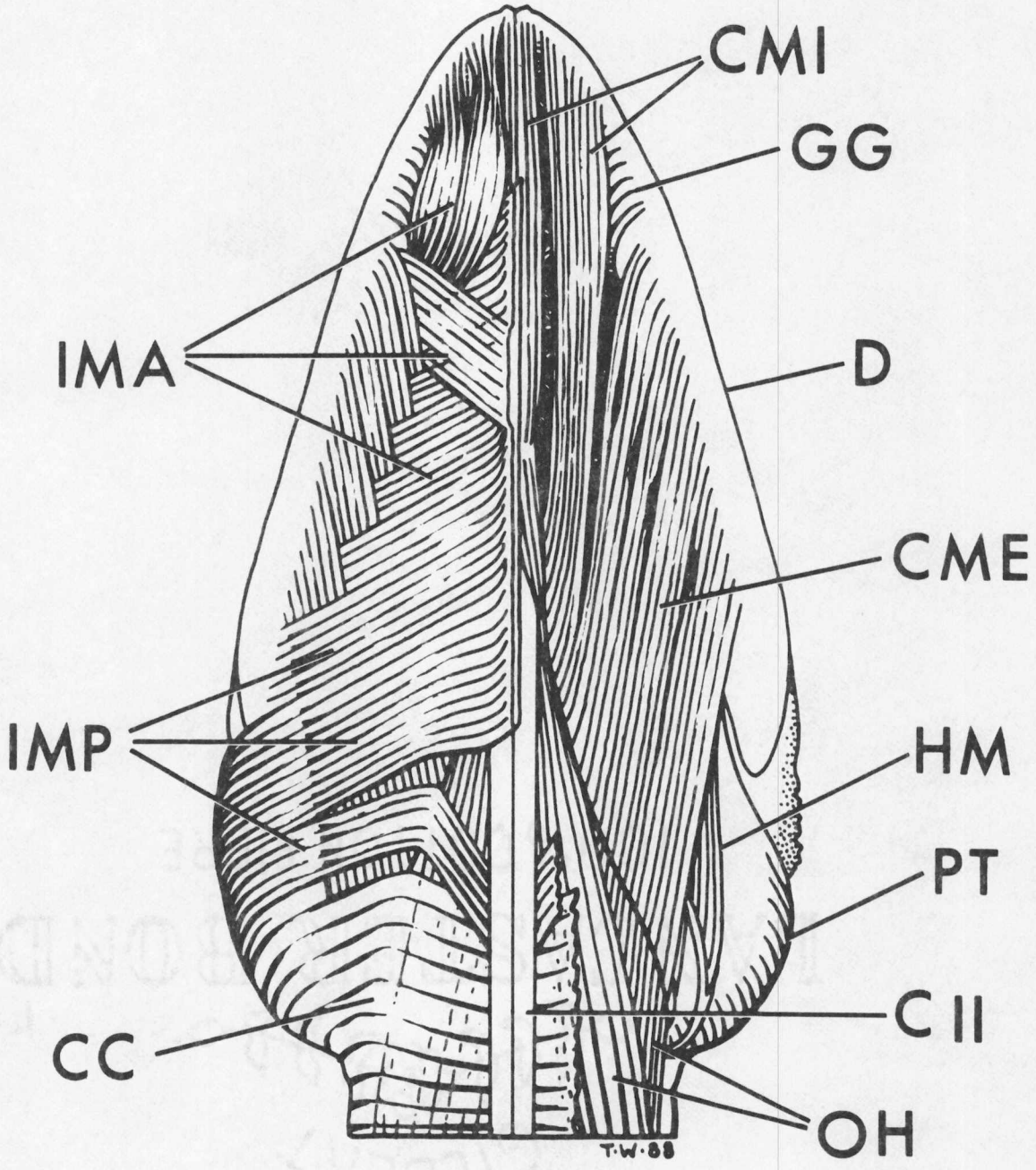

Figure 4. Muscles of the Buccal Floor, Superficial Layers. Mm. intermandibularis anterior and posterior and M. constrictor coli have been cut on the right to expose underlying muscles. For abbreviations used in this Figure see Appendix A, page 98.

attached to the symphyseal region of the mandible (Fig. 4). Beneath the body of the hyoid, a few superficial fibers from M. intermandibularis anterior insert on the underlying skin.

The M. intermandibularis posterior arises from ventral and ventrolateral borders of the mandible, superficial to M. pterygoideus. Caudally, a small bundle of fibers detaches from the main portion of M. intermandibularis posterior and passes dorsal to the second ceratobranchials of the hyoid apparatus to join fibers from the opposite side (Fig. 4). This bundle overlaps the anteriormost fibers of M. constrictor coli.

The M. intermandibularis anterior arises from the ventromedial border of the rostral half of the mandible, deep to the insertions of M. hyomandibularis and M. ceratomandibularis externus. It interdigitates with the latter muscle before reaching a superficial position on the ventral aspect of the buccal floor. The M. intermandibularis anterior can be divided into superficial and deep portions, the fibers of which run at almost right angles to each other.

The M. constrictor coli is the most superficial muscle of the neck. It originates on fascia of the skin in the cervical region and inserts along a midline raphe, dorsal to the second ceratobranchials. This muscle is exceedingly thin in Anolis equestris (Fig. 4).

Connective tissue, present between the superficial throat musculature and the skin in the gular region, may contribute to

the adherence of the skin to the buccal floor when the dewlap is withdrawn.

Suprahyoid Musculature

The suprahyoid musculature consists of three muscles: Mm. ceratomandibularis internus and externus, and M. hyomandibularis. The M. ceratomandibularis internus includes medial and lateral portions (Fig. 4). The medial portion is a thin, ribbon-like muscle bundle arising from the mandibular symphysis and the skin in the symphyseal region. It inserts on the hyoid apparatus, at the transition between the body and the second ceratobranchials. The lateral portion of M. ceratomandibularis internus arises from the ventral border of the mandible, lateral to the symphysis and deep to M. intermandibularis anterior, and inserts on the proximal one third of the ipsilateral first ceratobranchial. The M. ceratomandibularis externus arises as a broad sheet from the ventromedial border of the mandible, interdigitates with M. intermandibularis anterior, and inserts on the distal two thirds of the first ceratobranchial.

The origin of M. hyomandibularis is on the ventromedial border of the mandible, deep to M. ceratomandibularis externus. The muscle then courses posteriorly between M. ceratomandibularis externus and M. intermandibularis anterior, and inserts on the distal one third of the ipsilateral ceratohyal (Fig. 5).

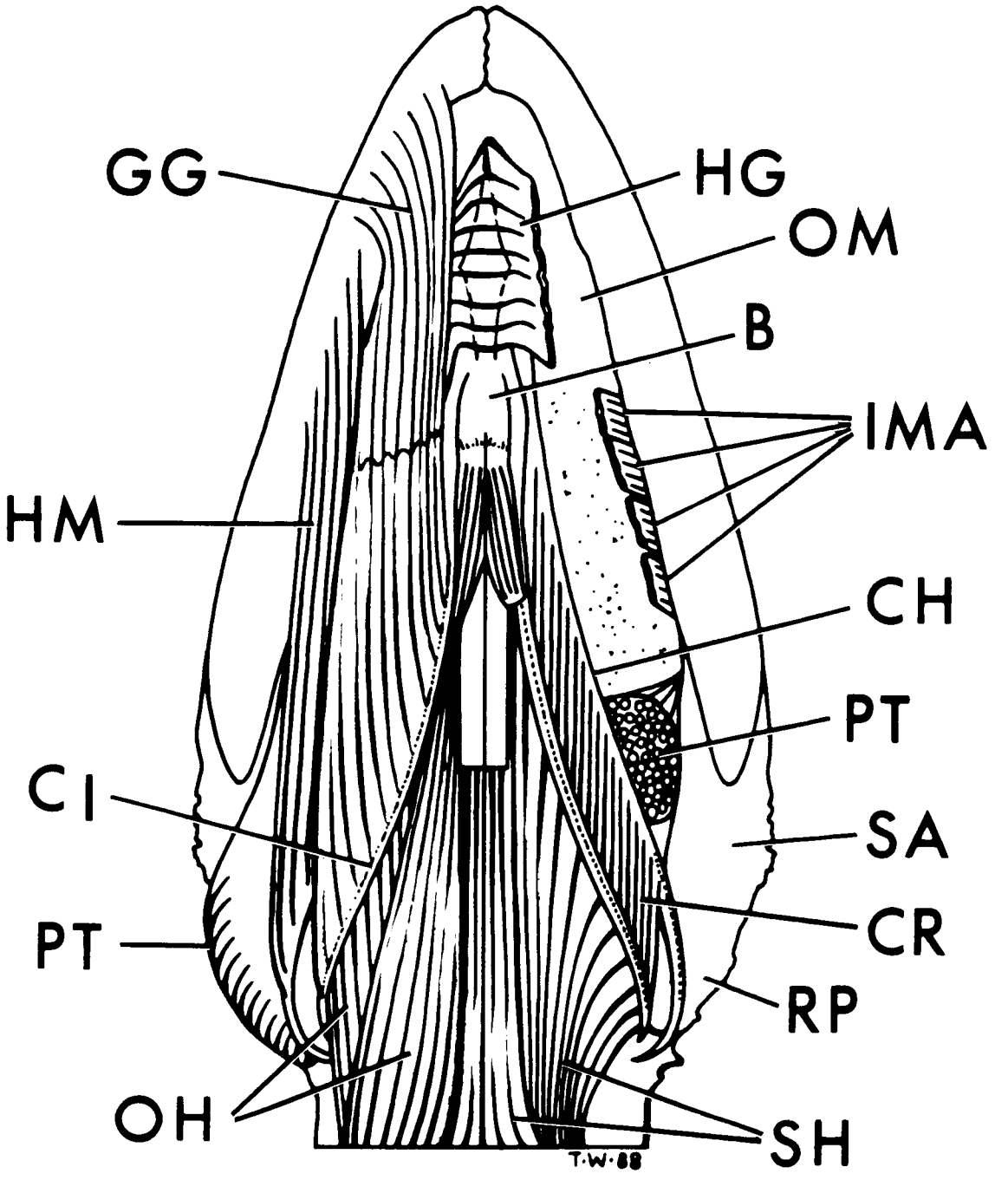

Figure 5. Deep View of the Muscles of the Buccal Floor. The superficial throat musculature and M. ceratomandibularis have been removed bilaterally. The Mm. genioglossus and hyomandibularis have been removed on the right to expose M. ceratohyoideus. For abbreviations used in this Figure see Appendix A, page 98.

Infrahyoid Musculature

Two muscles, M. omohyoideus and M. sternohyoideus, form the infrahyoid musculature (Fig. 5). The M. omohyoideus has medial and lateral portions originating from the shoulder girdle (clavicle and anterior border of the suprascapula) and inserting on the hyoid. The medial portion is broad near its origin and tapers as it extends anteriorly to insert at the base of the second ceratobranchial, caudal to the insertion of M. ceratomandibularis internus. The lateral portion of M. omohyoideus inserts along the ventral aspect of the first ceratobranchial of the hyoid apparatus. Fibers from M. intermandibularis posterior and M. constrictor coli conceal both portions of M. omohyoideus along most of their course.

The M. sternohyoideus is very thin in Anolis equestris. Like M. omohyoideus, it has medial and lateral portions (Fig. 5). The lateral portion (M. sternothyroideus of other authors; e.g., Gnanamuthu, '37) arises from the clavicle, interclavicle and sternum, and inserts along the first ceratobranchial, dorsal to the insertion of M. omohyoideus. A medial bundle can sometimes be distinguished running along both sides of the trachea to insert dorsally at the base of the ipsilateral second ceratobranchial, opposite the insertion of the medial portion of M. omohyoideus.

Intrinsic Hyoid Musculature

This musculature consists of a single paired muscle in A. equestris. The M. ceratohyoideus is a thin, parallel-fibered

muscle sheet extending between the first ceratobranchials and the ceratohyals on either side of the hyoid apparatus (Fig. 5).

Extrinsic Tongue Musculature

The extrinsic tongue muscles are M. genioglossus (tongue protractor) and M. hyoglossus (tongue retractor) (Fig. 5). The two fuse into a single muscle complex in lizards (Oelrich, '56). The M. genioglossus arises from the ventral border of the mandible, lateral to the symphysis, and inserts on the ventral surface of M. hyoglossus. Deeper fibers insert on the lateral edge of the tongue.

The fibers of M. hyoglossus originate along the distal two thirds of the first ceratobranchial, dorsal to the insertion of M. ceratomandibularis externus, and pass anteriorly around the body of the hyoid to merge with the intrinsic tongue musculature.

Muscle Stimulation

Stimulation of M. ceratohyoideus, but not of other hyoid muscles, brings the second ceratobranchials to a position perpendicular to the buccal floor. If the dewlap is intact, this movement simulates normal dewlap extension. Simultaneously, the ceratohyals slide posterio-laterally. The epihyals on their distal ends bend against the skin on the sides of the neck creating two small ridges below the angles of the jaws. The same movement pattern was observed when stimulating the ramus pharyngo-laryngeus.

Stimulation of suprahyoid muscles protracts the entire hyoid apparatus, whereas stimulation of infrahyoid muscles retracts it. Abduction of ceratohyals and first ceratobranchials is produced by stimulation of M. hyomandibularis and M. ceratomandibularis externus, respectively. Stimulation of M. omohyoideus, on the other hand, causes the first ceratobranchials to adduct about their joints with the body of the hyoid.

Electromyography of Key Hyoid Muscles

Activity in M. ceratohyoideus and M. omohyoideus (lateral portion) was assessed by electromyography. Simultaneous EMG and video recordings were made of 27 headbobbing displays given to conspecifics or mirrors. In addition, recordings were made of lizards feeding, drinking or moving about their cages.

Electrodes inserted in M. ceratohyoideus registered activity during the 14 headbobbing displays that incorporated dewlap extension. An original recording is shown in Figure 6 to illustrate the nature of muscle activity. Activity in M. ceratohyoideus starts at or immediately precedes the onset of dewlap extension. This activity continues while the dewlap is extended and often persists for a fraction of a second after the dewlap is seen to have folded back. However, precise timing of dewlap folding in relation to the EMG pattern was difficult to obtain because of the side-to-side movement of the head at the end of the display. Short bursts of activity occur in M. ceratohyoideus with each bout of lapping during drinking and

Figure 6. Electromyogram of Anolis equestris During Dewlap Extension. Representative data from experiments in which video recordings were synchronized with EMG recordings of two hyoid muscles during headbobbing displays in Anolis equestris. Upper graph depicts vertical displacement of the head (i.e., headbobs) through time, including the side-to-side movement of the head at the end of the display. Blackened block represents dewlap extension through time. Small vertical arrows enclose period of maximum dewlap extension. Note activity in M. omohyoideus (lateral portion) coinciding with the lateral head movement. Scale is in seconds.

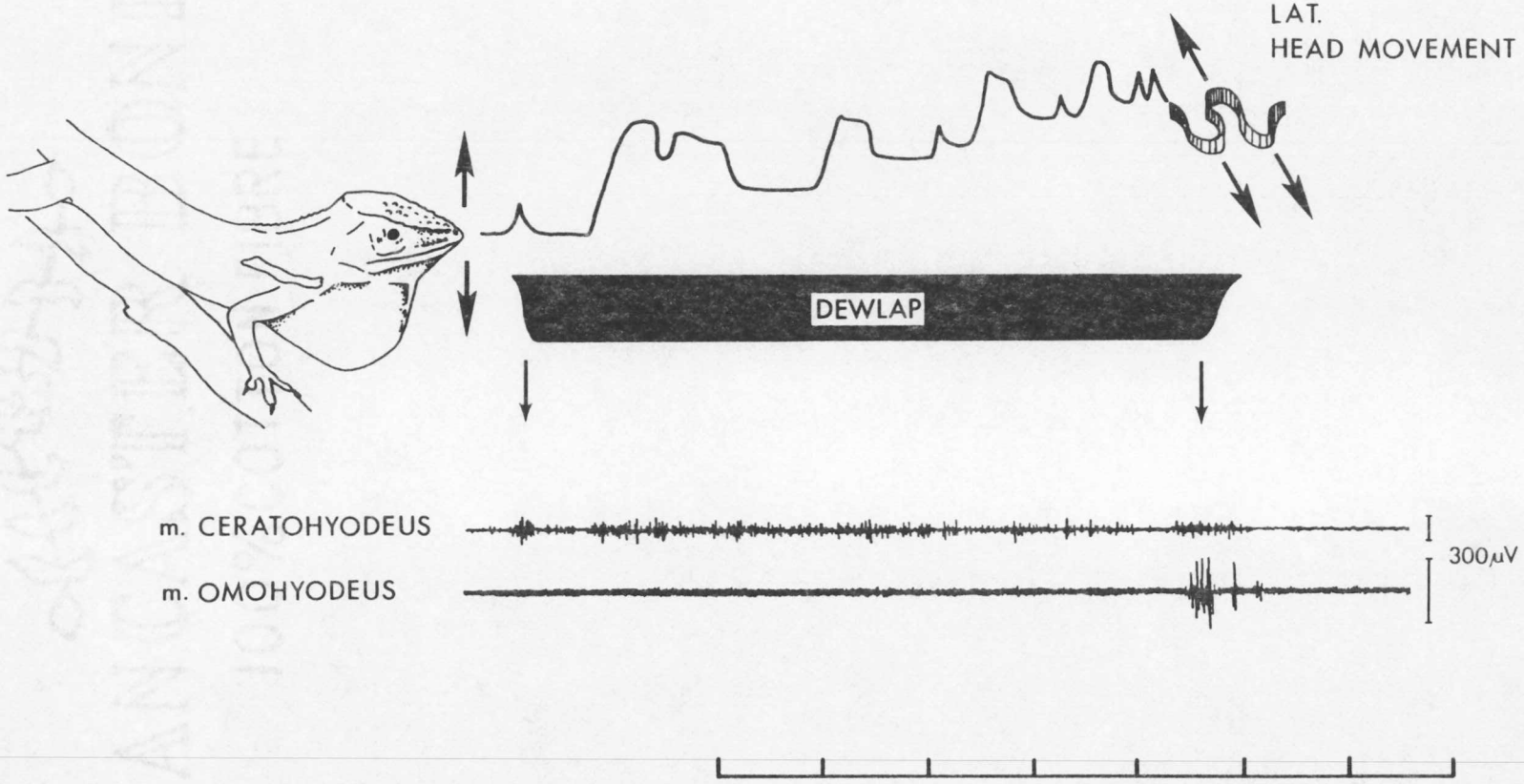
LAT.
HEAD MOVEMENT
DEWLAP
m. CERATOHYODEUS
m. OMOHYODEUS
300μV

Figure 6

chemosensory tongue protrusion. Activity is also present during all phases of feeding (for a description of the feeding cycle in lizards see Smith, '84).

The lateral portion of M. omohyoideus is active during feeding and locomotion, especially when bending the neck. During headbobbing displays this muscle shows bursts of activity coinciding with the lateral head movement at the end of the display (Fig. 6). Since this activity is also present in displays lacking dewlap extension, it suggests that the lateral portion of M. omohyoideus does not participate in dewlap extension or withdrawal. However, the medial portion of M. omohyoideus could contribute to the folding of the dewlap through its insertion on the second ceratobranchials (see below).

Effects of Denervation of M. Ceratohyoideus

Unilateral transection of the ramus pharyngo-laryngeus proximal to the M. ceratohyoideus on that side of the throat results in the dewlap extending obliquely, rather than perpendicular to the buccal floor. In every case the dewlap was angled toward the side that had its nerve supply interrupted. This would be expected if simultaneous contraction of both Mm. ceratohyoideus is required for normal dewlap extension.

Discussion

Hyoid Morphology

The hyoids of lizards differ both in the relative dimensions

of their parts and in the presence or absence of certain hyoid elements (for examples see Plates III-VI in Cope, 1892). Within anoline lizards, the hyoids are structurally similar (Etheridge, '59). They are elongated, rather than broad, and possess one pair each of ceratohyals, first ceratobranchials and second ceratobranchials. Epihyals and epibranchials are also present in some forms. The second ceratobranchials are long and slender, a feature presumably related to the possession of a dewlap. Elongated second ceratobranchials occur in other iguanid and agamid lizards that have dewlaps (Tanner and Avery, '82), and have been retained by all species of Anolis, including those, like A. agassizi and A. hendersoni, that have secondarily lost their dewlaps (Etheridge, '59).

The hyoid musculature of Anolis resembles that of other iguanid lizards (Kesteven, '44; Oelrich, '56; Avery and Tanner, '71). Elongated and roughly parallel ceratohyals and first ceratobranchials provide broad surface for insertion of a well developed M. ceratohyoideus. A M. ceratohyoideus in this position represents the primitive condition for lizards (Rieppel, '78). More recently evolved lizards (e.g., Varanus) have more than one pair of intrinsic hyoid muscles, contributing to complex movements of the hyoid during the feeding cycle (Mc Dowell, '72; Smith, '84, '86).

Previous Models of Dewlap Extension

The earliest interpretation of the mechanism of dewlap

extension is Bell's (1826). According to him, the dewlap of Anolis lineatus is extended ("when the animal is excited with anger or desire"; p. 192) by a backward pull of M. sternohyoideus on the hyoid apparatus. Since the distal ends of the second ceratobranchials attach to the skin in the abdomen, retraction of the hyoid would cause them to become arched, thus extending the dewlap "like the fabric that stretches over the ribs of an opened umbrella" (Bell, 1826; p. 193-194).

Chemin (1899) attributed dewlap extension in the agamid Calotes versicolor to contraction of M. sternohyoideus pulling the hyoid apparatus down, and to air swallowing. According to Chemin, M. ceratomandibularis internus forms a raphe with its counterpart of the opposite side. This "strap" of tissue prevents the entoglossal process from moving down, so that a downward pull by M. sternohyoideus would make the whole apparatus pivot on the body of the hyoid and extend the dewlap (Chemin, 1899).

These early models of dewlap extension were challenged by von Geldern ('19) and later Gnanamuthu ('30, '37), who noted that the second ceratobranchials are not fixed in the skin, nor does M. ceratomandibularis internus form a midline raphe in any of the species they studied. Moreover, contraction of M. sternohyoideus pulls the hyoid back rather than down and dewlap extension is independent of air swallowing.

In agreement with my results, von Geldern ('19) proposed that dewlap extension in Anolis carolinensis follows from

contraction of M. ceratohyoideus. Gnanamuthu ('30, '37) reviewed the anatomy of the buccal floor in a number of reptiles and concluded that in A. carolinensis, Calotes versicolor and Sitana ponticeriana, dewlap extension results from simultaneous contraction of M. ceratohyoideus and retraction of the tongue. A similar explanation was favored by Bellairs ('70; p.313). According to this interpretation, since M. hyoglossus (an extrinsic tongue muscle) inserts on the entoglossal process, tongue retraction would push the entoglossal upward and backward. Insofar as the entoglossal is continuous with the rest of the hyoid, this movement would supplement the action of M. ceratohyoideus and aid in dewlap extension. In fact, the entoglossal is not attached to the musculature of the tongue (Smith, '84, '88; present study). Furthermore, X-ray photographs demonstrate that, at least in A. equestris, the tongue is usually not retracted during dewlap extension. Instead, tongue protrusion and dewlap extension occur simultaneously during antipredator displays.

Mechanism of Dewlap Extension

The results of the present study suggest that dewlap extension in Anolis equestris occurs in two phases (Fig. 7). Initially, a slight depression of the buccal floor, possibly through relaxation of the intermandibular musculature, straightens the ceratohyals and brings them to a position dorsal and parallel to the first ceratobranchials (Fig. 7A). During the second phase of dewlap extension, contraction of M.

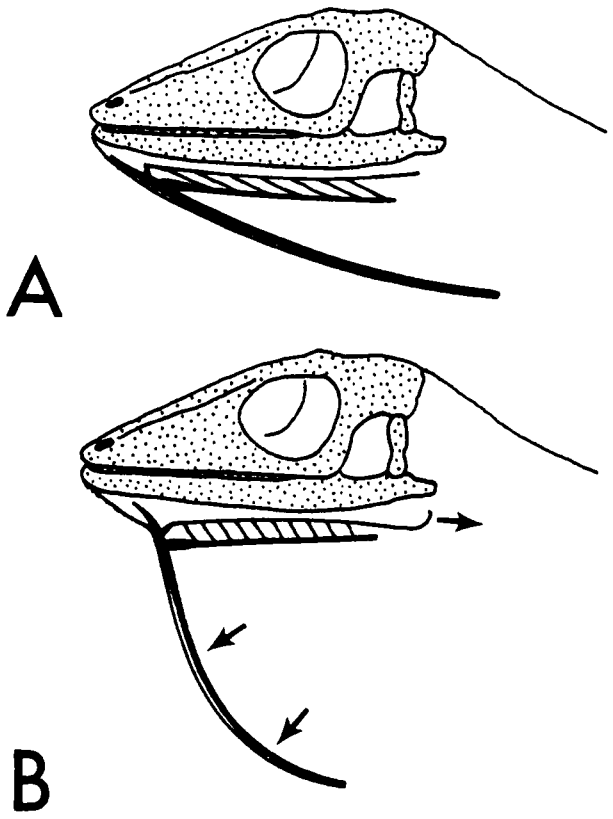

Figure 7. Proposed Mechanism of Dewlap Extension in Anolis equestris. For clarity, only one side of the hyoid is shown. The M. ceratohyoideus is drawn in situ as lines running between the ceratohyal and the first ceratobranchial. **A** Immediately preceding dewlap extension, the entire hyoid is slightly depressed, presumably through relaxation of intermandibular musculature. **B** Moment of maximum dewlap extension; arrows indicate direction of movement. Note bending on distal end of ceratohyal. Adapted from X-ray photographs.

ceratohyoideus pulls the ceratohyals posterio-laterally, narrowing the gap between ceratohyals and first ceratobranchials. The pull on the ceratohyals causes the hypohyals and the body of the hyoid to rotate dorsally around the first ceratobranchial/body joints while the second ceratobranchials swing ventrally extending the dewlap (Fig. 7B).

Thus, the hyoid functions as a first order lever. The hypohyals are the short or power arm, the second ceratobranchials are the weight arm, and the first ceratobranchial/body joints act as the fulcrum. This is in agreement with von Geldern's ('19) observations in A. carolinensis.

Although the Mm. ceratohyoideus on both sides of the hyoid usually work together, a relative independence is also possible. This permits some angling of the dewlap away from the mid-sagittal plane, as when an animal displays toward an antagonist positioned above or below its horizontal plane.

Since EMG activity was recorded from two muscles only, participation of hyoid muscles other than M. ceratohyoideus in the mechanism of dewlap extension cannot be evaluated. However the results of EMG, muscle stimulation, and denervation experiments demonstrate that M. ceratohyoideus plays a crucial role in dewlap extension in Anolis equestris.

A variation of this mechanism may be found in the South Indian flying lizard, Draco dussumieri. In this species, the medial bundle of M. ceratomandibularis internus is well

developed and inserts along the proximal one third of the second ceratobranchials (Gnanamuthu, '37; John, '72). This is unlike the condition in Anolis, where the muscle is thin and has a smaller insertion on the body of the hyoid. Contraction of M. ceratomandibularis internus may assist the action of M. ceratohyoideus in Draco by pulling forward on the second ceratobranchials (Gnanamuthu, '37; John, '72). This hypothesis is supported by the fact that the dewlap of Draco projects beyond the lizard's snout when extended (Hairston, '57), farther rostrally than in the majority of anoles. Draco dussumieri also has wattles, small appendages on either side of the neck that are extended during dewlap displays (John, '72) and are reminiscent of ridges (described above) in A. equestris and jowls in Iguana iguana (Distel and Veazey, '82). The wattles of D. dussumieri are extended by the first ceratobranchials of the hyoid apparatus, which in this species are longer than the ceratohyals (Gnanamuthu, '37; John, '72). The ridges on either side of the neck in A. equestris, on the other hand, are extended by the epihyals attached on the distal ends of the ceratohyals; their combined length exceeds that of the first ceratobranchials.

Dewlap folding or withdrawal is likely due to contraction of the medial portions of Mm. omohyoideus and sternohyoideus. Because they insert at the base of the second ceratobranchials, contraction of these muscles will pull the extended second ceratobranchials back to a horizontal position. Relaxation of

M. ceratohyoideus and the elastic nature of second ceratobranchials and dewlap skin also contribute to restoring the hyoid to a resting position.

Other Hyoid Displays

In addition to dewlap extension, displaying Anolis lizards often exhibit throat extension, a conspicuous ventral expansion of the buccal floor (Stamps and Barlow, '73; Greenberg, '77; Jenssen, '79). Throat extension is widespread among all orders of reptiles, including the rhynchocephalian Sphenodon punctatus (Carpenter and Ferguson, '77; Gans et al., '84). In A. equestris, throat extension involves simultaneous depression and protraction of the hyoid apparatus. Relative to other iguanid lizards, the entoglossal process of A. equestris is short and highly tapered and resembles the processes of agamid lizards (Smith, '88). When the hyoid moves forward, the entoglossal bends beneath the mandibular symphysis and the body of the hyoid pushes against the skin in the mental area, externally creating the appearance of a chin. According to Oelrich ('56), throat extension in Ctenosaura (Iguanidae) is accomplished by contraction of the suprahyoid musculature. X-ray photographs of A. equestris with extended throats show a curvature of the distal ends of the ceratohyals suggesting that M. hyomandibularis is actively pulling on them. Without additional information, participation of other suprahyoid muscles in the mechanism of throat extension is uncertain.

Throat and dewlap extension may occur simultaneously,

indicating a complex pattern of muscle activation. This is usually seen during defensive (i.e., antipredator) displays in which the second ceratobranchials sweep ventrally while the hyoid is held in a protracted position. The combination of throat and dewlap extension results in the dewlap being located further rostrally than when dewlap extension occurs alone.

The hyoid displays of some Australian agamids differ greatly from dewlap extension due to divergent hyoid morphology. Chlamydosaurus kingii and Amphibolurus barbatus lack second ceratobranchials of the hyoid apparatus, possessing instead elongated first ceratobranchials that support a conspicuous frill (Kent, 1895; Beddard, '05; Throckmorton et al., '85). Throckmorton et al. ('85) studied frill extension in A. barbatus and concluded that Mm. ceratomandibularis, ceratohyoideus, hyomandibularis, and hyoglossus/genioglossus participate in the mechanism of frill extension. Unlike the dewlap of Anolis and other lizards, which extends in a ventral direction, the frills of Amphibolurus and Chlamydosaurus extend laterally. In accordance with their predominant orientation, frills are used in concert with frontal, rather than lateral displays. The different appearance of frills and dewlaps attests to the plasticity of the hyoid and its potential for producing widely contrasting displays.

CHAPTER III

NEURAL PATHWAY FOR DEWLAP EXTENSION

Introduction

Communication signals are well suited for the study of neural and hormonal substrates of complex vertebrate behavior because they are often stereotyped, quantifiable, and controlled by discrete sets of neurons and muscles (Bass, '86). Research on central motor pathways used in communication has concentrated on acoustic and electric communication systems (e.g., Demski et al., '73; Nottebohm et al., '76; Konishi, '85; Grant et al., '86), while visual signals have been relatively neglected. This study is concerned with the neural pathway controlling a visual signal, the dewlap display of Anolis lizards.

The dewlap, an extendible flap of skin ordinarily folded under the throat, is a specialized display organ present in many iguanid and agamid lizards (Carpenter and Ferguson, '77). Dewlaps are usually brightly colored, yielding striking visual displays when extended. In the iguanid genus Anolis, dewlap displays are performed, often in concert with other bodily movements, in the course of inter- and intraspecific interactions (Jenssen, '77; Williams and Rand, '77). Dewlap displays may be functionally analogous to the advertisement calls of territorial birds (Rand, '67). In the North American green anole, A. carolinensis, dewlap display by the male has

been shown to be strongly influenced by testosterone (Crews et al., '78) and to facilitate follicular development in females (Crews, '75, '80). Recently, experimental evidence has been gathered for a role of dewlap display and dewlap coloration in species-recognition (Losos, '85) and female attraction (Sigmund, '83).

Previous research on the central control of dewlap displays indicated brain nuclei from which dewlap extension could be elicited by electrical brain stimulation in Crotaphytus collaris (Sugerman, '74; Sugerman and Demski, '78), Anolis sagrei (Sugerman, '80) and Iguana iguana (Distel, '78a, b). Tarr ('82) demonstrated sites (mainly telencephalic) in the brain of Sceloporus occidentalis from which species-typical displays, some including dewlap extension, could be elicited by electrical stimulation. Also, deficits in courtship and/or agonistic displays, which include dewlap extension, were observed after radiofrequency destruction of targets in the basal forebrain of A. carolinensis (Greenberg, '77; Greenberg et al., '79, '84; Wheeler and Crews, '78). However, these experiments suffer from the same difficulties of interpretation that afflict other lesion and stimulation studies (e.g., Distel, '78a; Peterson, '80), and by themselves provide an incomplete picture of neural mechanisms underlying dewlap display behavior.

The present study uses neuroanatomical tracers to characterize the motor outflow to musculature involved in dewlap extension in Anolis equestris (Sauria, Iguanidae). This

approach has been applied with success to a number of vertebrate behaviors (e.g., Wetzel et al., '80; Weerasuriya and Ewert, '81; Takei et al., '84; Wetzel et al., '85; Seiwert and Adkins-Regan, '87).

Materials and Methods

Animals

This report contains results from 14 adult Anolis equestris (12 male, 2 female; 44-98 g in body weight). This species was chosen because its large size facilitates the experimental manipulations. Lizards were obtained from commercial suppliers in Miami and maintained under standard laboratory conditions (for details see Chapter II). They were housed in 110 l glass terraria on a 14L:10D cycle and fed a diet of insects and baby mice and rats.

Experimental Approach

Dewlap extension in Anolis is accomplished by contraction of paired ceratohyoid muscles, which extend between the ceratohyals and the first ceratobranchials of the hyoid apparatus on either side of the throat (Fig. 8). Contraction of the ceratohyoid muscles causes the elongated second ceratobranchials of the hyoid apparatus to swing forward unfolding the dewlap (von Geldern, '19). Other buccal floor muscles play, if any, only a minor role in dewlap extension (see Chapter II). Innervation of the ceratohyoid muscles is through a branch of the ramus

Figure 8. Lateral View of the Hyoid Apparatus of Anolis equestris. For clarity, only half of the hyoid apparatus is illustrated. The ceratohyoid muscle (hatching) is depicted extending between the ceratohyals (top) and the first ceratobranchials (bottom) of the hyoid apparatus. During dewlap extension, contraction of the ceratohyoid muscle on either side of the throat causes the second ceratobranchials of the hyoid apparatus (shown along lower edge of dewlap) to swing forward and down unfolding a flap of gular skin. The ceratohyoid muscle is innervated by a branch of the ramus pharyngo-laryngeus (RPL), which receives contributions from several cranial nerves. Note that the RPL proceeds past the ceratohyoid muscle to innervate the laryngeal musculature and other structures in the pharyngeal floor. The arrow indicates where the RPL was cut for the HRP tracing experiments. Reconstructed from dissected specimens and X-rays of displaying animals. Inset shows X-ray of female A. equestris during a defensive display.

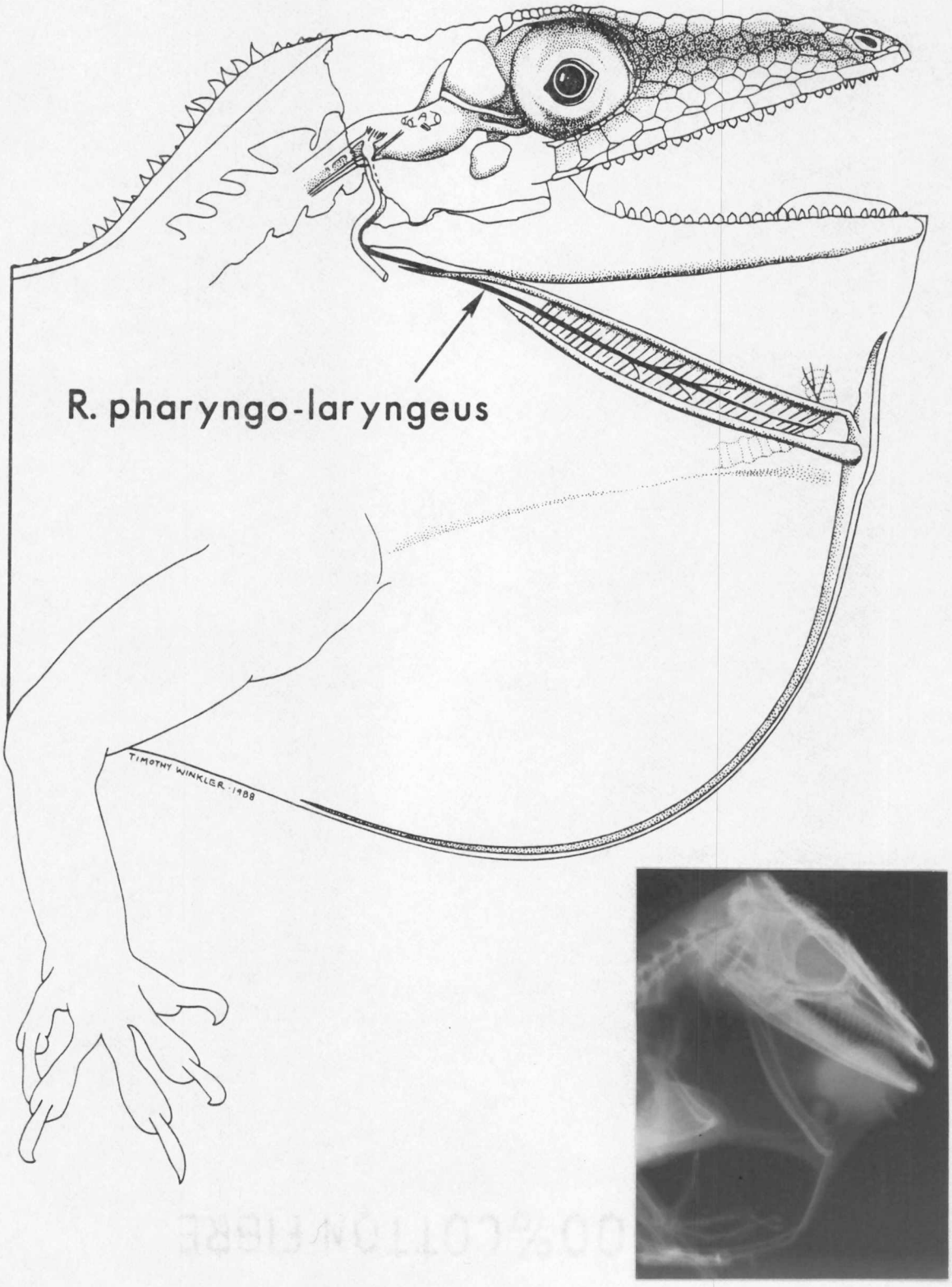

Figure 8

pharyngo-laryngeus (RPL), which carries fibers from several cranial nerves in the caudal medulla (Willard, '15).

Retrograde transport of horseradish peroxidase (HRP) was used to explore the brain stem origins of efferent fibers to the ceratohyoid muscles. All animals were anesthetized by intramuscular injection of ketamine hydrochloride (Ketaset, Bristol Laboratories) in doses of 200-336 mg/kg (Font and Schwartz, '89). In one animal, HRP[1] was applied to the ceratohyoid on one side of the throat using a root canal file (Klueber and Ontell, '84). In three lizards, the RPL on one side of the throat was surgically isolated and transected proximal to the ceratohyoid muscle (Fig. 8). A 10-40% solution of HRP (Miles Laboratories or Sigma type VI) in saline was delivered to the central end of the cut RPL through plastic microtubules. The cut nerve was suctioned into a small length of polyethylene tubing. The tubing was then attached to the nerve and surrounding tissue with Histoacryl adhesive, filled with HRP and sealed with paraffin. To study the overlap between the nerve supply to the ceratohyoid muscles and other structures in the buccal floor, two additional lizards received HRP injections in the tongue and one in the musculature of the larynx.

In a second experiment, tracer was applied in six lizards to the vagal part of nucleus ambiguus (Amb X), the main source of

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[1]A complete list of abbreviations used in this Chapter may be found in Appendix B, page 100.

efferents to the ceratohyoid muscles as identified in this study. Micropipettes drawn from 1 mm capillary glass tubing were used to pressure inject nanoliter amounts of 10% HRP (Sigma type VI) or 3% wheat germ agglutinin-HRP conjugate (WGA-HRP, Sigma) into Amb X. The animals were placed dorsal side up on a modified Kopf small animal stereotaxic headholder. Ear bars fitted with pencil rubber erasers were pressed against the temporal region on either side of the head, rostral to the tympanic membrane. A metal bar taped across the arms of the headholder was used to hold the lizard's snout down, thus maintaining a fixed angle between head and body. An incision was made in the skin of the back of the neck taking care not to damage the highly vascularized tissue of the erectile nuchal crest. The neck muscles were then separated along the midline by blunt dissection and the hindbrain accessed through the foramen magnum. This approach eliminated the need for drilling or removing parts of the skull. After dura and pia membranes were teased away, micropipettes (internal tip diameter 8-22 μm) were lowered into Amb X using blood vessels and other external brain landmarks for guidance. Use of a pneumatic drive and pressure regulator (Alheid et al., '81) permitted accurate delivery of 20-150 nl of tracer.

Histochemistry

After survival times of 42-69 h, the lizards were reanesthetized and perfused through the heart with saline, followed by a mixture of 1.25% glutaraldehyde and 1%

paraformaldehyde in 0.2 M phosphate buffer (pH 7.4) at room temperature, and a phosphate buffer-sucrose postperfusion rinse at 4°C. The brains and cervical spinal cords were removed from the crania, stripped of the surrounding membranes and embedded in 10% gelatin (300 Bloom) with 20% glycerin. Gelatin-glycerin blocks were hardened in a 40% formaldehyde solution for 1 h and cut into transverse sections of 60 μm on a Vibratome (Oxford). The sections were treated for HRP reaction product with a modification of Mesulam's ('82) tetramethylbenzidine method using ammonium heptamolybdate as a stabilizing agent (Olucha et al., '85). All sections were counterstained with a 1% neutral red solution and the location of labelled cells noted using an Olympus BH microscope equipped for photomicrography. The brain of an untreated lizard was processed in the same manner as those of experimental animals to control for endogenous peroxidase activity.

Results

Labelling of Medullary Neurons that Control the Ceratohyoid Muscles

In Anolis, paired ceratohyoid muscles are the effectors in the neuroeffector pathway controlling dewlap extension. A branch of the ramus pharyngo-laryngeus (RPL) innervates the ceratohyoid on either side of the throat (Willard, '15). After giving many small branches to the ceratohyoid, the RPL continues its rostral course to innervate the constrictor and dilator

muscles of the larynx (Fig. 8). A few small branches are also given to the pharyngeal floor. Proximal to the ceratohyoid muscle, the RPL joins the hypoglossal nerve and the two turn around the retroarticular process at the posterior end of the lower jaw. Emerging from the neck, this combined cranio-cervical trunk (RPL + hypoglossal nerve) is joined by a sympathetic nerve arising from the cervical plexus. A short distance above this junction, the hypoglossal nerve leaves the cranio-cervical trunk and enters the brain stem as a series of rootlets on its ventral side. The RPL then proceeds rostrally, splitting into two branches at the level of the petrosal ganglion. One branch, the superior laryngeal ramus, joins the vagus nerve. The other branch continues as the glossopharyngeal nerve, which gives off fibers to the facial nerve (rami communicantes internus et externus) before entering the dorsal side of the brain stem rostral to the entrance of the vagus nerve (Willard, '15; Oelrich, '56; Barbas-Henry and Lohman, '84).

The topography of medullary neurons that supply axons to the ceratohyoid muscles was investigated by retrograde transport of HRP. Identification of labelled cell bodies was facilitated by comparison with results obtained by Barbas-Henry and Lohman ('84) in Varanus exanthematicus and Szekely and Matesz ('88) in Lacerta agilis. Following HRP application to the ceratohyoid or RPL two types of labelled cells were observed ipsilaterally in the caudal medulla. Large polygonal cells were consistently

found in the lateral tegmentum in a column extending from the obex region to the cervical spinal cord (Figs. 9A-C, 10). Cells in this column are situated near cells of the hypoglossal nuclear complex (Fig. 11B), with which they sometimes intermingle. This column corresponds in location and neuronal morphology to the vagal part of nucleus ambiguus (Amb X) identified in V. exanthematicus by Barbas-Henry and Lohman ('84). Large polygonal cells were also found in a ventrolateral group extending from the level of the rostral pole of the dorsal motor nucleus of the vagus to the level of the posterior root of the vestibulocochlear nerve (Fig. 9G-M). These neurons constitute the glossopharyngeal part of nucleus ambiguus (Amb IX). Labelled neurons in the latter nucleus were less abundant and less heavily labelled than neurons in Amb X.

Small spindle-shaped neurons were labelled in three locations. A small group was located immediately rostral to the large neurons in Amb X (Fig. 9D). A second group of spindle-shaped neurons was situated in a slightly more ventral position, rostral to the obex and directly above the cells in Amb IX (Fig. 9F-I). Finally, spindle-shaped neurons were observed scattered around the rostral pole of the dorsal motor nucleus of the vagus, ventral to the nucleus of the solitary tract (Fig. 9E-G, 11C). According to the terminology of Barbas-Henry and Lohman ('84), the first two groups are the parasympathetic cells of Amb X (Amb X ps) and the inferior salivatory nucleus (IX Sal inf), respectively. The third group of spindle-shaped neurons (DMX r)

Figure 9. Locations of Labelled Cells after Application of HRP to the Ramus Pharyngo-Laryngeus. All labelled neurons are ipsilateral to the side of HRP application. Large dots indicate large polygonal motoneurons, small dots indicate spindle-shaped (parasympathetic) neurons. For abbreviations used in this Figure see Appendix B, page 100.

HRP in r. PHARYNGO-LARYNGEUS

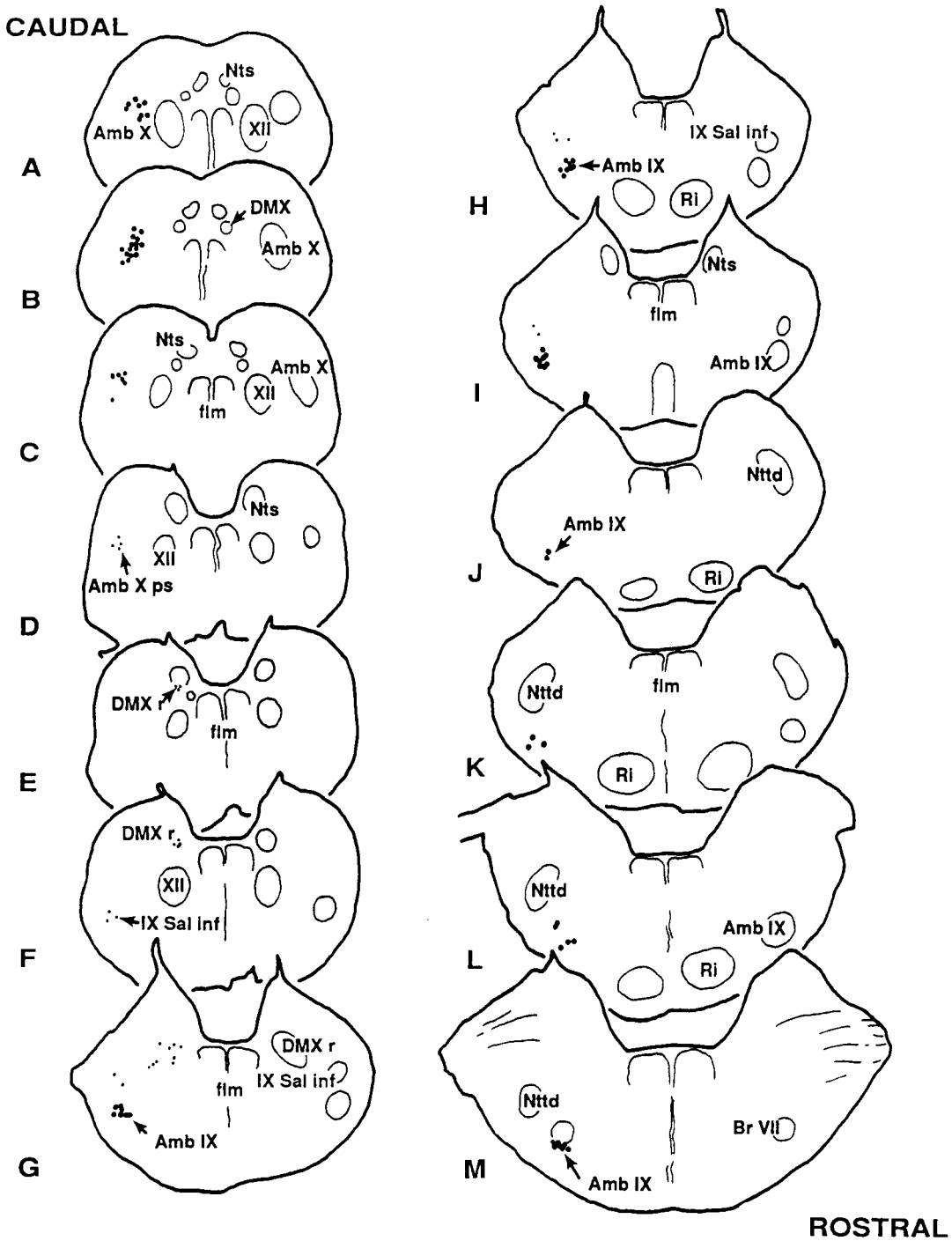

Figure 9

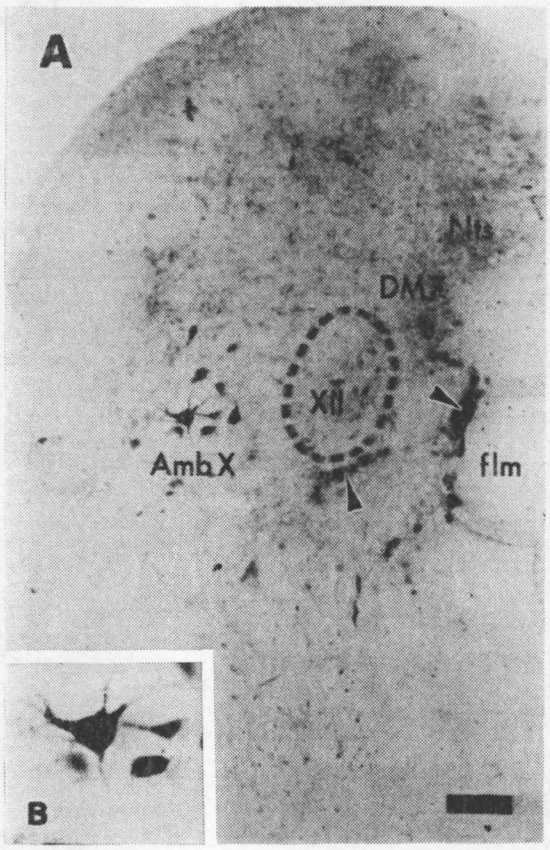

Figure 10. Photomicrographs of Labelled Cells in Amb X after Application of HRP to the Ramus Pharyngo-Laryngeus. **A** Photomicrograph of transverse section through the medulla of Anolis equestris showing HRP-labelled motoneurons in nucleus Amb X. Nonlabelled cells of the hypoglossal nucleus (XII, outlined) are visible to the right of Amb X. Arrowheads point to two large blood vessels. Scale bar, 100 μm. **B** Detail of labelled motoneuron in Amb X. For abbreviations used in this Figure see Appendix B, page 100.

Figure 11. Photomicrographs of HRP-Labelled Cells. **A** Labelled neurons in Amb X after injection of HRP into the larynx. Medial is to the left. **B** Labelled neurons in the hypoglossal nucleus (XII) after injection of HRP into the musculature of the tongue. The location of Amb X (outlined) is indicated. Arrowhead points to blood vessel in the area of the nucleus of the tractus solitarius. **C** Labelled cells in the rostral part of the dorsal motor nucleus of the vagus (DMX r) following HRP application to RPL. **D-F** Labelled neurons in Pv, EW and Vmes after injection of WGA-HRP into the caudal medulla. Arrowhead in E points to blood vessel. Scale bar, 100 μm. For abbreviations used in this Figure see Appendix B, page 100.

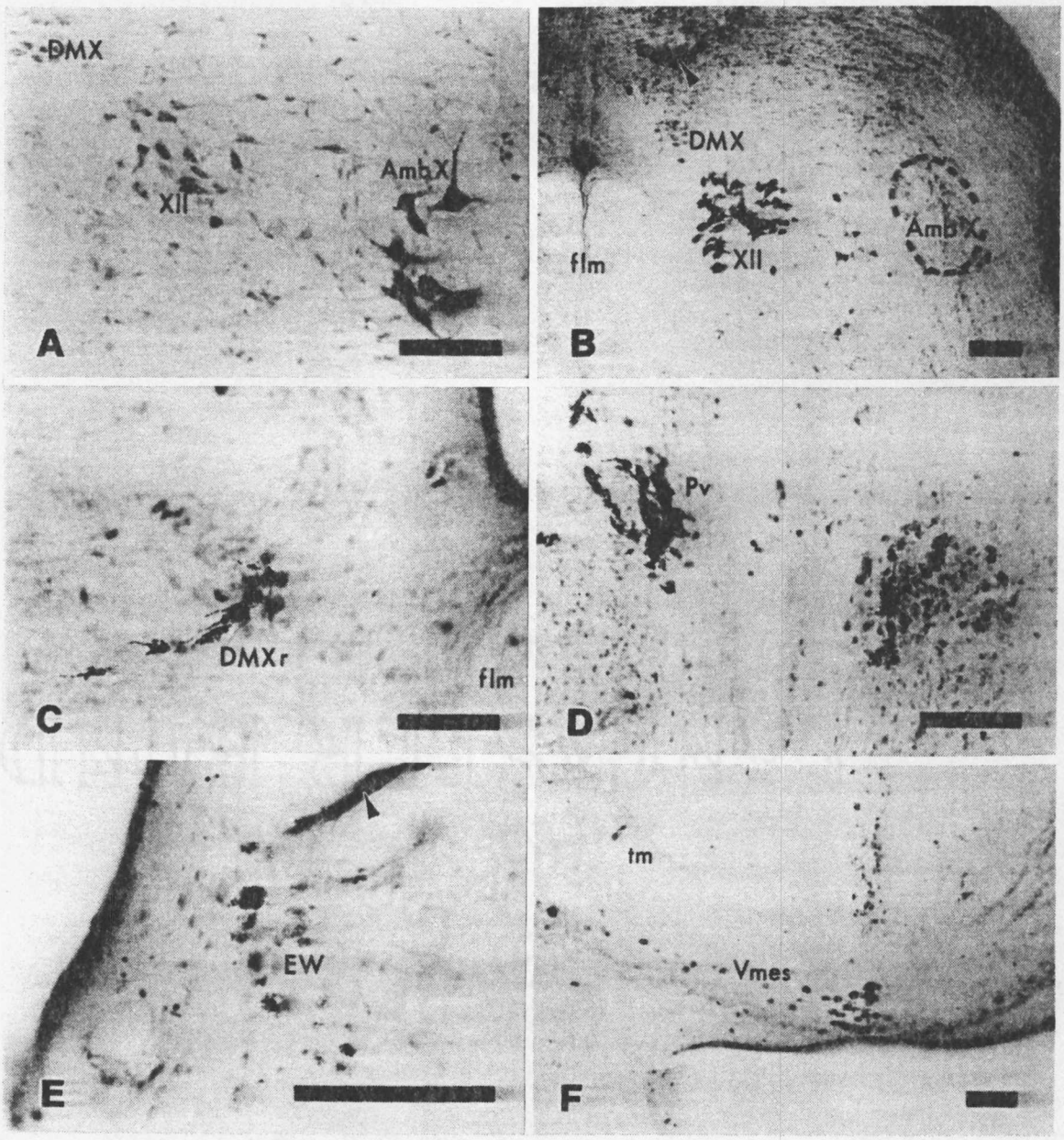

Figure 11

comprises cells in the rostral extension of the dorsal motor nucleus of the vagus as well as "scattered" cells of the lateral visceromotor area as described by Szekely and Matesz ('88) in Lacerta agilis. Barbas-Henry and Lohman ('84) reported labelled cells in a similar location after application of HRP to the glossopharyngeal nerve in Varanus exanthematicus.

On the basis of cell size and form, all three groups of spindle-shaped neurons are part of the general visceral efferent (autonomic preganglionic) column of the brain stem. They supply epithelial glandular structures in the pharynx and larynx. Willard ('15) described a small ganglion beneath the epithelium surrounding the larynx in Anolis carolinensis. This ganglion may contain cell bodies of the corresponding postganglionic cells. The large polygonal cells in Amb X and Amb IX belong to the special visceral efferent (branchiomotor) column that innervates musculature of branchial derivation (Ariens Kappers et al., '36; Nieuwenhuys, '74). Thus, in A. equestris cranial nuclei Amb X and Amb IX contain the motoneuronal pool supplying the ceratohyoid muscles.

Occasionally, a few lightly labelled cells were found in the dorsal trigeminal motor nucleus, dorsal motor nucleus of the vagus (DMX), and hypoglossal nucleus (XII). In one animal, extraperikaryal label was also present in the nucleus of the solitary tract (Nts).

The pattern and distribution of labelled cells was substantially similar after application of HRP intramuscularly

or through the cut RPL, although the latter yielded more robust labelling. This is probably due to larger amounts of enzyme being available for transport when HRP is applied to the nerve.

Since RPL continues past the ceratohyoid to innervate the larynx and the pharyngeal floor (Fig. 8, p. 50), it is possible that some of the cells labelled following HRP application to the RPL send axons to targets other than the ceratohyoid muscle. This question was addressed by applying HRP to the laryngeal musculature. Bilateral injection of HRP into the larynx yielded retrogradely labelled cells in Amb X, Amb IX, Amb X ps and DMX r, but not in IX Sal inf (Figs. 11A, 12). As in experiments in which HRP was applied to the RPL, most labelled cells were found in Amb X (145 neurons in this nucleus contained heavy to moderate accumulation of reaction product). These results suggest extensive overlap between the nerve supply to the ceratohyoid and to the laryngeal musculature.

Labelling of Neurons in Supramedullary Regions

Descending connections to nucleus Amb X, the major source of motor fibers to the ceratohyoid muscle as identified in this study, were investigated by HRP histochemistry. In four animals, small (less than 60 nl) injections of HRP or WGA-HRP placed in or near Amb X produced inconsistent results. Only a few faintly labelled cells could be identified and their locations varied from brain to brain (see also Wetzel et al., '85). In two animals, larger injections (ca 150 nl) yielded heavily labelled perikarya in several nuclei in the

Figure 12. Locations of Labelled Cells after Injection of HRP into the Larynx. Distribution of labelled neurons in the brain stem of Anolis equestris following injection of HRP into the musculature of the larynx (bilateral). Large dots indicate large polygonal motoneurons, small dots indicate spindle-shaped (parasympathetic) neurons. For abbreviations used in this Figure see Appendix B, page 100.

HRP in LARYNX

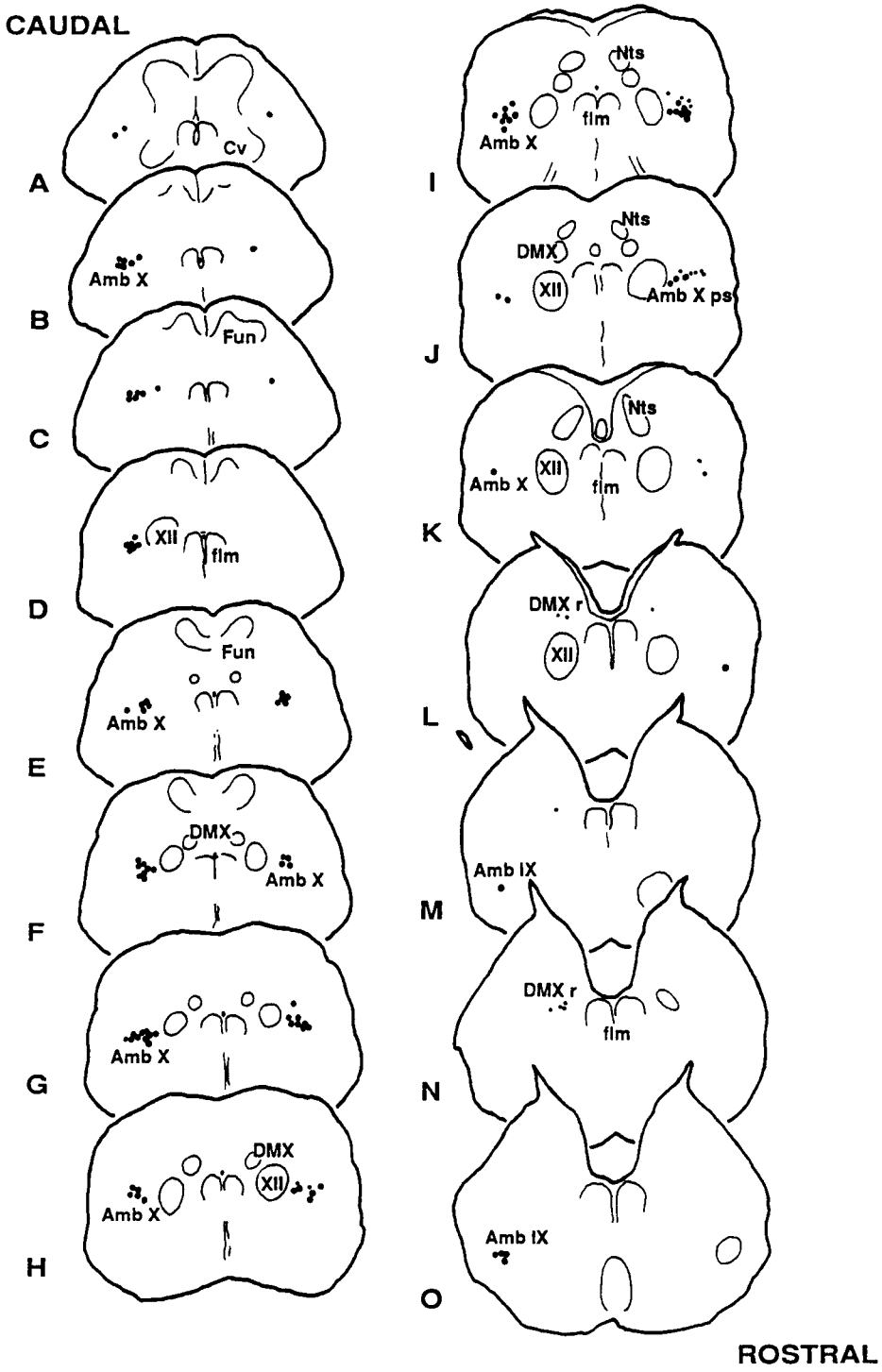

Figure 12

rhombencephalon, mesencephalon and diencephalon, but involved areas adjacent to Amb X (e.g., Fig. 13A; see Discussion).

In the diencephalon, heavily labelled cells were observed ipsilateral to the side of injection in the nucleus paraventricularis (Pv, Fig. 13H) and nucleus periventricularis hypothalami (Ph, Fig. 13G, H). A few labelled cells were also found in Pv on the contralateral side (Fig. 11D). In the mesencephalon, HRP-filled neurons were seen ipsilaterally in the interstitial nucleus of the fasciculus longitudinalis medialis (Iflm, Fig. 13E, F), nucleus of Edinger-Westphal (EW, Figs. 11E, 13D, E), laminar nucleus of the torus semicircularis (Torl, Fig. 13C) and mesencephalic trigeminal nucleus (Vmes, Figs. 11F, 13C). Cells were also labelled in the contralateral nucleus ruber (Rub, Fig. 13E, F). A prominent bundle of labelled axons observed in the lateral tegmentum probably corresponds to the crossed rubrospinal tract (Fig. 13C-E). The tectum mesencephali (tm) contained a few labelled cells on both sides (Fig. 13F). In the rhombencephalon, retrogradely labelled cells were found ipsilaterally in the locus coeruleus (Lc, Fig. 13C), parabrachial region (Pb, Fig. 13C), nucleus of the descending trigeminal tract (Nttd, Fig. 13B) and vestibular nuclear complex (Vevl). Many large HRP-positive cells were present throughout the magnocellular reticular formation, including the contralateral nucleus reticularis isthmi (Ris, Fig. 13D), ipsilateral nucleus reticularis superior (Rs, Fig. 13C), ipsilateral nucleus reticularis inferior (Ri, Fig. 13B) and in

Figure 13. Locations of Labelled Cells after Injection of WGA-HRP into the Caudal Medulla. Dots and broken lines represent retrogradely labelled cells and fibers, respectively. The size of the dots is roughly proportional to the size of the labelled cells. Inset shows a parasagittal section through the brain of Anolis equestris in which the levels and angle of sections A-H are indicated. Asterisk marks the approximate level of injection.

The pattern of retrograde labelling was substantially similar in two lizards receiving injections of tracer into the caudal medulla. In the case depicted here, the injection site (shaded area in A) extended from the upper cervical spinal cord to the rostral pole of the hypoglossal nucleus (from the level of section A to the level of section N in Fig. 12) and included, in addition to the latter, the following nuclei: Amb X, Amb X ps, Amb IX, IX Sal inf, DMX, DMX r, Nts, Ri and caudal portions of Nttd. The other case (not pictured here) had a slightly smaller injection site (D through L in Fig. 12) affecting the same nuclei but sparing the most lateral portions of the lateral funiculus. For abbreviations used in this Figure see Appendix B, page 100.

HRP in CAUDAL MEDULLA

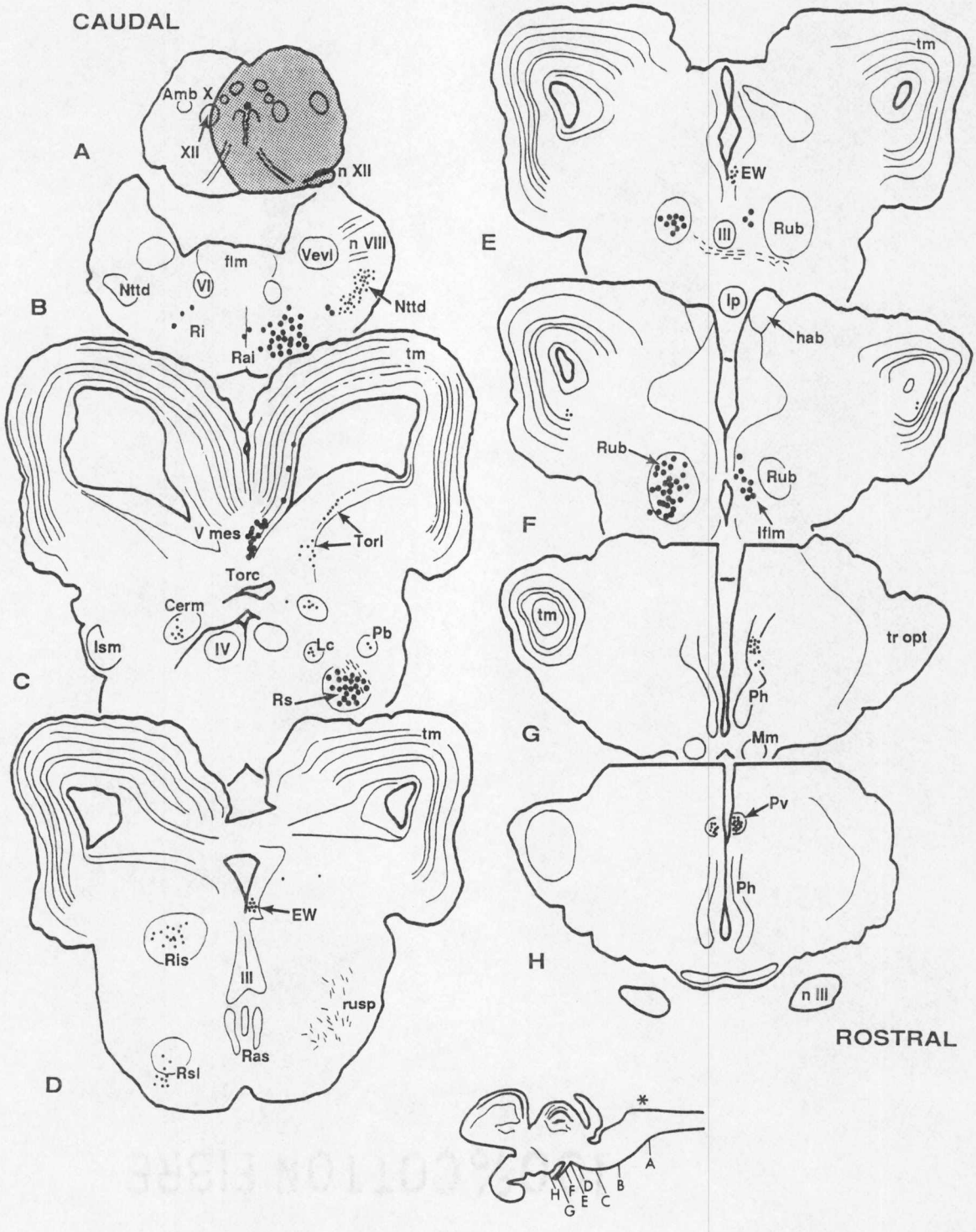

Figure 13

the midline nucleus raphes inferior (Rai, Fig. 13B). Smaller neurons were labelled contralaterally in the lateral reticular zone in a nucleus termed the lateral part of Rs (Rsl, Fig. 13D) following ten Donkelaar et al. ('87). A few labelled cells were also present bilaterally in a deep cerebellar nucleus (Cerm, Fig. 13C).

No labelled cells were seen in the brain of the untreated control, ruling out the possibility that labelled cell groups in the experimental animals are the result of endogenous peroxidase activity (Mesulam, '82).

Discussion

Results of the present study indicate that motoneurons which innervate the ceratohyoid muscles in Anolis equestris are localized ipsilaterally within the vagal (Amb X) and glossopharyngeal (Amb IX) parts of the medullary nucleus ambiguus. A few large neurons found in other cranial nerve nuclei (e.g., trigeminal motor nucleus) probably reflect the presence of crossovers or collaterals of fibers innervating the ceratohyoid muscles. Sugerman and Krogh ('86) described heavily labelled cells in two cranial nuclei after HRP injection of the ceratohyoid in collared lizards, Crotaphytus collaris. The labelled nuclei were identified as the glossopharyngeal part of nucleus ambiguus (Amb IX) and the hypoglossal nucleus (XII). The latter, however, corresponds in location to Amb X of the present study. HRP localization experiments show the

hypoglossal nucleus of lizards to be in close proximity to Amb X, yet the two form distinct cellular aggregates (Fig. 10, p. 59; Fig. 11A, B, p. 60; Barbas-Henry and Lohman, '84). My HRP material also shows a division of the hypoglossal nucleus of A. equestris into dorsal and ventral subnuclei. This differs from the results of a previous study using retrograde degeneration in Anolis sp (Gillaspy, '54), but agrees with work on other lizards (e.g., Varanus exanthematicus, Barbas-Henry and Lohman, '84).

In lizards, cells in Amb IX are rostrally continuous with cells of the branchiomotor nucleus of the facial nerve (Br VII, Fig. 9M, p. 57; Gillaspy, '54; Schwab, '79; Barbas-Henry and Lohman, '84; Szekely and Matesz, '88). Nucleus Amb X consists of scattered large and medium-sized polygonal neurons ranging from the level of the obex to the upper cervical spinal cord (Barbas-Henry and Lohman, '84; Szekely and Matesz, '88; Kennedy, '81). There are species differences in the position of Amb X. In Gekko gecko (Kennedy, '81) and Lacerta agilis (Szekely and Matesz, '88) Amb X is located dorsal to the hypoglossal nucleus (XII) and lateral to the dorsal motor nucleus of the vagus (DMX). In Varanus exanthematicus (Barbas-Henry and Lohman, '84), as in Anolis equestris (present study), Amb X is found lateral to the hypoglossal nuclear complex (Fig. 10, p. 59; Fig. 11A, B, p.60).

The lacertilian larynx is doubly innervated through the RPL and through the recurrent (inferior) laryngeal ramus of the vagus nerve (Willard, '15; Oelrich, '56). HRP injected into the

larynx of Anolis equestris labelled cells in Amb X, Amb X ps, Amb IX and DMX r. In a study of central projections of the recurrent laryngeal nerve of Tokay geckos, Gekko gecko, Kennedy ('81) reported labelled cells in Amb X. In fact, his illustrations reveal a second group of labelled neurons rostral to the obex. Based on location, this second group corresponds to the rostral extension of the dorsal motor nucleus of the vagus (DMX r) found in this study and those of Szekely and Matesz ('88) and Barbas-Henry and Lohman ('84). Since cells were not labelled in Amb X ps or Amb IX after HRP application to the recurrent nerve (Kennedy, '81), it is likely that axons from neurons in these nuclei travel to the larynx via the RPL.

The present results in Anolis equestris indicate that Amb IX and Amb X neurons supply motor fibers to the ceratohyoids as well as to constrictor and dilator laryngeal muscles (see also Kennedy, '81). The distribution of the two motoneuronal pools provides no evidence of topographical organization. Thus, unlike its mammalian counterpart (Wetzel et al., '80; Hopkins et al., '86), the nucleus ambiguus of lizards does not appear to be functionally subdivided.

Nucleus ambiguus participates in neuroeffector pathways subserving a communication function in many vertebrate taxa. In Siamese fighting fish, Betta splendens, neurons in cranial nerve nuclei IX and X-XI (parts of which may be homologous to Amb IX and Amb X) send efferent projections to specialized muscles that control branchiostegal membrane extension, a component of

aggressive display behavior in this species (Augenbraun and Gorlick, '87). In frogs, Amb X and Amb IX form a single column in the ventrolateral tegmentum (Matesz and Szekely, '78; Simpson et al., '86). Cells in this column innervate laryngeal bipinnate muscles used to produce vocalizations in African clawed frogs, Xenopus laevis (Kelley, '80). In lizards, ambiguus motoneurons innervate laryngeal musculature responsible for the production of vocalizations (Kennedy, '81), as well as musculature associated with the dewlap display (present study). In rats, nucleus ambiguus contains the cells of origin of laryngeal nerves subserving ultrasonic vocalization (Wetzel et al., '80).

Because of methodological limitations, results of HRP or WGA-HRP injections into Amb X can only suggest candidate brain nuclei that contribute afferents to this nucleus. First, only the largest injections yielded consistent retrograde labelling, so that cells may have been labelled due to transport from nontarget areas (i.e., areas adjacent to Amb X). Second, agreement between locations of labelled cells in this study and others investigating descending supraspinal pathways (Robinson, '69; ten Donkelaar, '76a, b; ten Donkelaar and de Boer-van Huizen, '78, '84; ten Donkelaar et al., '80; Cruce and Newman, '81) suggests that the presently described labelling may be due to uptake by fibers passing through rather than terminating within the site of injection (i.e., fibers of passage). Use of

anterograde tracers such as [^{3}H] leucine or PHA-L should clarify descending pathways to Amb X.

Five nuclei in the reticular formation contained labelled cells after injection of HRP or WGA-HRP into the caudal medulla (including Amb X) of Anolis equestris. A projection from reticular formation nuclei to nucleus ambiguus is also present in frogs (Wetzel et al., '85) and rats (Travers and Norgren, '83). The reticular formation of the brain stem has been implicated in motor pattern generation in anurans (Nieuwenhuys and Opdam, '76; Weerasuriya, '83; Wetzel et al., '85), lizards (Northcutt, '78), birds (Dubbeldam, '84) and mammals (Kaneko et al., '81; Jean, '84; Lowe, '84). This suggests that the reticular formation of Anolis may contain premotor neurons contributing to the generation of the dewlap display. Indeed, electrical stimulation of sites in the reticular formation yielded dewlap extension in Crotaphytus collaris (Sugerman, '74; Sugerman and Demski, '78) and Iguana iguana (Distel, '78a, b).

The same stimulation studies identified additional sites from which dewlap extension could be readily elicited in the vicinity of the nucleus laminaris of the torus semicircularis (Torl). This nucleus, also containing labelled cells in the present study, is believed to be homologous to the mammalian periaqueductal gray matter (Kennedy, '75; Foster and Hall, '78). Several reports in reptiles have proposed a role for Torl in the generation of communication signals (Butler and Bruce, '81; Kennedy and Browner, '81; Browner and Baruch, '84; Ingle and

Crews, '85). Electrical stimulation of Torl elicited aggressive vocalizations in Gekko (Kennedy, '75). Likewise, electrical stimulation of homologous neuronal populations elicited species-typical vocalizations in fish (Demski et al., '73), birds (Seller, '83) and mammals (Hunsperger, '56; Jurgens and Ploog, '70).

Further support for a role of Torl in the generation of behaviors used in communication derives from histochemical studies. Cells in Torl bind sex steroids in Anolis carolinensis (Martinez-Vargas et al., '78; Morrell et al., '79) and Pseudemys scripta (Kim et al., '81). Gonadal hormones have major influences in the behavior of reptiles, including facilitation of sexually dimorphic displays (Crews et al., '78; Crews and Greenberg, '81). Male Anolis have larger dewlaps and display more frequently than females. Therefore, the presence of steroid binding in Torl cells provides a possible substrate for interaction of nervous and hormonal mechanisms affecting dewlap extension.

The Torl of reptiles receives somatosensory afferents from the spinal cord (Ebbesson, '67, '69; Foster and Hall, '78) and projects to the anterior dorsal ventricular ridge (ten Donkelaar and de Boer-van Huizen, '88), to the dorsal thalamus (Hoogland, '82), to the rhombencephalon (ten Donkelaar and de Boer-van Huizen, '81) and to the cervical spinal cord (ten Donkelaar and de Boer-van Huizen, '78, '84; ten Donkelaar et al., '80; Butler and Bruce, '81). Descending projections to the spinal cord are

of particular interest as they suggest a possible route for Torl to exert its influence on lower motoneurons, presumably including those in Amb X. Alternatively, Torl could project to motor pattern generators in the reticular formation and these in turn project to appropriate motoneuronal pools in the caudal medulla (Fig. 14; see Weerasuriya, '83). The available information is, however, insufficient to distinguish between these two hypotheses. Confirmation of pathways descending to motoneurons controlling the dewlap display of Anolis must await further work.

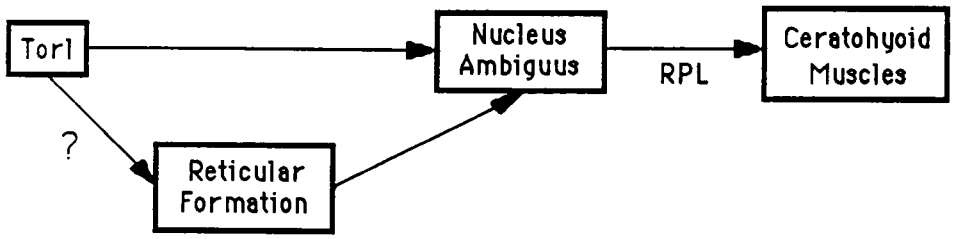

Figure 14. Proposed Neural Pathway for Dewlap Extension. Electrical stimulation of the midbrain laminar nucleus of the torus semicircularis (Torl) in lizards yields dewlap extension and defensive posturing (e.g., sagittal trunk compression). Torl may serve to couple specific motivational states to their corresponding emotional expressions (Jurgens and Ploog, '70). Its influence on lower motoneuronal pools may be mediated by nuclei in the reticular formation. These nuclei probably contain motor pattern generators for behaviors used in defensive and social displays. Descending projections to Torl that would relay input from higher levels of the neuraxis have not been identified. Torl projects to the caudal medulla and upper cervical cord, possibly including nucleus ambiguus. The latter nucleus contains motoneurons supplying the ceratohyoid muscles *via* the ramus pharyngo-laryngeus (RPL) as well as motoneurons for supply of laryngeal muscles.

CHAPTER IV

CONCLUDING REMARKS

The main findings of this study can be summarized as follows:

1. Dewlap extension is an element of defensive and headbobbing displays of Anolis equestris. Defensive displays elicited by a threatening human consist of lateral presentation, sagittal trunk compression, nuchal crest erection, gaping, throat and dewlap extension. Similar displays in the wild are addressed to potential predators (Ruibal, '64). In the laboratory, headbobbing displays are elicited by conspecifics or mirrors. They consist of up-and-down movements of the head usually accompanied by a single pulse of dewlap extension.

2. During defensive and headbobbing displays, dewlap extension is effected by contraction of ceratohyoid muscles extending between the ceratohyals and the first ceratobranchials of the hyoid apparatus. Contraction of the ceratohyoid muscles causes elongated second ceratobranchials of the hyoid apparatus to swing forward unfolding a flap of gular skin. Participation of other muscles in the mechanism of dewlap extension is unlikely.

3. Brain stem motoneurons for supply of the ceratohyoid muscles are located in the vagal and to a lesser extent in the

glossopharyngeal part of nucleus ambiguus. This nucleus also contains motoneurons that innervate the laryngeal musculature.

4. Putative sources of afferent input to the vagal part of nucleus ambiguus are found in the diencephalon, mesencephalon and rhombencephalon. These anatomical data, together with the results of previous experiments using electrical stimulation of the brain (Sugerman, '74, '80; Distel, '78a, b; Sugerman and Demski, '78) suggest a neural network for control of dewlap extension.

Speculations on the Evolutionary Origins of the Dewlap and its Neural Control

The notion that displays used in communication evolved from noncommunicative behavioral progenitors or "proto-ethotypes" (Weldon, '83) was already implied in Darwin's The Expression of the Emotions in Man and Animals (1872). In his seminal work, Darwin contended that expressive movements derive from incomplete or preparatory phases of initially noncommunicative behaviors, or from involuntary activities. In many animals, he noted, excitement is accompanied by hurried breathing, tachycardia, peripheral vasoconstriction, increased muscle tension, sweating and piloerection. These responses, which Darwin attributed to "the disturbed state of the sensorium" (1872; p. 78), are now believed to be a result of activation of the autonomic nervous system. More recently, Morris ('56) has

stressed the importance of autonomic, as well as somatic responses as sources of ritualized displays.

The increased muscle tone and deep breathing attending a state of arousal are often cited as precursors of vocalizations and facial expressions in mammals and other taxa (Buck, '84; Scherer, '85). Darwin remarked that "... when the sensorium is strongly excited, the muscles of the body are generally thrown into violent action; and as a consequence, loud sounds are uttered, however silent the animal may generally be, and although the sounds may be of no use" (1872; p. 83). Many lizards, for instance, produce hissing sounds when threatened or during intraspecific fighting, presumably by forcefully expelling air from the lungs (Gans and Maderson, 1973).

Heightened muscle tension during arousal or excitement occurs either throughout the body or in localized areas, most prominently the neck (e.g., tension headaches) and around the mouth (Buck, 1984). Many of the affected muscles are branchiomeric, rather than myotomic, and derive from the muscles in the branchial arches of primitive fishes. The branchial musculature of higher vertebrates comprises muscles in the throat as well as the specialized muscles of mastication and facial expression (Romer, 1956). Branchiomeric muscles are indistinguishable from ordinary voluntary striated muscles, and only their innervation (through special visceral motoneurons) gives away their visceral nature. Their activation during autonomic arousal may also be a reflection of their visceral

derivation. Possibly because of their involvement in respiration and preparatory biting patterns, branchiomeric muscles have been incorporated into affective or emotional displays (e.g., branchiostegal and opercular displays of fishes, throat pulsation in frogs, facial expressions of mammals, vocalizations). In lizards, branchiomeric muscles play an important role in display behavior through their participation in a diversity of gular displays (e.g., throat and dewlap extension, frill erection). I suggest that these displays may have evolved from noncommunicative muscle tension in the throat during arousal.

A final point of consideration is the similarity between the proposed neural pathway for dewlap extension in Anolis and neural pathways subserving sound production in vertebrates. Central networks for vocalization share common control centers in all vertebrate taxa. Peripheral effector organs used to produce those vocalizations are the larynx in many tetrapods, the syrinx in birds, and the swim bladder or the body wall in teleosts. Nucleus ambiguus innervates laryngeal muscles used to produce vocalizations in frogs (Kelley, '80), geckos (Kennedy, '81), rats (Wetzel et al., '80) and squirrel monkeys (Jurgens and Pratt, '79). The syrinx of birds, on the other hand, is innervated by the hypoglossal nucleus (Nottebohm et al., '76; Seller, '83) and the sonic muscles of the swimbladder of toadfish (Opsanus beta) are controlled by the sonic nucleus of the spinal cord (Demski et al., '73). Midbrain vocal control

centers are homologous in all vertebrates. Electrical stimulation of the midbrain periaqueductal gray elicits species-specific vocalizations in many mammals (reviewed in Jurgens and Pratt, '79). Likewise, stimulation of areas homologous to the periaqueductal gray elicits vocalizations in birds (Seller, '83), geckos (Kennedy, '75), frogs (Schmidt, '66) and toadfish (Demski et al., '73). Thus, while the peripheral effector organs are independently derived, the central structures that supply them are remarkably similar.

It has been suggested that the similarities among central networks controlling vocalization in different taxa may be related to their equivalent functional needs: "Equivalent systems, even if independently derived, must interface with equivalent control areas if they are to work effectively" (Wilczynski, '84; p.759). In Anolis, ambiguus motoneurons supply muscles responsible for dewlap extension. Further, stimulation of areas surrounding the laminar nucleus of the torus semicircularis, believed to be homologous to the mammalian periaqueductal gray, results in dewlap extension (Sugerman, '74, '80; Distel, '78a, b; Sugerman and Demski, '78). This evidence implies that vocal control systems and the neural pathway for dewlap extension are essentially similar. Visual and acoustic signals are equivalent adaptations and serve equivalent functions. The similarities between the neural pathways subserving visual and acoustic signals may reflect their

equivalent neural processing requirements, without regard of the channel employed for signal transmission.

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APPENDIXES

APPENDIX A

LIST OF ABBREVIATIONS USED IN CHAPTER II

B	Body of the hyoid
b	Bone and marrow cavity
c	Hyaline cartilage
CC	M. constrictor coli
CH	Ceratohyal
CME	M. ceratomandibularis externus
CMI	M. ceratomandibularis internus
CR	M. ceratohyoideus
CI	First ceratobranchial
CII	Second ceratobranchial
D	Dentary
EBI	First epibranchial
EH	Epihyal
EMG	Electromyography
EP	Entoglossal process
GG	M. genioglossus
HG	M. hyoglossus
HH	Hypohyal
HM	M. hyomandibularis
IMA	M. intermandibularis anterior
IMP	M. intermandibularis poterior
M, m; Mm	Muscle; muscles
OH	M. omohyoideus

OM	Oral mucosa
p	Perichondrium
PT	M. pterygoideus
RP	Retroarticular process
RPL	Ramus pharyngo-laryngeus
s	Synovial capsule
SA	Supra-angular
SH	M. sternohyoideus

APPENDIX B

LIST OF ABBREVIATIONS USED IN CHAPTER III

Amb IX	Glossopharyngeal part of nucleus ambiguus
Amb X	Vagal part of nucleus ambiguus
Amb X ps	Parasympathetic cells in nucleus Amb X
Br VII	Branchiomotor nucleus of the facial nerve
Cerm	Nucleus cerebellaris medialis
Cv	Ventral horn
DMX	Dorsal motor nucleus of the vagus
DMX r	Rostral part of DMX
EW	Edinger-Westphal nucleus
flm	Fasciculus longitudinalis medialis
Fun	Nucleus of the dorsal funiculus
hab	Habenula
HRP	Horseradish peroxidase
Iflm	Interstitial nucleus of the flm
Ip	Nucleus interpeduncularis
Ism	Magnocellular part of nucleus isthmi
Lc	Locus coeruleus
Mm	Mamillary nucleus
Nts	Nucleus of the tractus solitarius
Nttd	Nucleus of the descending trigeminal tract
n III, n VIII, n XII	Cranial nerves III, VIII, XII
Pb	Parabrachial region

Ph	Nucleus periventricularis hypothalami
PHA-L	Phaseolus vulgaris leucoagglutinin
Pv	Nucleus paraventricularis
Rai	Nucleus raphes inferior
Ras	Nucleus raphes superior
Ri	Nucleus reticularis inferior
Ris	Nucleus reticularis isthmi
RPL	Ramus pharyngo-laryngeus
Rs	Nucleus reticularis superior
Rsl	Lateral part of Rs
Rub	Nucleus ruber
rusp	Rubrospinal tract
tm	Tectum mesencephali
Torc	Central nucleus of the torus semicircularis
Torl	Laminar nucleus of the torus semicircularis
tr opt	Optic tract
Vevl	Nucleus vestibularis ventrolateralis
WGA-HRP	Wheat germ agglutinin conjugated with HRP
III, IV, VI, XII	Motor nuclei of cranial nerves III, IV, VI, XII
Vmes	Mesencephalic trigeminal nucleus
IX Sal inf	Inferior salivatory nucleus

VITA

Enrique Font was born on February 26, 1959 in Valencia (Spain), where he grew up. In 1981 he graduated from The University of Valencia with a B.Sc. and a year later was awarded a M.Sc. degree for his work on the behavior and ecology of urban stray dogs.

He began studies at The University of Tennessee in September of 1982 and shortly thereafter joined the Graduate Program in Ethology. He was awarded a Doctor of Philosophy degree with a major in Life Sciences/Ethology in December of 1988.

The author is a member of the Animal Behavior Society, Sociedad Espanola de Etologia, Society for Neuroscience, J.B. Johnston Club for Comparative/Evolutionary Neurobiology, Society for the Study of Amphibians and Reptiles, and Herpetologists' League. When not engaged in academic activities (a rare event), he plays loud electric guitar, listens to 60's music or talks to animals.