ed at the Society for the Study of Reproduction meeting, Madison, Wis., 1982.
10. Fluids from 25 fetuses were pooled for radioimmunoassay. Group means (± standard error of the mean) are based on ten pools for females and five pools each for 0M and 2M males. The data for females were reported previously (*3*). Concentrations in the amniotic fluid (picograms per fetus) were, for testosterone: all females, 115.1 ± 4.8; 0M males, 115.1 ± 8.5; 2M males, 110.2 ± 6.5; for progesterone: all females, 259.3 ± 14.0; 0M males, 253.4 ± 6.2; 2M males, 253.1 ± 18.9; concentrations of testosterone in the blood were, for all females (nanograms per milliter), 0.98 ± .05; 0M males, 2.89 ± .15; 2M males, 3.13 ± .20.
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Hypothalamic γ-Aminobutyric Acid Neurons Project to the Neocortex

Abstract. *Three groups of γ-aminobutyric acid–containing neurons were found in the mammillary region of the posterior hypothalamus. The groups correspond to the tuberal, caudal, and postmammillary caudal magnocellular nuclei. Many cells in these nuclei were retrogradely labeled with fast blue after the injection of this fluorescent dye into the neocortex. Immunohistochemical experiments showed that these same neurons also contained the γ-aminobutyric acid–synthesizing enzyme glutamate decarboxylase. These results provide morphological evidence for a γ-aminobutyric acid pathway arising in magnocellular neurons of the posterior hypothalamus and innervating the neocortex.*

For many years it was thought that the neocortex receives its entire subcortical input through the thalamus. However, electrophysiological evidence has suggested the existence of a "reticular activating system" arising in the brainstem and innervating the neocortex (*1*). With the demonstration of brainstem monoamine systems that project to the neocortex, this idea was given a morphological basis (*2*). In addition, recent evidence indicates the presence of a cholinergic input to the cortex from magnocellular neurons of the basal forebrain (*3*). In this report we present evidence from retrograde tracing and immunohistochemical studies that γ-aminobutyric acid (GABA)–containing neurons of the posterior hypothalamus also directly innervate widespread regions of the neocortex.

The synthetic enzyme glutamate decarboxylase (GAD) is a specific marker for GABA neurons. We used well-characterized antibodies raised against purified mouse brain GAD (*4*) to demonstrate GABA neurons in the rat hypothalamus by indirect immunofluorescence (*5*). Sections of Formalin-fixed brain were cut on a cryostat to a thickness of 14 μm and incubated with antiserum to GAD diluted 1:100 overnight at 4°C. The sections were then rinsed and incubated with rhodamine-conjugated swine antiserum to rabbit immunoglobulin G (IgG) (Dako), rinsed again, and examined in a fluorescence microscope. Serum from unimmunized rabbits served as a control. To enhance the immunoreactivity of GAD in GABA cell bodies, some animals received an intraventricular injection of colchicine (120 μg in 20 μl of saline) 24 hours before perfusion.

A dense network of GABA fibers and many GABA cell bodies were found in many regions of the hypothalamus (*6*). Of particular significance was the observation of three distinct clusters of magnocellular, GAD-positive neurons in the mammillary region of the posterior hypothalamus. These cell groups correspond to the caudal (Fig. 1, A and C), tuberal (Fig. 1D) and postmammillary caudal (Fig. 2G) magnocellular nuclei in the atlas of Bleier *et al.* (*7*). Almost all of the closely packed magnocellular neurons in these three nuclei were GAD-positive.

To determine the projection areas of these hypothalamic GABA neurons, numerous rats were injected at various sites with 50 to 200 nl of a 3 percent solution of the retrogradely transported fluorescent dye fast blue (*8*). Injections were stereotaxically placed in the frontal cortex (25 rats), motor cortex (four rats), occipital cortex (eight rats), striatum (six rats), amygdala (four rats), olfactory bulb (four rats), and cerebellum (four rats). After 2 to 5 days the rats were injected with colchicine, and 24 hours thereafter they were perfused. The brains were removed and soaked overnight in 5 percent sucrose and then sectioned on a cryostat. The hypothalamus

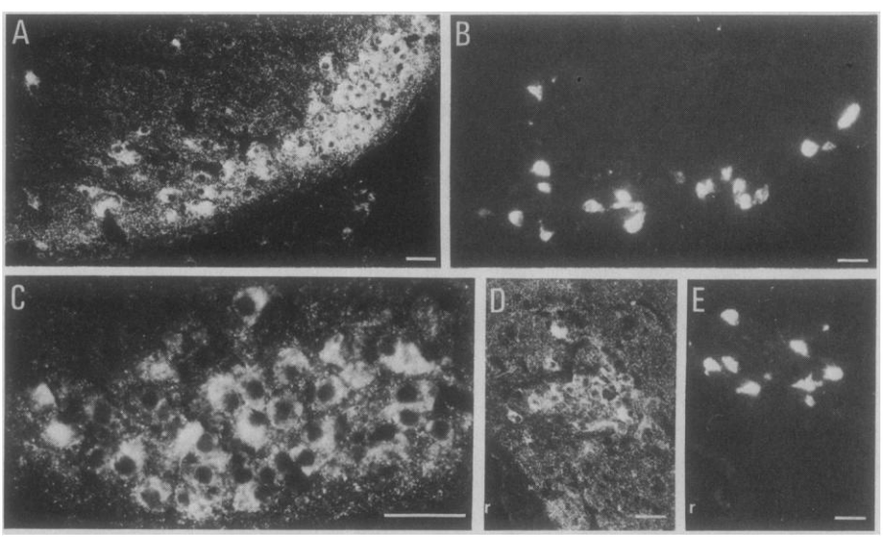

Fig. 1. Fluorescence micrographs of magnocellular neurons of the posterior hypothalamus immunohistochemically stained for GAD (A, C, and D) or retrogradely labeled by injections of fast blue into the frontal cortex (B and E). The GAD-positive neurons that comprise the caudal magnocellular nucleus lie along the ventral surface of the hypothalamus (A), and their characteristic shape can be seen in an enlargement (C). Many cells in this particular region are retrogradely labeled by fast blue injections into the frontal cortex (B). GAD-positive neurons are also found in the tuberal magnocellular nucleus, which lies at the dorsal edge of the mammillary recess (*r*) (D). This nucleus also contains many retrogradely labeled neurons (E). Scale bars, 50 μm.

was examined and fast blue–positive cells in the magnocellular nuclei of the posterior hypothalamus were photographed.

Many cells in the posterior magnocellular nuclei were retrogradely labeled after the dye was injected into the cortex (Fig. 1, B and E), the striatum, or the amygdala. No cells were labeled in these nuclei after cerebellar injections, and only a few cells in the caudal magnocellular nucleus were labeled after large injections into the olfactory bulb. The cortical area in which the injection was placed did not appear to affect the number or distribution of the labeled neurons. In all cases the retrograde labeling occurred bilaterally in all three nuclei, although the side contralateral to the injection contained only about one-third as many labeled cells as the ipsilateral side.

To demonstrate that the labeled cells were in fact GABA neurons, we photographed cells labeled with fast blue and then stained the sections for GAD immunoreactivity. After the injection of fast blue into the frontal cortex, many neurons that were retrogradely labeled in these caudal magnocellular nuclei were also shown to be GAD-positive when subsequently stained immunohistochemically (Fig. 2).

In previous retrograde transport studies, labeled neurons were found in the magnocellular nuclei of the basal forebrain and in the lateral hypothalamus after cortical injections of horseradish peroxidase (*9*). However, a direct projection from the magnocellular nuclei of the posterior hypothalamus to the cortex has not, to our knowledge, been previously reported. The diffuse nature of this pathway suggests that it represents another example of a nonspecific projection to the cortex. This widespread input to the cortex from the hypothalamus might provide a direct pathway by which limbic, emotional, and visceral information can reach many regions of the neocortex.

Biochemical experiments have shown that the cortex contains high levels of GABA and GAD and have indicated that cortical undercutting does not decrease cortical GABA or GAD (*10*). This suggests that the vast majority of cortical GABA is intrinsic. In fact, many GABA neurons and fibers have been detected in the neocortex (*11*). Apparently the projection from the magnocellular GABA neurons of the posterior hypothalamus provides only a small fraction of the GABA in the cortex.

In conclusion, at least two separate GABA systems appear to exist in the cortex, suggesting that this amino acid transmitter may subserve several functions in this brain area. The major contribution is found in the interneurons, which occur in all cortical layers and which presumably have local inhibitory actions. In contrast, the ascending GABA neurons of the hypothalamus represent a diffuse system innervating widespread cortical regions.

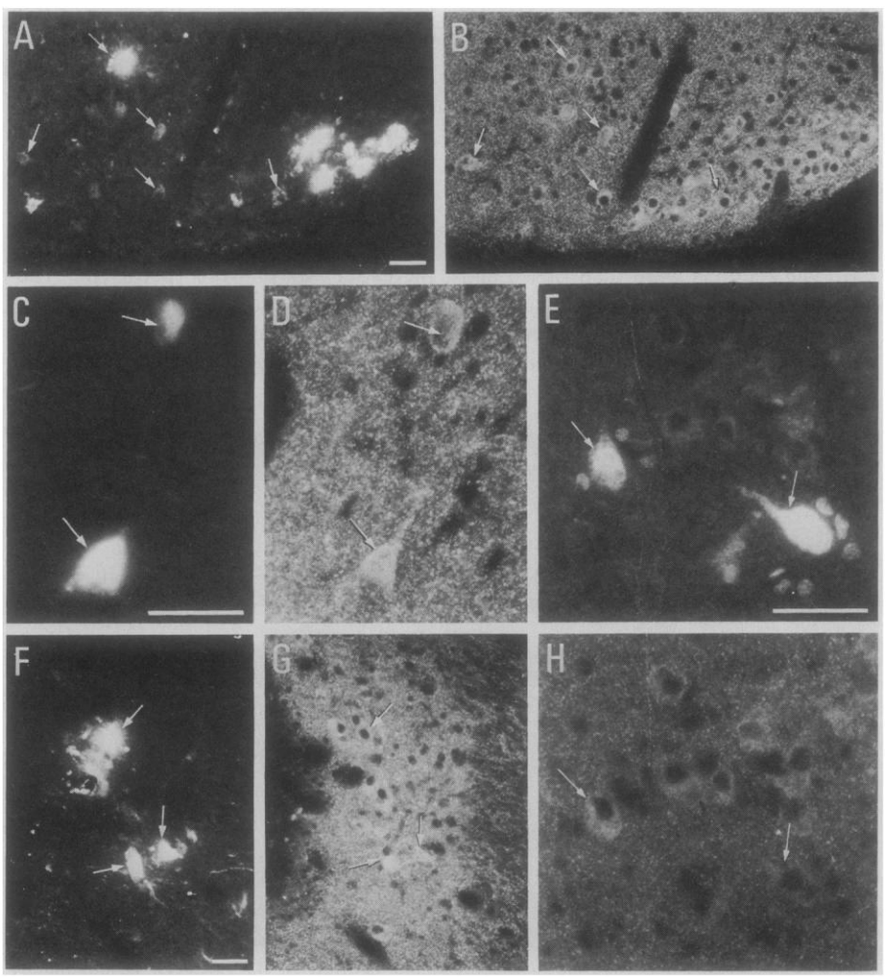

Fig. 2. Fluorescence micrographs of sections through the caudal magnocellular nuclei of the hypothalamus, showing retrogradely labeled neurons (A, C, E, and F) that are also stained with antibodies to GAD (B, D, G, and H). All dye injections were made into the frontal cortex. Panels (A) and (B) show the same section of tissue and illustrate retrogradely labeled (A) and GAD-positive (B) neurons in the caudal magnocellular nucleus. Similar cells are visible in higher power micrographs of this nucleus (C, D, E, and H). Panels (F) and (G) show the same section of postmammillary caudal magnocellular nucleus and illustrate GAD-positive (G) and retrogradely labeled neurons (F). Scale bars, 50 μm.

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Regenerative Impulses in Taste Cells

Abstract. *Taste cells and nongustatory epithelial cells in the isolated lingual mucosa from the mud puppy* Necturus maculosus *were impaled with microelectrodes. The taste cells, but not surrounding epithelial cells, were electrically excitable when directly stimulated with current passed through the recording electrode. Action potentials produced by taste cells had both a sodium and a calcium component.*

Little is known about the cellular mechanisms of chemosensory transduction in vertebrate taste buds. Taste cells are small and relatively inaccessible; hence, they have been difficult to study with intracellular microelectrodes. It has been established that taste receptors differentiate from surrounding stratified squamous epithelium (*1*), form synaptic contacts with gustatory nerve fibers (*2*), and convert chemical stimulation by sapid agents into signals that can be transmitted to the central nervous system (*3*). Furthermore, it has been held that taste cells have relatively low resting potentials and linear (ohmic) membrane resistance and that they respond passively, with graded receptor potentials, to chemical stimulation (*3–5*). In this report, I describe a preparation for studying intracellular responses in vertebrate taste cells—the isolated lingual epithelium from the mud puppy *Necturus maculosus*—and provide evidence that these cells have high resting potentials, very high input resistances, and generate sodium and calcium impulses.

I selected *Necturus maculosus* because the taste cells are much larger than those in other vertebrates (*6*). Isolating a thin sheet of lingual epithelium and stretching it flat in a shallow chamber containing Ringer solution allowed individual taste cells and epithelial cells to be distinguished with remarkable clarity, especially with Nomarski optics. The isolated preparation is quite stable, and pharmacological agents added to the chamber gain ready access to taste cells.

Adult mud puppies were kept in well-aerated aquariums filled with recirculated water at about 21°C. Animals were decapitated and pithed, the top of the head and upper jaw were removed, and the lower jaw was pinned firmly on a dissection board, exposing the tongue. A transverse cut through the mucosal epithelium was made with fine scissors, and the epithelial sheet was freed from the underlying connective tissue with blunt dissection. A 1-cm^2 region was removed from the anterior of the tongue, transferred to a recording chamber (*7*), and pinned down, mucosal surface uppermost. During the dissection, the preparation was frequently flushed with cold Ringer solution containing 112 m*M* NaCl, 5 m*M* $CaCl_2$, 3 m*M* KCl, and 3 m*M* Hepes buffer (*p*H 7.2). The Ringer solution in the chamber was identical to that above. Intracellular glass micropipettes were filled with 2.5*M* KCl and had resistances between 50 and 150 megohms.

Stable penetrations with resting potentials up to −90 mV could be obtained reliably from taste cells (*8*). Measurement accuracy was assured by recording resting potentials at the end of an impalement when the microelectrode was abruptly withdrawn from the cell. The input resistance of taste cells was measured by injecting current pulses through the recording electrode (Fig. 1). In a sample of 11 taste cells in which both the resting potential and input resistance were measured, the mean ± standard error (S.E.M.) of the resting potential was −43 ± 6 mV and of the input resistance was 200 ± 30 megohms. These values are likely to be underestimates of the true resting potentials and input resistances, since impaling a cell with a glass micropipette and passing currents across the membrane noticeably injured the taste cells (*9*). Nevertheless, the values for resting potentials and input resistances obtained in these experiments are significantly higher than those reported previously for this species (*5*) or other vertebrate taste cells (*3*).

The most striking finding was that brief depolarizing currents injected into taste cells produced regenerative impulses (Fig. 2) (*10*). These impulses had a relatively low threshold and a brief duration, suggesting that they were mainly sodium action potentials. Occasionally, a slight inflection on the falling phase of the impulses (arrow in Fig. 2A) suggested that there might be other components of the responses, such as calcium currents. Low doses of tetrodotoxin (TTX, 1 μ*M*) rapidly (within 5 to 10 minutes) and reversibly blocked the regenerative impulses. Nevertheless, even in the presence of 1 μ*M* TTX, regenerative responses could be restored if delayed potassium rectification was blocked by

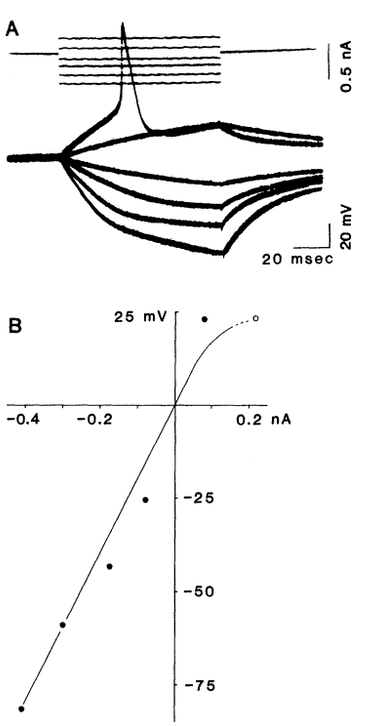

Fig. 1. Current-voltage relation of a taste cell in the mud puppy. (A) Current pulses (upper traces) were passed through the intracellular recording electrode via a bridge circuit. (B) The relation between applied current pulses and changes in membrane potential at the end of the current pulse was plotted and a linear regression line was drawn through the points. The slope of the line yields the input resistance, which in this case was 201 megohms for the linear region (closed circles, r = .977). The open circle in (B) represents the threshold level for impulse generation. The resting potential of this taste cell recorded at the end of the impalement was −73 mV. The constant current pulses did not always produce an exponential rate of change in the membrane voltage, particularly in the hyperpolarizing direction. This finding was made frequently, especially for large hyperpolarizations. Since it was not explained by a fluctuation in the microelectrode resistance when currents were passed, the nonexponential rate of potential change suggests that the passive membrane properties of taste cells from the mud puppy may be more complex than a simple ohmic resistance with parallel capacitance.