seawater bath temperature and applied to the oral veil with a Pasteur pipette. Squid homogenate was taken from the same batch used in conditioning.

14. Most of the command cell penetrations were held long enough (~ ½ hour) to enable us to apply a complete sequence of stimuli. Several penetrations were held long enough (about 1 hour) to enable us to fully replicate the stimulus sequence in order to test the stability of the responses. The responses to the second sequence were generally identical to the first.
15. Of the six command neurons in naive animals that were inhibited by food stimuli, four were from two animals that refused to feed during the experiment, and two were from the last half of an experiment in which the animal had ingested large quantities of seawater during analysis of the first two command neurons, visibly distending the gut and inducing satiety-like effects. Apparently, when specimens are unwilling to eat for any reason, command neurons are inhibited rather than excited by food stimuli.
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Biologically Active Pituitary Hormones in the Rat Brain Amygdaloid Nucleus

Abstract. *While an attempt was being made to identify the source of the growth hormone releasing factor present in cerebral spinal fluid of man, it was discovered that cells of the rat amygdaloid nucleus, grown in tissue culture, produce a material that is immunologically and chromatographically identical to growth hormone found in the pituitary. Immunoperoxidase staining revealed dense accumulation of the peroxidase-antibody to growth hormone complex in amygdala cells. Significant amounts of growth hormone and adrenocorticotropin could be extracted from this limbic structure. Extracts containing immunoequivalent amounts of growth hormone were measured by bioassay in hypophysectomized rats. Stimulation of the growth of epiphyseal cartilage by extracts of the amygdala was comparable to the stimulation by extracts of anterior pituitary glands. The stimulatory effect of amygdala extracts on adrenal and gonadal size and weight and on growth of thyroid follicular epithelium was also comparable to that of pituitary extracts.*

While investigating the source of a growth hormone releasing factor present in human cerebral spinal fluid (*1*), we first studied the amygdaloid nucleus of the limbic system because of the many reports linking that brain structure to endocrine function of the anterior pituitary gland (*2*). Unexpectedly, we have discovered significant amounts of immunoreactive growth hormone in this structure and in media harvested from these cells grown in tissue culture. Significant amounts of immunoreactive adrenocorticotropin (ACTH) are also present in the amygdaloid nucleus of the rat brain, confirming a similar report of the extrapituitary presence of ACTH in discrete regions of the central nervous system (*3, 4*). Likewise, bioassay of rat amygdaloid nucleus extracts in hypophysectomized animals reveals significant bioactivity similar to the bioactivity of growth hormone, ACTH, gonadotropin, and thyroid stimulating hormone, as well.

Adult male and female Sprague-Dawley rats (approximately 200 g) were quickly decapitated and the brain was removed. A coronal slice was made at the level of the chiasmal crossing, and the amygdaloid nucleus quickly removed. This tissue was extracted and frozen for subsequent studies or immediately prepared for tissue culture by enzyme dispersal of single cells by means of short-term incubation with Pronase. Rat growth hormone was extracted by homogenization of the tissue in 0.01*N* sodium hydroxide and phosphate-saline buffer (*5*). Rat growth hormone, either from the anterior pituitary or from portions of the rat brain, was measured by solid phase radioimmunoassay (*6*) adapted in our laboratory for rat growth hormone. Rat ACTH was immunoassayed with an antiserum to 1-24 ACTH, which has a molecular weight of 2800; but we based our calculations on 1-39 ACTH which has a molecular weight of 4500. With these approaches, rat amygdaloid nucleus contained approximately 250 ng of growth hormone per gram of wet tissue. The ACTH content was approximately 4.1 ng per milligram of tissue or roughly 5 percent of pituitary content. Chromatography of this growth hormone-like material on Sephadex G-100, cycled with a Veronal buffer at *p*H 8.4, revealed a profile identical to that of ^{125}I-labeled rat growth hormone. Sections stained immunohistochemically with conjugated peroxidase–antibody to growth hormone complex revealed numerous cells of the amygdala containing a dense accumulation of the granular precipitate (*7*). Preliminary examination of other brain areas revealed the presence of a material similar to the immunoreactive growth hormone in the cortex, hippocampus, and hypothalamus, but none was present in the caudate nucleus or in the cerebellum. On the basis of wet weight of tissue, the amygdaloid nucleus contained the largest amount of this material.

Because of the possibility that this growth hormone might be of pituitary rather than of brain origin, we examined the effects of injecting radioactively labeled growth hormone into the systemic circulation. The ^{125}I-labeled growth hormone, 11×10^6 count/min, was injected into the tail vein of lightly anesthetized animals, and portions of muscle, kidney, fat, and brain were examined for the presence of radioactivity. No radioactivity was found in any part of the brain examined, whereas kidney and liver contained large amounts of radioactivity.

To provide further evidence that amygdaloid growth hormone was derived from this extrapituitary source, we also examined rats subjected to hy-

Table 1. Mean and standard deviations derived from bioassay of extracts of anterior pituitary (RAP) and amygdaloid nucleus (RAM) from adult rats 14 days after hypophysectomy. Four to five animals were included in each group. Experimental animals received comparable amounts of immunoreactive growth hormone (IRGH) in four daily divided doses administered intraperitoneally. A minimum of ten width measurements were made of individual silver nitrate–stained tibial epiphyses by means of an ocular micrometer, where 1 micrometer unit (m.u.) is equivalent to 0.005 mm.

Extract and dose (μg IRGH/ per rat)	Sex	Tibia epiphyseal width (m.u.)	Testis (mg)	Ovaries (mg)	Adrenals (mg)
Saline-0	M	8.3 ± 0.5	154 ± 0.7		7.6 ± 0.4
RAP-113	M	10.7 ± 0.4*	193 ± 4.0*		11.3 ± 0.9†
RAM-73	M	10.3 ± 0.2*	204 ± 6.2*		10.5 ± 2.8†
Saline-0	F	11.1 ± 2.0		15.2 ± 3.2	11.2 ± 1.6
RAP-95	F	13.6 ± 3.2†		19.1 ± 4.9†	13.2 ± 1.9†
RAM-90	F	15.1 ± 3.2*		29.7 ± 4.5*	16.7 ± 2.7*

*$P < .01$. †$P < .05$.

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pophysectomy. An initial 50 to 75 percent reduction in extractable growth hormone 7 days after hypophysectomy was followed by an actual increase in extractable immunoreactive growth hormone over that which obtained in intact animals. Further, when cells of the amygdaloid nucleus were successfully grown in tissue culture, progressive production of immunoreactive growth hormone was noted (Fig. 1) even when the medium was completely replenished every 3 to 5 days; amygdala cells from hypophysectomized animals showed a comparable phenomenon.

Finally, immunoequivalent amounts of extracted growth hormone from either the amygdaloid nucleus or the anterior pituitary gland were injected (intraperitoneally once daily for 4 days) into 28-day-old female and male rats 14 days after they had been hypophysectomized. Significant stimulation of target gland growth and epiphyseal cartilage width was noted (Table 1). Histologic examination of thyroids from hypophysectomized animals receiving extracts of the amygdaloid nucleus showed stimulation of follicular epithelial height comparable to that obtained with anterior pituitary extracts.

These data indicate the presence of immunoreactive pituitary hormone-like substances in the amygdaloid nucleus of the brain and show that this remarkable limbic structure also contains factors with biologic activity identical to or nearly identical to pituitary trophic hormones. These observations, although surprising, are not entirely unexpected. Thyrotropin releasing hormone (*8*), somatostatin (*9*), gonadotropin releasing factors (*10*), and beta lipotropin (*11*) have already been shown to be widely distributed in the brain. Prolactin-like immunoreactivity in nerve terminals of the rat hypothalamus has been reported (*12*). Krieger *et al.* (*3*) have recently reported extrapituitary localization of ACTH in a variety of rat brain areas, including the medial basal hypothalamus, median eminence, hippocampus, cortex, and cerebellum.

Although it seems unlikely that these brain-based, pituitary-like hormones are of pars distalis origin, reports based on neuroanatomical studies have suggested that hormones from the pars distalis may reach the brain by retroflow through the neurohypophysis and portal vessels, or by retrograde ependymal tanocyte transport to the cerebral spinal fluid with selective absorption by specific areas of the brain (*13*). However, the observation that cells of the amygdaloid nucleus,

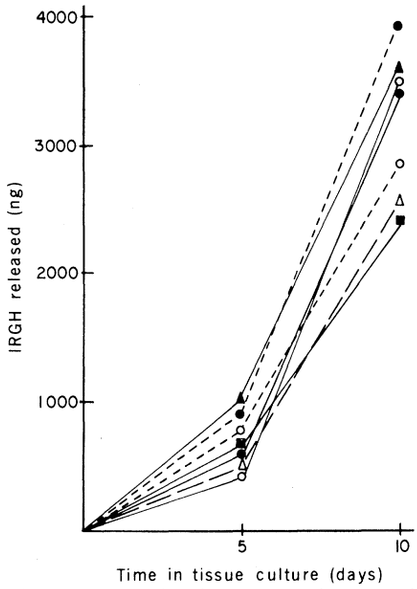

Fig. 1. Cumulative immunoreactive growth hormone (*IRGH*) released from cells of the adult rat amygdaloid nucleus grown in tissue culture. In these seven experiments, medium was completely exchanged at 5, 8, and 10 days. Each symbol represents growth hormone release from approximately 2×10^5 viable cells, enzymatically dispersed, washed, and plated into Falcon culture flasks containing basic Eagle's medium with 10 percent fetal calf serum.

from both intact and hypophysectomized animals, produce growth hormone in tissue culture for up to 16 days, argues for the synthesis of hormones in situ by this special central nervous system structure and probably by other areas of the brain, as well. Furthermore, Krieger *et al.* (*3*) failed to note any clear diminution in extrapituitary or extrahypothalamic ACTH in animals that had been hypophysectomized 10 days previously, thus strengthening the case for believing that pituitary hormones are, in fact, produced within the central nervous system.

Particularly impressive was the increase in the immunoreactive growth hormone content of the amygdaloid nucleus 30 days after hypophysectomy. Baker and Yen (*14*) reported a marked reduction in immunoperoxidase staining for growth hormone inhibitory factor or somatostatin in the rat median eminence 133 days after hypophysectomy. Whether this particular observation might account for the increase in growth hormone in the amygdaloid nucleus of long-term hypophysectomized rats is not known. Given this observation, we are at a loss to explain why an initial decline in growth hormone was noted 7 days after hypophysectomy, but suspect it is related to the abrupt change in the endocrinologic milieu that occurs after hypophysectomy. Indeed, it may be that brain levels of somatostatin initially rise in response to the loss of pituitary growth hormone and its metabolic products.

The finding of pituitary hormones synthesized by specific neurons of the central nervous system may lend additional credence to the "APUD" theory (amine, precursor uptake, L-dopa decarboxylase) that endocrine secretory cells are ectodermally derived (*15*). Others have suggested that neurosecretory axons of the brain may actually influence receptive cells to assume the micromorphologic and secretory functions we traditionally associate with specific cell groups within the anterior pituitary (*16*). Whether or not brain-derived pituitary-like hormones might function in such a manner, it is apparent that central nervous system (CNS)-derived substances cannot reach the systemic circulation because the absence of circulating pituitary trophic hormones clearly characterizes the hypopituitary state. Thus, we might speculate that these CNS-derived products, seemingly incapable of reaching the systemic circulation under normal conditions because of the intactness of the blood brain barrier, may, nevertheless, serve as neurotransmitter agents affecting both regulation of neurosecretory influences on pituitary function and affective behavior (*17*).

S. T. PACOLD, L. KIRSTEINS
S. HOJVAT, A. M. LAWRENCE
Research and Medical Services,
Veterans Administration Hospital,
Hines, Illinois 60141

T. C. HAGEN
Medical Service,
Wood Veterans Administration Center,
Milwaukee, Wisconsin 53193

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Serengeti Ungulates: Feeding Selectivity Influences the Effectiveness of Plant Defense Guilds

Abstract. *Association of* Themeda triandra, *a palatable grass species, with less palatable plants protected it from grazing by two comparatively unselective herbivores, wildebeest and African buffalo. Grazing on* T. triandra *by two more selective herbivores, Thomson's gazelle and zebra, was not related to the relative abundance of less palatable plants. The differential effectiveness of plant defense guilds against different ungulates may contribute to the high species diversity of the East African grazer fauna.*

Consumption of palatable forage plants by domestic ungulates is reduced when the palatable species are spatially associated with unpalatable plants in pastures (*1*). Atsatt and O'Dowd (*2*) proposed that such functional interdependence among plant species constitutes plant defense guilds. This report presents the first evidence, from studies in the Serengeti ecosystem, that such defense guilds may influence consumption by large wild herbivores. However, the protection from grazing afforded to a palatable species by association with unpalatable plants was not sustained as herbivore feeding selectivity increased.

East African ecosystems are well known for high biomasses and diversities of large, wild, grazing ungulates (*3*). The proportion of net above-ground primary productivity consumed by these herbivores is higher than has been reported for any other terrestrial grazing food web (*4*). This suggests that herbivore impact upon forage plants is intense, and places a premium on effective strategies of plant "escape" from grazing. In addition, the general ungulate fauna is one of substantial antiquity (*5*), suggesting a long period of plant-herbivore coevolution that might distinguish relations between animals and their food plants in these ecosystems from those involving domestic ungulates and their food plants. To examine the effectiveness of plant defense guilds in such an ecosystem, experiments were done in Tanzania's Serengeti National Park and Kenya's Masai-Mara Game Reserve during the dry seasons of 1974 and 1975. Forage quality falls below maintenance requirements of the animals during the dry season (*6*), and therefore acceptable forage should be strongly sought after by grazers during this period. Consumption of a highly palatable (*7*) grass, *Themeda triandra* Forsk., was determined in stands where the proportion of such less palatable (*7*) species as *Cymbopogon excavatus* (Hochst.) Stapf, *Loudetia kagerensis* (K. Schum) Hutch., and *Pennisetum mezianum* Leeke varied. An exclosure was erected in a test stand, and consumption was estimated by comparing fenced and unfenced areas before and after standard grazing periods ranging from 4 to 14 days, depending on grazing intensity (*8*). Although genotypic variations in palatability within plant species are well known (*9*), I assume that unpalatable genotypes of *T. triandra* were not preferentially associated with other plant species of generally low palatability.

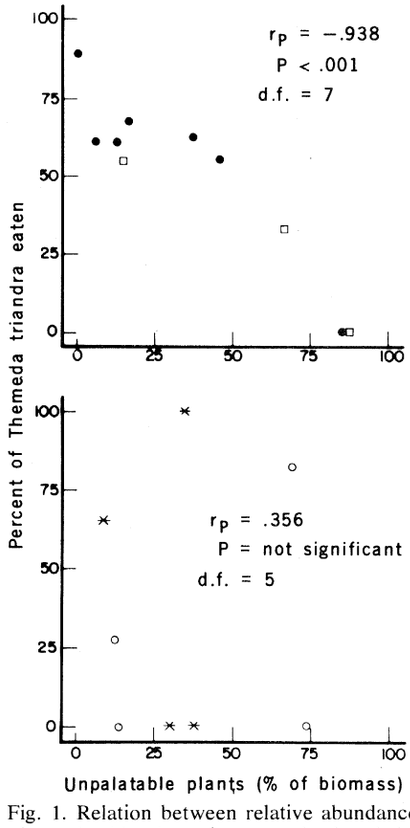

Fig. 1. Relation between relative abundance of unpalatable plants in a grassland and the percentage of *Themeda triandra* that was eaten by four herbivore species: (top) wildebeest (•) and buffalo (□); (bottom) zebra (○) and Thompson's gazelle (*). r_p is the partial correlation coefficient with *T. triandra* relative abundance held constant. Each point is the mean of four replicate samples in a single study stand.

I studied four species of large grazing ungulates: wildebeest (*Connochaetes taurinus albojubatus* Thomas), Thomson's gazelle (*Gazella thomsonii* Gunther), zebra (*Equus burchelli* Gray), and African buffalo (*Syncerus caffer* Sparrman). These species constitute the vast majority of the grazing biomass in the Serengeti ecosystem (*10*). Spatial segregation between these grazers during the dry season is pronounced (*11*), and hence study stands were isolated where grazing was confined to a single animal species (*12*). Previous studies suggest that zebra and Thomson's gazelle are more selective feeders than wildebeest and buffalo (*13*). I calculated feeding selectivity directly from randomly placed study sites, rather than those utilized in this experiment, as

$$S = \Sigma|p_s - p_c| /2$$

where p_s was the proportional abundance of a plant species in fenced control areas, p_c was the proportional abundance of that species in consumption by a given herbivore population (*8*), and the absolute value of the differences are summed for all plant species. This index will be zero if animals consume species in the same proportions as their abundances in the plant community, and will approach one as consumption becomes the inverse of species relative abundance. I found mean dry season selectivities to be 0.189 for wildebeest, 0.215 for buffalo, 0.315 for zebra, and 0.335 for Thomson's gazelle ($F_{3,14} = 10.470$ for $P < .001$). The least significant difference for a three-way comparison ($P = .05$) was .036. So, during the dry season, the species fall into two groups, the less selective species being wildebeest and buffalo, and the more selective species being zebra and Thomson's gazelle. Therefore, I combined data for analyses of the effect on consumption of *T. triandra* of association with unpalatable plants.

One potentially serious design defect in studies of defense by association is the

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