of observers. The nestlings usually consumed this food quickly.

13. The unprovisioned foster broods performed an average of 1.07 fights per dyad per day, while the provisioned broods actually fought slightly more (mean = 1.56) (Mann-Whitney U test: $U = 14$, not significant).
14. Egret chicks fed by egret parents begin direct feeds on day 7 and use that method in 90 percent of their boluses by day 24. Similarly, fostered heron chicks began using it regularly on about day 9 and reached 90 percent on day 24. By contrast, neither heron young fed by heron parents nor fostered egret chicks reached a frequency of 20 percent.
15. See J. B. Nelson, *The Sulidae* (Oxford Univ. Press, Oxford, 1978); R. S. Miller, *Wilson Bull.* **85**, 436 (1973); B. Meanley, *ibid.* **67**, 84 (1955); D. F. Werschkul, *Auk* **96**, 116 (1979); D. Mock and B. J. Ploger, in preparation; M. Fujioka, unpublished data. Data on reddish egret from R. T. Paul (personal communication).
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Learned Histamine Release

Abstract. *Most of the effort directed at understanding the problems of allergy has focused on the interacting components of the immune system. The possibility that histamine may be released as a learned response has now been tested. In a classical conditioning procedure in which an immunologic challenge was paired with the presentation of an odor, guinea pigs showed a plasma histamine increase when presented with the odor alone. This suggests that the immune response can be enhanced through activity of the central nervous system.*

Although there is evidence that learning may modify immunosuppression (*1–3*), its effect in activating the immune system has not been demonstrated. There have, however, been many anecdotal reports of associative learning in allergic reactions. Suggestions of this phenomenon existed in the 19th century; for example, an asthmatic patient who had an allergic reaction to roses experienced an attack when exposed to an artificial rose (*4*). However, the lack of data on the mechanisms of such a response has hindered the acceptance of associative learning as a factor in immune responses. Histamine is a mediating factor in the immune system (*5, 6*) and has been found in most tissues (*7*). It is released in a variety of pathological conditions thought to be stress related, such as duodenal ulcers (*8*), asthma (*9*), cluster headaches (*10*), and premenstrual stress (*11*). This suggested to us that histamine may be released as a learned response to the pairing of a neutral stimulus with an immunologic challenge. We showed that guinea pigs (*Cavia cobaya*) had increased plasma histamine levels in response to a neutral stimulus (an odor) in a classical conditioning procedure in which an immunologic challenge was paired with presentation of the odor.

Table 1. Experimental sequence. During training, CS+ was given with the antigen, and CS− was given without the antigen. During test trials no antigen was present.

Trial No.	Stimulus
Training trials	
1	CS+
2	CS−
3	CS−
4	CS+
5	CS+
6	CS−
7	CS−
8	CS+
9	CS−
10	CS+
Test trials	
1	CS+
2	CS−
3	CS+

Four weeks before training was begun, eight adult male guinea pigs (Hartley strain) were immunologically sensitized to bovine serum albumin (BSA) by injecting into a footpad 2.5 mg of BSA in a 0.1-ml mixture of equal volumes of Freund's adjuvant and normal saline. A 5 percent solution of BSA subsequently served as the unconditioned stimulus for histamine release. All animals were handled and gentled three times weekly while they were weighed and their cages were cleaned. This also served to reduce stress to the animal caused by being handled during the testing procedures.

A classical discrimination conditioning design was used to train the animals, with each animal acting as its own control. In this design, one odor (CS+) was always paired with the unconditioned stimulus (BSA), and the other (CS−) was paired with saline. One percent solutions of dimethylsulfide (sulfur smelling) and triethylamine (fishy smelling) were used as the conditioned stimuli. For half the animals, CS+ was dimethylsulfide and CS− was triethylamine and for the other half, CS+ was triethylamine and CS− was dimethylsulfide. Each animal was given ten presentations, five trials with the CS+ and five trials with the CS−. Training trials were given 1 week apart to allow the animal to recover from any allergic reaction. The order of the presentation of the CS+ and the CS− was randomized (Table 1).

At the start of each training trial the animal was placed in a glass-walled container and a cotton-tipped swab soaked in either the CS+ and BSA or in the CS− and saline was placed on the animal's nose for 3 seconds. Ten minutes after exposure to the stimulus, the animal was anesthetized with ether, and 2 to 4 ml of blood were drawn from the retro-orbital sinus (*12*). The blood was immediately centrifuged and the plasma was tested for histamine by a radioenzymatic assay (*13*). Test trials began 2 weeks after the training. In the first trial the CS+ odor was presented without BSA. Two weeks later the CS− odor was presented. After another 2 weeks the CS+ was presented again. All other procedures were identical to those during training. The experimenter, in all phases of the study, was unaware of the order and pairing of the stimuli.

Plasma histamine levels during the test trials (extinction) were analyzed with a repeated measures analysis of variance, which achieved an overall F value of 45.57 ($P < 0.0005$). One-tailed planned comparisons between specific test conditions revealed significant differences between the CS− and the first CS+ ($P < 0.001$). All eight animals had greater histamine release in response to the CS+ (mean ± S.E.M. = 147.5 ± 28.7 ng/ml) than to the CS− (49 ± 7.7 ng/ml) (Table 2). A smaller difference was found between the CS− and the second CS+ (54.2 ± 17.0 ng/ml) ($P < 0.055$). Only one animal had a lower histamine level in response to the second CS+ than to the CS−. No significant difference was found between the CS− trial and the

Table 2. Results of test trials with the conditioned stimuli in the absence of antigen. Results are given as means ± S.E.M.

Trial	Plasma histamine (ng/ml)
Baseline	18.3 ± 4.9
Test trials	
CS+	147.5 ± 28.7
CS−	4.9 ± 7.7
CS+	54.2 ± 17.0

pretraining baseline ($P = 0.75$). As a final verification that the histamine release on the first test trial was not a generalized effect from the preceding antigen-paired CS+ training trial, we compared the first CS+ training trial with the next CS− trial. The histamine release in response to the first CS+ training trial was greater in all eight animals ($t = 2.94$; $P < 0.025$). Generalized sensitivity to the previous histamine release does not explain our result. An additional comparison of data from the first experience with an antigen to data from the first test trial—the first test trial was CS+ with no antigen present—showed an increase in plasma histamine comparable to that experienced from an allergen (means of 140 and 142 ng/ml, respectively).

These results indicate that the animals had experienced a significant increase in plasma histamine as a function of associative learning. We believe we have conditioned a histamine release of a magnitude similar to that found in a physiologic (*7*) reaction for our animals. The difference in the levels of histamine between the first and second CS+ trials also indicates that this learned response may be extinguished through repeated unpaired exposures to the stimulus odor, as would be expected of a classically conditioned response.

Through learned associations between allergic reactions and environmental stimuli, a specific allergic response may be generalized to a number of environmental elements. Associative learning should be included in understanding the development and treatment of allergies.

MICHAEL RUSSELL
KATHLEEN A. DARK
ROBERT W. CUMMINS
GEORGE ELLMAN
ENOCH CALLAWAY
HARMAN V. S. PEEKE

Brain-Behavior Research Center, Sonoma Developmental Center, University of California, Eldridge 95431

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Processing of Proenkephalin Is Tissue-Specific

Abstract. *Most neuropeptides are synthesized as large precursor proteins. These precursors undergo a maturation process involving several proteolytic events that generate the biologically active peptides. The enzymatic mechanisms underlying this processing are still largely unknown. The processing of the precursor protein proenkephalin was studied in two different bovine tissues, the hypothalamus and adrenal medulla. The high molecular weight enkephalin-containing peptides that accumulate in these two tissues were found to be different, indicating the existence of two processing pathways for this neuropeptide precursor.*

Neuropeptides are widely distributed throughout the central and peripheral nervous systems and participate in diverse neuronal functions. The major events underlying the biosynthesis of neuropeptides are not yet fully understood. Most neuropeptides appear to be synthesized initially as large precursor proteins, which undergo proteolytic processing to produce the active peptides (*1*). One example of such a precursor is proenkephalin, a 27.3-kD protein that contains four copies of [Met]enkephalin and one copy each of [Leu]enkephalin, the heptapeptide [Met]enkephalin-Arg^6-Phe^7, and the octapeptide [Met]enkephalin-Arg^6-Gly^7-Leu^8 (*2*) (see Fig. 1).

Several large enkephalin-containing peptides (ECP's) thought to represent intermediates in the processing of proenkephalin have been purified from the bovine adrenal medulla (*3*). The characterization of these intermediates has led to the proposal that the processing of proenkephalin in the adrenal medulla involves several proteolytic cleavages that start in the carboxyl-terminal region of this precursor (*4*).

The processing pathway of proenkephalin in brain tissue has not yet been described. The major obstacle in characterizing such a pathway has been the lack of a brain region in which the processing intermediates accumulate as they do in the adrenal gland. We observed that high molecular weight ECP's are present in the bovine hypothalamus (*5*), where the enkephalins and oxytocin are localized in the magnocellular neurons of the supraoptic nucleus (*6*).

The well-characterized anatomy of the hypothalamic magnocellular neuronal system projecting to the neurohypophysis provides a convenient model to study the axonal transport and processing of neuropeptide precursors (*7*). Dissection of the cell bodies located in the supraoptic nucleus, the axons traveling through the pituitary stalk, and the nerve terminals located in the neurohypophysis has allowed a separation of the different stages in the maturation of the precursor of the pituitary hormone vasopressin (*7*). By exploiting these anatomical features, we found that proenkephalin is processed during axonal transport through the hypothalamo-neurohypophysial system. Furthermore, the processing intermediates that accumulate in the supraoptic nucleus are different from those found in the adrenal medulla, indicating different processing pathways in these two tissues.

The amounts of free [Met]enkephalin and total [Met]enkephalin-containing material were determined in the supraoptic nucleus, pituitary stalk, and neurohypophysis (Table 1). The [Met]enkephalin content of each tissue was measured by radioimmunoassay (RIA) both before and after sequential digestion with trypsin and carboxypeptidase B. This proteolytic treatment releases [Met]enkephalin sequences from larger precursors in which they are flanked by basic amino acid residues (*8*). In the cell bodies of the supraoptic nucleus, the [Met]enkephalin immunoreactivity released by this enzymatic treatment represents 75 percent of the total [Met]enkephalin content. In the axons passing through the pituitary stalk the proportion of [Met]enkephalin immunoreactivity in ECP's is reduced to 56 percent of the total. The neuronal terminals of the neurohypophysis contain almost exclusively free [Met]enkephalin, with an increase of only 11 percent after digestion. Thus, the amount of [Met]enkephalin present in larger peptides decreases with increasing distance from the cell body.

The molecular size of the various [Met]enkephalin-containing peptides throughout the hypothalamo-neurohypophysial system was determined by gel