the effect on homing performance, is in agreement with similar observations by Keeton (5) in his magnet tests in overcast conditions.

12. Our results also rule out inertial navigation as suggested by J. S. Barlow [*J. Theor. Biol.* **6**, 76 (1964)], and the short vanishing intervals of the experimentals without magnets (median, 242 seconds) make a scanning of map gradients highly improbable, as discussed theoretically by H. G. Wallraff [*Das Navigationssystem der Vögel* (Oldenbourg, Munich, 1974), p. 64]; compare also W. T. Keeton [in *Advances in the Study of Behavior*, D. S. Lehrman *et al.*, Eds. (Academic Press, New York, 1974), vol. 5, pp. 47–132].
13. Keeton (5) demonstrated that inexperienced pigeons were disoriented by magnets in sunny conditions. Recent clock-shift experiments show that very young and inexperienced pigeons do not use the sun compass but are nevertheless well oriented (R. Wiltschko and W. Wiltschko, *Behav. Ecol. Sociobiol.*, in press). Together these findings suggest that the early navigational abilities include the magnetic compass.
14. All computer work for this study was carried out by the Hochschulrechenzentrum of the Universität Frankfurt a.M. We thank W. Mann, U. Kotzur, and all the other helpers who assisted in maintaining and testing the birds. Thanks are also due to C. Walcott, State University of New York at Stony Brook, for critically reading an early draft of this report and to W. T. Keeton for methodological assistance. This study was supported by the Deutsche Forschungsgemeinschaft in program SFB 45.

27 March 1981; revised 26 May 1981

Modification of the Discharge of Vagal Cardiac Neurons During Learned Heart Rate Change

Abstract. *Visually conditioned heart rate change in the pigeon has been developed as a vertebrate model system for the cellular neurophysiological analysis of associative learning. In previous studies of the "final common path," it was shown that both the vagal and sympathetic cardiac innervations contribute to this response. The present experiments indicate that, prior to any behavioral training, the visual stimulus elicits a small decrease in the discharge of vagal cardiac neurons. During conditioning, this stimulus evokes a progressively greater decrease in discharge that parallels the acquisition of the conditioned cardioacceleration. In contrast, nonassociative control animals show habituation of the initial decrease in discharge. These data confirm the involvement of the vagal cardiac innervation in conditioned heart rate change, indicate that the vagal innervation acts synergistically with the sympathetic to produce cardioacceleration, and suggest that a short-latency pathway mediates the conditioned response.*

The neuronal mechanisms of information storage remain one of the principal challenges in contemporary neurobiology. Over the past decade, however, the development of effective model systems has significantly advanced our understanding of the cellular basis of nonassociative learned behaviors. This progress has resulted largely from the exploitation of "simple" invertebrate models, but few effective systems are available for cellular analysis of associative learning or of learning in vertebrates (*1*).

Over the past 15 years we have been developing one such model that permits cellular analysis of both nonassociative and associative learning in a relatively simple vertebrate system (*2*). The associative learning is established with a conventional Pavlovian procedure in which whole-field retinal illumination (the conditioned stimulus, CS), is paired with foot shock (the unconditioned stimulus, US), to produce a learned change in heart rate (the conditioned response, CR). Behaviorally, this system is now well characterized and has many attractive properties for cellular neurophysiological analysis (*3*). For example, in the pharmacologically immobilized animal, stable conditioning develops in 30 minutes and asymptotic performance in approximately 2 hours (*4*). Moreover, considerable effort has been devoted to identifying the relevant neural circuitry, and a first approximation to a necessary pathway from the eye to the heart is now available (*2*).

As part of this effort to map the neural circuitry, we established that the CR is mediated entirely by the cardiac nerves and that both vagal and sympathetic innervations participate. Moreover, the cells of origin of this "final common path" have been localized and criteria established for their electrophysiological identification in the behaving animal (*5*).

We now describe the discharge characteristics of vagal cardiac neurons during CR development. The objectives of investigating these motoneurons were (i) to describe the temporal properties of the informational flow along the identified pathways, unconfounded by delays at the motor periphery, and (ii) to characterize more precisely the vagal and sympathetic contributions to conditioned heart rate change.

We studied 23 experimentally naïve white Carneaux pigeons (*Columba livia*), ranging in age from 2 to 6 months and weighing 450 to 650 g. Under pentobarbital anesthesia, the posterior cerebellum was removed to expose the floor of the fourth ventricle. Five to ten days later cellular neurophysiological experiments were undertaken. The animals were immobilized with α-bungarotoxin (*4*), artificially ventilated, and placed in a stereotaxic apparatus in an acoustic chamber. Electrodes for monitoring the electrocardiogram and delivering the foot shock were inserted (*3*), and the left pupil was dilated and a contact lens placed over the cornea (*4*). Under lidocaine anesthesia, the midcervical, right vagus nerve was exposed and placed over a bipolar Ag-AgCl electrode for stimulation. A 4*M* NaCl micropipette (8 to 12 megohm) was advanced into the brainstem, and single vagal cardiac units were isolated and identified (*5, 6*).

After an adaptation period, either conditioning (N = 13) or sensitization (N = 10) training was initiated. For both procedures, the visual stimulus was a 50 foot-lambert (1 foot lambert = 3.4263 cd/m^2), monocular presentation of 6-second whole-field illumination; this stimulus was delivered through a fiber-optic bundle and an electronically controlled shutter (*4*). The foot shock consisted of a 500-msec train of biphasic pulses delivered at 60 Hz with a constant-current

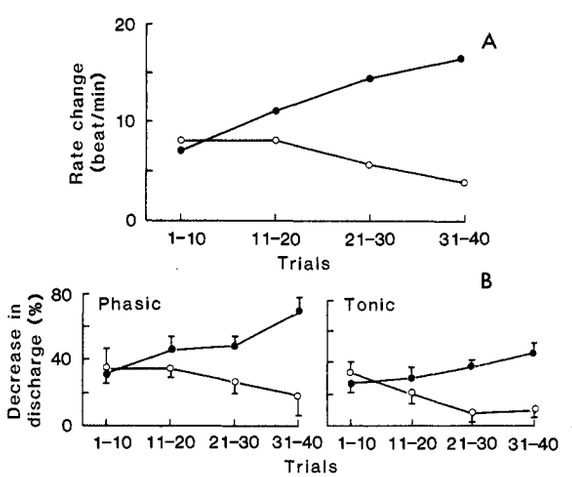

Fig. 1. (A) Mean differences between heart rates during the light and preceding control periods of birds receiving conditioning (●) (N = 13) or sensitization (○) (N = 10) procedures. Each point represents a group mean for a block of ten trials. (B) Mean discharge changes of vagal cardiac neurons during the phasic and tonic response periods during conditioning (N = 10) and sensitization (N = 8). Each point represents a group mean for a block of ten trials, and the error bars represent 1 standard error of the mean. Units were recorded from the same birds as in (A).

0036-8075/81/1016-0345$01.00/0

stimulator. Conditioning animals received 40 light presentations, each immediately followed by the foot shock. The mean intertrial interval was 3.7 minutes. Sensitization animals also received 40 lights and 40 foot shocks, but they were unpaired (*3*).

Heart rate changes (*7*) are presented in Fig. 1A. Consistent with previous studies (*3*), the response to the light increased during conditioning and attenuated with unpaired presentations [$F(3, 62) = 3.94$, $P < .02$]; the absolute response levels differed during the final two ten-trial blocks [$t(21) = 1.79$, $P < 0.5$; $t(21) = 2.11$, $P < .025$]. Baseline heart rates neither differed between groups nor were affected differentially by the training procedures. Thus, associative learning was clearly established.

With respect to the neuronal data, vagal cardiac neurons showed a short-latency (unconditioned) decrease in discharge in response to the initial light presentation, which was maintained for the duration of the visual stimulus. The magnitude of this light-evoked decrease in discharge was affected differentially by the two training procedures, increasing over conditioning and attenuating over sensitization. For quantitative assessment of these changes, the 6-second visual stimulation period was divided into phasic and tonic response periods, and the change in unit activity relative to spontaneous activity before the stimulus was calculated (*8*). The decrease in discharge was greater during conditioning than sensitization in both phasic [$F(1, 17) = 5.69$, $P < .05$] and tonic [$F(1, 17) = 10.3$, $P < .01$] periods (Fig. 1B). Further analysis indicated that, analogously to the heart rate responses, the neuronal responses in the two behavioral procedures differed during the last two ten-trial blocks in both phasic and tonic response periods ($P < .05$ for all t-tests). Comparison of A and B of Fig. 1 suggests a close relationship between the heart rate and cellular responses. As with heart rate, the baseline activity of the vagal cardiac neurons did not differ between groups and was not affected differentially by training.

To estimate the central processing time for the CR, the response latencies were analyzed further. Unfortunately, precise definition of the latencies of individual units was precluded by their low maintained activity. Consequently, histograms of pooled data after the stimulus were constructed with 40-msec bins. The data for each bin were expressed as a standardized score relative to maintained activity just before the stimulus in order to normalize the responses and allow direct assessment of probability values.

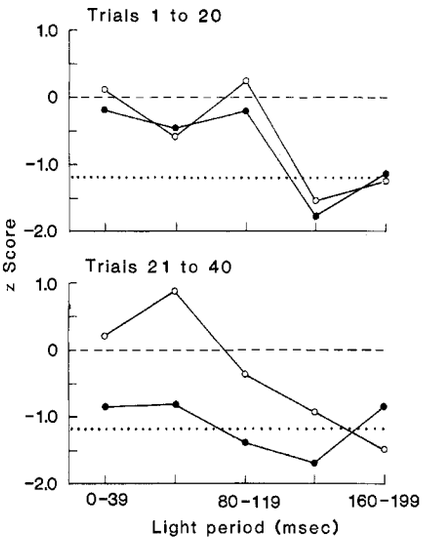

Fig. 2. Change in the short-latency, light-evoked discharge of vagal cardiac neurons during conditioning (●) and sensitization (○). Each point represents a mean standardized score for two ten-trial blocks. Points below the dotted line ($z = -1.17$) are significantly different from baseline ($P < .05$).

Early in training (trials 1 to 20), when the groups did not differ significantly with respect to change in either light-evoked heart rate or neuronal discharge rate, the response latencies were similar, first reaching significance at ≤ 120 to 159 msec. Late in training, however, (trials 21 to 40), when the light-evoked heart rates and discharge rates of the two groups had diverged, response latency in the conditioning group decreased to ≤ 80 to 119 msec and increased to ≤ 160 to 199 msec in the nonassociative control group. Thus, the shortest latency changes in discharge of vagal cardiac neurons appear to be affected differentially by training.

The data confirm our previous observation, based on selective cardiac denervation, that the vagal cardiac innervation contributes to the cardioacceleratory CR (*5*). Moreover, the response of the vagal cardiac neurons to the CS, a decrease in discharge rate, indicates that this component of the final common path acts synergistically with the sympathetic cardiac innervation.

An unexpected finding was that the vagal cardiac neurons responded to the light before training. Since the cardiac sympathetic postganglionic neurons also showed an unconditioned response to the light, the pathway mediating the CR seems initially responsive to light, and the effect of associative training is to increase the probability of occurrence and the magnitude of these light-evoked responses. In contrast, nonassociative training attenuates the initial light-evoked change in discharge. Thus, the response enhancement during conditioning depends on systematic light-shock pairing and reflects an associatively learned response. Indeed, the properties of the vagal discharge over training parallel the CR development.

The response latencies of the vagal cardiac neurons are of interest, since they indicate that the information transfer along the pathway is rapid. In later training these latencies may be < 80 msec, and since the shortest latency responses of the retinal ganglion cells to the CS are approximately 20 msec (*2, 9*), the minimum central processing time for the vagal component of the CR may be ≤ 60 msec. This suggests a relatively simple system and also eliminates feedback from the cardiovascular periphery as contributing to discharge changes during the phasic response period.

Our results do not address the question of whether "plastic" change occurs at the level of the vagal cardiac neurons, since the modification of their discharge over training may merely reflect changes occurring along more rostral segments of the pathway. Identifying sites of training-induced modification is more easily pursued by analyses beginning at the input segment of the system (*2, 9*). However, the results, particularly when combined with those for the sympathetic cardiac innervation and retinal ganglion cells, establish temporal boundary conditions for the short-latency component of the neural activity and define a time window within which other structures along the identified pathways must respond to the CS.

MICHAEL R. GOLD*
Department of Physiology, University of Virginia, Charlottesville 22903
DAVID H. COHEN
Department of Neurobiology and Behavior, State University of New York, Stony Brook 11794

References and Notes

1. See E. R. Kandel, in *Handbook of Physiology*, section 1, *The Nervous System*, vol. 1, *Cellular Biology of Neurons*, E. R. Kandel, Ed. (American Physiological Society, Bethesda, Md., 1977), p. 1137.
2. D. H. Cohen, in *Limbic and Autonomic Nervous System Research*, L. V. DiCara, Ed. (Plenum, New York, 1974), p. 223; in *Neural Mechanisms of Goal-Directed Behavior and Learning*, R. F. Thompson, L. H. Hicks, V. B. Shvyrkov, Eds. (Academic Press, New York, 1980), p. 283.
3. ——— and D. G. Goff, *Physiol. Psychol.* **6**, 127 (1978).
4. M. R. Gold and D. H. Cohen, *Brain Res.* **216**, 163 (1981).
5. D. H. Cohen and L. H. Pitts, *ibid.* **9**, 15 (1968); J. S. Schwaber and D. H. Cohen, *ibid.* **147**, 79

(1978); D. H. Cohen and A. M. Schnall, *J. Comp. Neurol.* **140**, 321 (1970); ——, R. L. Macdonald, L. H. Pitts, *ibid.*, p. 299.
6. Care was taken with infiltration anesthesia to minimize discomfort. Successful conditioning performance demands the animals be in no distress, since discomfort is invariably reflected in high baseline heart rates and a lack of conditioning.
7. The heart rate response was measured by subtracting the number of beats in the 6-second period immediately preceding the light (baseline period) from the number of beats during the light period. This difference was expressed in beats per minute (*3*).
8. The rationale for separating the stimulus period into a 500-msec (phasic) period and a subsequent 5500-msec tonic period for analytic purposes is that neurophysiological analyses of other segments of the system have indicated distinct transient responses occurring within the first few hundred milliseconds (*9*). This is particularly prominent for the cardiac sympathetic postganglionic neurons, in which the light-evoked response is almost entirely phasic. In fact, the CR may be largely mediated by this short-latency component (*2*).
9. C. M. Gibbs and D. H. Cohen, *Soc. Neurosci. Abstr.* **6**, 424 (1980); J. Wall *et al.*, *ibid.*, p. 424.
10. Supported by NSF grants BNS-72-20537 and BNS-80-16396 and NIH grant P01 NS 14620. M.R.G. was supported by NIH training grant T32 HL07284.. We thank C. Schack for her secretarial assistance.
* Present address: Department of Physiology, University of Colorado School of Medicine, Denver 80262.

6 February 1981; revised 8 June 1981

Supraoptic Nucleus of the Brattleboro Rat Has an Altered Afferent Noradrenergic Input

Abstract. *The distribution of fluorescent varicosities in the supraoptic nucleus of Brattleboro rats was compared to that in normal rats. The Brattleboro rat, which is characterized by a genetic absence of vasopressin, had fewer fluorescent varicosities in apposition to the vasopressin-deficient perikarya. The oxytocin-producing neurons in the same nucleus were hyperinnervated. These data suggest that the target neuron peptide (vasopressin) is necessary for the maintenance of normal noradrenergic innervation patterns.*

Oxytocin and vasopressin are magnocellular neurosecretory peptides of the mammalian hypothalamo-neurohypophyseal system. They play an important role in the onset of parturition and milk ejection and help regulate water and electrolyte balance within intra- and extracellular fluid compartments. These hormones are synthesized in and transported by neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus (*1*). In addition, the hypothalamic nuclei receive a dense input from brainstem noradrenergic neurons of the locus coeruleus and pontomedullary reticular formation (*2*). These noradrenergic neurons, through their hypothalamic innervation, are thought to play an important role in regulating the release of vasopressin and oxytocin (*3*).

A new approach for examining putative neuronal interactions (*4*) has been used to study the distribution of noradrenergic varicosities and their peptidergic target neurons in the SON and PVN (*5*). From these studies it appears that the densest accumulation of fluorescent varicosities occurs in the most ventral regions of the SON, where vasopressinergic cells predominate. On the basis of a recent ontogenetic study wherein peptide staining of neurosecretory neurons of the SON and PVN appeared before fluorescence of noradrenergic varicosities, it was postulated that the magnocellular neurosecretory products act as neurotrophic agents, providing a target for ingrowing noradrenergic axons (*6*). Examining a different central target, however, Lauder and Bloom (*7*) found, through the use of autoradiography and fluorescence histochemistry, that noradrenergic neurons of the locus coeruleus begin their growth and development several days before their probable target neurons in the cerebellum and hippocampus begin to differentiate. This suggested that monoamine neurons regulate the onset of neuronal differentiation in their projection areas, perhaps in a neurotrophic manner.

To further test these two hypotheses, we studied the Brattleboro rat, which lacks a specific target neuron peptide (vasopressin) but not the target neuron itself. This rat is genetically incapable of vasopressin synthesis and is therefore chronically dehydrated (*8*). Analysis of noradrenergic innervation patterns of the vasopressin-deficient SON might help determine whether peptide content is necessary for the maintenance of functional noradrenergic interactions.

Table 1. Number of varicosities apposed to perikarya in the SON of normal and Brattleboro rats. Each value is the mean for three rats (*15*).

SON region	Strain	Varicosities per neuron	P*
Dorsal	Control	1.25	
	Brattleboro	1.76	< .0005
Ventral	Control	1.97	
	Brattleboro	1.64	< .001

*One-way analysis of variance.

Six adult male Long-Evans homozygous Brattleboro rats and five normal male Long-Evans rats of the same age were decapitated and processed for formaldehyde-induced fluorescence of catecholamines (*9*). All tissue blocks were serially sectioned (6 μm) and every tenth section was mounted and stained with Luxol fast blue and cresyl violet to provide anatomical orientation. Sections throughout the rostral, middle, and caudal levels of the SON were pressed onto glass slides and photographed in a Leitz Dialux fluorescence microscope with mercury-vapor lamp, narrow-band excitation, and K460 barrier filters. Sections adjacent to those used for fluorescence microscopy were mounted on gelatin-coated glass slides and stained for rat neurophysin by the peroxidase-antiperoxidase procedure (*10*). The sections stained for neurophysin were photographed, and photomontages were assembled to reconstruct the SON at its various levels. Using clear acetate overlays, we traced all the neurons of the SON, marking those which stained for neurophysin and, in the Brattleboro rat, marking those which did not stain for neurophysin. Each overlay was then transferred to the photomontage of the adjacent section processed for fluorescence microscopy, and the varicosities abutting both neurophysin-positive and neurophysin-negative perikarya were marked. With this approach the pattern of fluorescent varicosities in the SON of three normal and three Brattleboro rats was quantitatively assessed. We counted the number of varicosities apposing each of a total of 1415 neurons (Table 1).

The SON of control animals contained a dense distribution of catecholamine varicosities. The distribution was densest in the ventral region of the nucleus and became progressively less dense in the more dorsal regions (Fig. 1). Most of the ventrally distributed varicosities appeared to appose magnocellular perikarya and their ventrally directed processes. The majority of these neurons have been immunocytochemically identified as vasopressinergic, whereas most of the dorsal SON neurons are oxytocin-containing (*11*).

The distribution of noradrenergic fibers in the SON of the Brattleboro rats appeared different from that in the controls. Ventral regions of the nucleus

0036-8075/81/1016-0347$01.00/0