11. The measurement was made by N. Langerman at Scripps Institution of Oceanography, La Jolla, Calif., on 25 and 26 July 1977 with the methods of K. H. Nealson, T. Platt, and J. W. Hastings [*J. Bacteriol.* **104**, 313 (1970)].
12. Data were calculated from recordings similar to those in Fig. 1B and made by J. Morin at the University of California, Los Angeles, on 29 August 1977. Bioluminescence was produced by mechanical stimulation of an animal or house placed in a vial in a lighttight chamber over a side window photomultiplier tube (RCA 1P21). The specimens were stimulated by injecting 1 to 3 ml of seawater into the vial through a small hole in the chamber lid. The output voltage from the photomultiplier tube was proportional to light intensity (in arbitrary units) and was displayed on a chart recorder (Clevite Brush). The response time of the circuit and the recorder were well above the recorded flash frequencies.
13. J. Morin, personal communication.
14. J. Case, personal communication.
15. Animals freshly collected at Long Beach, Calif., were immediately removed from their houses and washed in two changes of Millipore-filtered (0.45 μm) seawater. They were then allowed to build new houses, which they discarded after a 30- to 60-minute occupancy. Invariably these isolated, "clean" houses flashed repeatedly upon mechanical stimulation, and microscopic examination revealed almost no adherent or filtered particulate matter.
16. J. W. Hastings and K. H. Nealson, *Annu. Rev. Microbiol.* **31**, 549 (1977).
17. N. Langerman and M. Haygood performed the bacterial luciferase assay at Scripps Institution of Oceanography, La Jolla, Calif., on 25 and 26 July 1977 with the methods of J. W. Hastings, W. H. Riley, and J. Massa [*J. Biol. Chem.* **240**, 1473 (1965)].
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19. H. H. Seliger and W. D. McElroy, *J. Mar. Res.* **26**, 244 (1968).
20. Puerto Escondido is a small, enclosed bay (0.7 by 0.3 km, maximum depth 14 m) connected on its south side to the Gulf of California by a narrow channel. I made in situ observations in shallow water along the southwest side of the bay and in the channel shortly before or after high tide on clear, moonless nights between 2100 and 2400 hours on 14 to 16 June 1977. Water temperature and salinity were 25°C and 36 parts per thousand, respectively.
21. Although I have no in situ measurements of intensity, several colleagues at the site confirmed my subjective impression that the light was at least as brilliant as that of temperate coastal displays caused by dinoflagellates, whose intensity is established (*2*). Subjective, visual estimates by me and several colleagues of the instantaneous density of flashes during extreme agitation are of the order of 10^4 to 10^5 per cubic meter; we estimated the instantaneous density of spontaneous flashes to be about 10^2 to 10^3 per cubic meter.
22. *Oikopleura dioica* secretes and discards four to seven houses per day at 13°C [G.-A. Paffenhöfer, *Mar. Biol.* **22**, 183 (1973)].
23. Seawater filtered through a 35-μm screen did not luminesce with or without agitation.
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26. Luminescence has been observed by me and my colleagues in *O. dioica* at Long Beach and La Jolla, Calif., and at Friday Harbor, Wash.; in *O. labradoriensis* at Friday Harbor; by Lohmann (*6*) in *O. albicans* at Messina, Italy; it was reported in *O. vanhoeffeni* in the Okhotsk Sea [N. Tarasov, *Marine Luminescence* (Academy of Science U.S.S.R., Moscow, 1956) (in Russian)]. Several colleagues observed luminescence with similar properties (abundant, long, intense flashes from relatively large sources) at Puerto Escondido during the 8-day period before this study, during August 1977, and in June 1976 (A. Miller, personal communication).
27. A. Bückmann, *Mar. Biol.* **21**, 349 (1973). Juveniles of *O. dioica*, often the most abundant age class (*25*) and 40 percent of my larvacean catch, have a body width of only 60 to 80 μm, and so could pass through most plankton nets. The houses of such animals are about 0.5 mm in diameter and would produce a light flash similar to that of *Noctiluca* [R. Eckert, *Science* **147**, 1140 (1965)].
28. Supported in part by a grant-in-aid from the Office of Graduate Studies and Research, California State University, Long Beach. I thank Lt. A. Encinas, Long Beach Marine Department, for access to collecting sites; A. Miller and his subtidal ecology class for introducing me to Puerto Escondido and for corroborating certain field observations; J. Ford, E. Horvath, S. Keck, and P. McIlvaine for collections; P. Brehm, G. Brown, M. Haygood, and N. Langerman for discussions and technical assistance; T. McCoppin and D. Nelson for translations; P. Anderson, J. Case, A. Herrera, and T. Pietsch for criticism of the manuscript, and J. Morin and L. Stuart for discussion, laboratory and field assistance, and criticism.

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Fluorescence-Immunocytochemistry: Simultaneous Localization of Catecholamines and Gonadotropin-Releasing Hormone

Abstract. *Gonadotropin-releasing hormone and dopamine were identified simultaneously in the same block of tissue from the median eminence of the rat brain. Two distinct bands of dopamine terminals were found in the lateral median eminence: an inner band which overlapped the gonadotropin-releasing hormone terminals and an outer band which appeared juxtaposed to portal capillaries.*

The concept that monoamines are involved in the secretion of gonadotropins from the anterior pituitary gland was proposed initially more than 30 years ago (*1*). Data now suggest that both catecholamines and indoleamines participate in the release of luteinizing hormone and follicle-stimulating hormone from the anterior pituitary (*2, 3*). Direct morphological studies of monoamines and releasing hormones have been facilitated by the development of specific histofluorescence (*4*) and immunocytochemical (*5*) techniques. However, certain technical limitations have prevented the direct morphological study of both monoamines and peptide hormones within a single tissue block. Here we describe the correlative distribution of the catecholamines norepinephrine (NE) and dopamine and the hypothalamic peptide gonadotropin-releasing hormone (GnRH) in the median eminence of the rat brain. The distributions were determined by means of a fluorescence-immunonocytochemical technique that allows the simultaneous visualization of monoamines and neuropeptides within adjacent tissue sections.

Six adult male albino Sprague-Dawley rats (200 to 300 g) were killed by decapitation. The calvaria was removed, the brain was excised, and the diencephalon was dissected. Each tissue block was submerged in Freon-22 cooled to −100°C by liquid nitrogen and freeze-dried in a

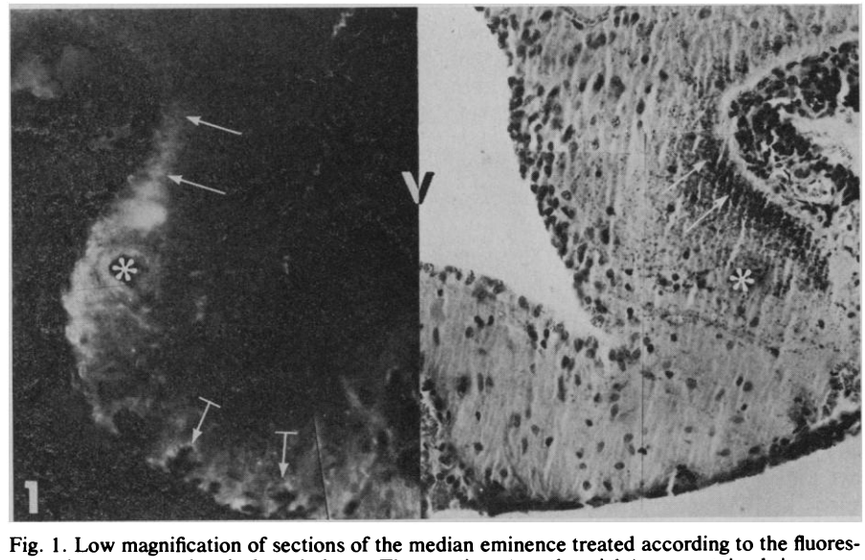

Fig. 1. Low magnification of sections of the median eminence treated according to the fluorescence-immunocytochemical technique. The section (on the right) was stained immunocytochemically and was turned over so that it could be compared with the exactly contiguous surface of the adjacent tissue section (on the left) that was treated for monoamine histofluorescence. Catecholamine and GnRH terminals (arrow) were found in the lateral regions of the median eminence. Dopamine terminals were also identified in the contact zone of the ventral and medial regions of the median eminence (arrows with bar) where only a few GnRH fibers were seen. The blood vessels in each section are identical and provide useful antomical landmarks (asterisks); *V*, third ventricle (×187).

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Sladek-Kontes freeze-drying glassware apparatus for 2 weeks. After being dried, each tissue block was treated with paraformaldehyde vapor for 2 hours at 80°C and sectioned serially at 10 μm. Every 15th section was stained with Luxol fast blue-Cresyl violet for anatomical orientation.

Sections used for fluorescence microscopy were mounted on glass slides and examined in a Leitz Dialux fluorescence microscope equipped with a mercury vapor lamp (HBO 200) and narrow-band (BG3, S405) excitation and K460 barrier filters. Adjacent sections to those used for fluorescence examination were stained immunocytochemically for GnRH according to the procedure of Sternberger (*5*). Deparaffinized sections were rehydrated in 0.01*M* phosphate-buffered saline (PBS), *p*H 7.1. Rabbit antiserum to synthetic GnRH was used as the initial step in the immunoperoxidase technique. In order to discount any variations in staining that might be caused by differences in the antiserums, we used two antiserums: antiserum 38 and antiserum F. The primary antiserum (either 38 or F) was incubated with the tissue at 4°C. The antiserum F to GnRH was incubated at a dilution of 1:1000 for 24 hours, whereas antiserum 38 was used at a dilution of 1:300 for the same amount of time. Sheep antiserum to rabbit gamma globulin, rabbit horseradish peroxidase antibody to peroxidase complex (PAP), and tetrahydrochloride 3,3′-diamino-benzidine with 0.003 percent H_2O_2 were used in the subsequent steps. Some sections were counterstained with Cresyl violet. Control sections were incubated with PBS instead of the primary antiserum while the rest of the procedure remained unchanged.

Absorption experiments for specificity of the immunoreactive stain were carried out on freeze-dried tissue: 10 μg of synthetic GnRH was added to 0.1 ml of the 1:300 dilution of antiserum 38 and 3 μg of GnRH was added to 0.1 ml of the 1:1000 dilution of antiserum F for 24 hours before application to the section. Sections were examined in a second Leitz Dialux microscope and were compared directly to adjacent sections treated for monoamine histofluorescence through a Leitz comparator bridge. The latter interconnected the images from each microscope and allowed either split-screen viewing or superimposition of two selected fields.

Brown reaction product indicating the presence of the antiserums against GnRH was most heavily concentrated in the lateral regions of the median eminence adjacent to the tuberoinfundibular sulcus (Fig. 1). Adjacent sections used for histofluorescence examination revealed an intense band of dopamine fiber terminals (*6*, *7*) in the same general area (Fig. 1). However, when the simultaneous localization technique was used, a distinct difference in the precise distribution between dopamine and GnRH could be discerned. In the dorsal region of the median eminence (Fig. 2), GnRH terminals were associated closely with a diffuse inner band of dopamine varicosities (Fig. 3). These varicosities appeared somewhat medial to GnRH terminals. Discrete dopamine terminals were seen also in the outermost region of the contact zone juxtaposed to the portal capillaries. Both dopamine layers morphologically are within the contact zone of the median eminence. In the ventral region of the median eminence, only a few GnRH fibers were found in the zona externa, in contrast to an extensive accumulation of dopamine terminals seen therein.

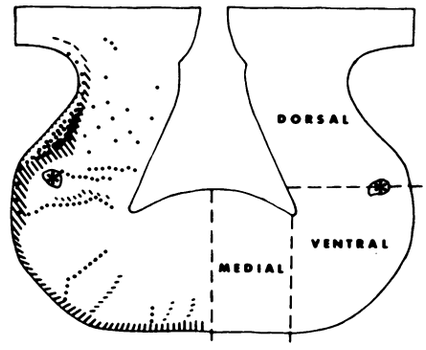

Fig. 2. Schematic representation of Fig. 1, depicting the distribution of catecholamines and GnRH in the median eminence. The median eminence is divided into a lateral and medial region at the most lateral aspect of the infundibular recess. The lateral region is subdivided into a dorsal, GnRH-rich region, and a ventral, GnRH-poor region. In the dorsal region, dopamine (line shading) is found in a diffuse inner band and in distinct terminals in the contact zone. Terminals of GnRH fibers are seen in the contact zone of this region (dots). In the ventral region, catecholamines are seen in the contact zone and along tanycytic profiles, and beaded GnRH fibers also are seen spanning the median eminence. Asterisks indicate blood vessel.

Strings of beaded GnRH fibers were seen in the fibrous and ependymal layers in the ventral region of the median eminence. Some of these fibers could be followed from the ventricular interface to the contact zone. Also, some fibers appeared to be associated with dopamine terminals. In contrast to the lateral region, the medial region of the median eminence was devoid of GnRH fibers, whereas intense accumulations of catecholamine terminals (*8*) were seen in the contact zone. Control sections prepared with absorbed antiserums or PBS as the primary antiserum were completely devoid of immunoreactive stain. However, endogenous peroxidase of red blood cells and connective tissue adjacent to the median eminence continued to stain.

Our study provides direct morphological evidence of a dual distribution of dopamine in the endocrine hypothalamus. The precise location of dopamine varicosities and GnRH teminals in identical regions of the median eminence lends support to the concept of a potential axo-axonic inhibitory regulatory mechanism of dopamine on GnRH release (*9*). Dopamine fibers in the dorsal region of the median eminence may terminate directly on GnRH processes or may send collateral branches to interact with the GnRH terminals as they course to the contact zone in the ventral region.

The finding of extensive concentra-

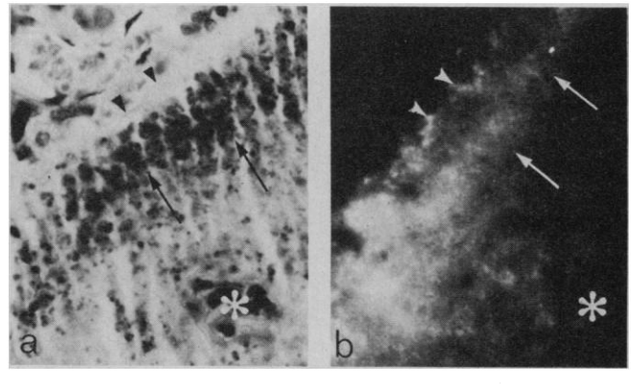

Fig. 3. High magnification of adjacent sections demonstrating (a) GnRH and (b) catecholamines. Terminals of GnRH fibers (black arrows) in the contact zone are associated with an inner layer of catecholamine varicosities (white arrows). Distinct dopamine terminals (white arrowheads) are seen in the outermost region of the contact zone not associated with GnRH (black arrowheads). The blood vessels indicated by asterisks in each section are identical (×375).

tions of dopamine in the contact zone of the ventral region of the median eminence, not directly associated with GnRH terminals, suggests that dopamine is available for direct release into portal blood to influence the anterior pituitary gland. A recent report (*10*) has shown high concentrations of dopamine in portal blood during different stages of the estrous cycle, which may support the idea that dopamine influences the release of prolactin from the anterior pituitary gland (*11*). It is also possible that dopamine in these regions of the median eminence may be involved directly in the regulation of other hypothalamic releasing hormones.

Thus, the correlative fluorescence-immunocytochemical technique provides for the simultaneous demonstration of monoamines and GnRH within the same tissue block and potentially offers a means for examining transmitter-hormone interactions microscopically at a given point in time. These data support the concept that dopamine may affect the release of both hypothalamic GnRH and anterior pituitary hormones. This technique may help to elucidate the role of neurotransmitters and neuropeptides in brain function by allowing the examination of simultaneous alterations in neuronal peptides and transmitters during different functional stages.

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12. We thank T. M. Nett for the antiserum 38; S. Sorrentino for antiserum F; L. Sternberger for the rabbit antiserum to horseradish peroxidase; E. A. Zimmerman and A. G. Robinson for the estrogen-stimulated neurophysin that was used to produce the section shown on the cover; and B. Putnam Buell for technical assistance in the preparation of this manuscript. The work was supported by PHS grant NS 11642.

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Behavior and Phylogeny: Constriction in Ancient and Modern Snakes

Abstract. *Comparative analyses of behavior have an underappreciated potential for revealing the role of ethoecological factors in the origins of higher taxa. Twenty-seven species (13 genera) in the advanced family Colubridae exhibited 19 patterns of coil application; one or two patterns were usually consistent within a genus. Forty-eight species (26 genera) in the primitive families Acrochordidae, Aniliidae, Boidae, and Xenopeltidae usually used a single pattern, despite differences in age, size, shape, habitat, and diet. This implies the shared retention of an action pattern used by their common ancestor no later than the early Paleocene. Constriction must have been used as a prey-killing tactic very early in the history of snakes and might have been a behavioral "key innovation" in the evolution of their unusual jaw mechanism.*

Three methods have been used to study the evolutionary history of behavior: structural correlates in extinct taxa, such as the head ornaments of some dinosaurs and the surface relief of cranial endocasts (*1*); fossil trackways and other artifacts (*2*); and comparative analyses of extant forms (*3*). Rigorous studies of the third type are infrequent, perhaps because of recent skepticism regarding behavioral homologies and because it is difficult to obtain large enough samples of taxa to be informative (*4*). However, comparisons across taxa can have important consequences for evolutionary biology. Given a fossil record of separate lineages in a group, an estimate of the minimum age of the behavior can be obtained. The behavior can then be correlated with morphology and paleoecology to suggest selective factors in the adaptive radiation of the group. We now report the modal action patterns used for constricting prey by 75 species of snakes in five families (*6–8*). We also specify an operational rationale for evaluating the origins of similar behavior in different species. Our results contribute to an understanding of ethoecological aspects of the origin of a highly unusual and widespread group of vertebrates and thus illustrate an underappreciated potential for comparative studies of animal behavior.

Constriction is a behavior pattern in which prey is immobilized by pressure exerted from two or more points on a snake's body (*9, 10*). Each portion of the

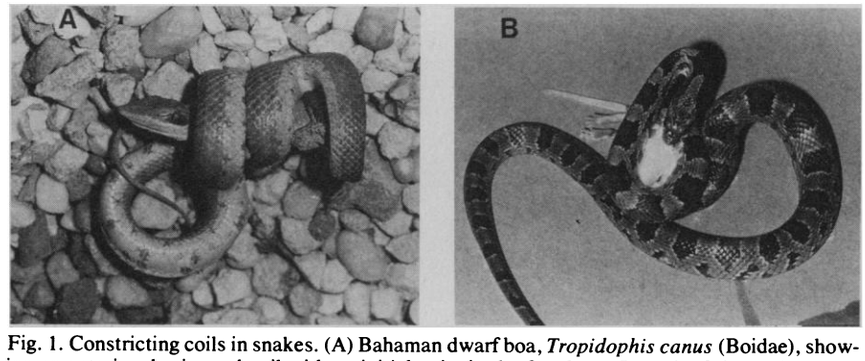

Fig. 1. Constricting coils in snakes. (A) Bahaman dwarf boa, *Tropidophis canus* (Boidae), showing an anterior, horizontal coil with an initial twist in the first loop. Length of the snake, ~ 22 cm. The prey is a lizard (*Anolis carolinensis*). (B) North American corn snake, *Elaphe guttata* (Colubridae), showing an anterior, vertical coil without an initial twist. Length, ~ 40 cm. The prey is a laboratory mouse.

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