from the ipsilateral ear, but from the contralateral ear (*6*). In any case, the data for cats and dogs suggest that the neurons most sensitive to acoustic signals are E-E, as found in the mustache bat. However, the functional significance of the aural representation found in cats and the sensitivity representation found in dogs is not yet clear. In the mustache bat, orientation sounds consist of two distinct components, which are used for the extraction of certain information about a target, and the auditory cortex is apparently organized for effective processing of such information. Since the auditory system has evolved together with the vocalization system that generates the acoustic signals used by the species, the functional organization of the auditory system should be studied in terms of receiving and processing acoustic signals that are frequently used by the species and are important for survival.

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References and Notes

1. The frequency of the CF component can be slightly different among *Pteronotus parnellii rubiginosus* living in different parts of Central America (*2, 3*). *Pteronotus parnellii rubiginosus* was previously called *Chilonycteris rubiginosa*.
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9. The term column is used for convenience to describe our observations.
10. Response patterns and thresholds of single neurons studied within 2 to 4 hours after an injection of sodium pentobarbital (30 mg/kg) were similar to those 9 to 12 hours after the injection. When the animal moved too much for us to study the properties of single neurons, one-third of the initial dose was administered. There was no noticeable difference between the response pattern and threshold of neurons just before and those after such an injection. It appeared very unlikely that the anesthetic changed the mode of binaural interaction observed. It has been demonstrated that the tonotopic and amplitopic representations in the Doppler-shifted-CF processing area are not altered by the anesthetic (*6*). If sodium pentobarbital at more than 30 mg/kg was administered, there might be a noticeable effect on response properties of neurons within a few hours.
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Immunoreactive Vasopressin and Oxytocin: Concentration in Individual Human Hypothalamic Nuclei

Abstract. *Individual hypothalamic nuclei were microdissected from brain tissue of ten human subjects who had died suddenly while in apparent good health. Appreciable amounts of vasopressin and oxytocin immunoreactivity were found by specific radioimmunoassay in six hypothalamic nuclei including supraoptic and paraventricular nuclei. Vasopressin and oxytocin are presumed to be synthesized in supraoptic and paraventricular nuclei for axonal transport to the posterior pituitary for storage and release. Vasopressin and oxytocin in other hypothalamic nuclei may be a part of this system of neurosecretion or may serve some other function.*

Neurosecretion of vasopressin and oxytocin is known from studies in animals to involve biosynthesis of hormone in the hypothalamus with transport by axons to the posterior pituitary for storage and release (*1*). Both hormones have been found in supraoptic and paraventricular nuclei of the hypothalamus in numerous animal species including humans (*2*). By means of a microdissection technique and specific radioimmunoassays both hormones were found recently in the rat in six hypothalamic areas in addition to supraoptic and paraventricular nuclei (*3*). In the study reported here I determined the concentrations of immunoreactive vasopressin and oxytocin in specific anatomical areas of human hypothalamus using microdissection and radioimmunoassay.

Human brain tissue was obtained from ten subjects who died suddenly while in apparent good health. The subjects included eight males and two females (ages 17 to 58 years, mean age 37 years), and the time interval from death to removal and freezing of hypothalamic tissue was 3 to 12 hours with a mean of 9 hours. The causes of death were acute coronary occlusion (three subjects), trauma (five subjects), stabbing (one subject), and gunshot (one subject). Hypothalamic tissue was removed and frozen in Dry Ice. Alternating 300-μm and 100-μm frozen sections were cut in the frontal plane in a cryostat at − 10°C. The 100-μm sections were stained and the hypothalamic nuclei were located according to Peele (*4*). Tissue samples were removed from adjacent 300-μm frozen sections with stainless steel cannulas, 500 μm in diameter, with the aid of a stereomicroscope (*5*). This diameter is well within the diameter of the hypothalamic nuclear areas. Tissue from each mircodissected area from each human brain was homogenized in 0.4 ml of 0.1*N* HCl, and the protein content was determined by the method of Lowry *et al.* (*6*). The amount of protein per sample was between 10 and 50 μg. The remainder of each tissue sample was diluted, neutralized with buffer, and assayed for vasopressin and oxytocin (*3*). The radioimmunoassay for each hormone was sensitive to 4 pg and not interfered with by 1000 pg of the other hormone. The interassay coefficient of variation of both radioimmunoassays was less than 10 percent. All samples were assayed in at least three serial dilutions to ensure immunologic identity with pure hormone.

Similar concentrations of immunoreactive vasopressin were found in supraoptic and paraventricular nuclei, with lesser concentrations in arcuate, dorsomedial, ventromedial, and posterior hypothalamic nuclei, median eminence,

Table 1. Hormone concentration (mean picograms per microgram of protein ± standard error of the mean) in human brain (*N* = number of subjects).

Brain area	*N*	Vasopressin	Oxytocin
Nucleus supraopticus	10	440.0 ± 156	28.0 ± 4.9
Nucleus paraventricularis	10	393.0 ± 158	52.0 ± 7.9
Nucleus arcuatus	10	17.0 ± 4.7	5.2 ± 3.0
Median eminence	10	247.0 ± 60	50.0 ± 13
Nucleus dorsomedialis	4	26.0 ± 15	7.6 ± 1.4
Nucleus ventromedialis	4	19.0 ± 13	4.3 ± 1.2
Nucleus hypothalamicus posterior	4	2.2 ± 1.0	0.13 ± 0.2
Mammillary body	4	3.4 ± 0.5	2.0 ± 0.5
Pituitary stalk	2	577.0 ± 66	86.0 ± 5.2
Nucleus preopticus lateralis	4	<1	<1
Nucleus preopticus medialis	4	<1	<1
Frontal cortex	4	<1	<1
Parietal cortex	4	<1	<1
Occipital cortex	4	<1	<1
Cerebellum	4	<1	<1
Brain stem	4	<1	<1

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and mamillary body (Table 1). Pituitary stalk had a larger concentration of hormone. Two additional hypothalamic nuclei and five areas of brain outside the hypothalamus were assayed and did not contain detectable vasopressin. Immunoreactive oxytocin was found only in the hypothalamic areas that contained vasopressin and was present in lesser amounts. Posterior pituitary glands from four subjects were available for assay and contained 5120 ± 1780 ng of vasopressin (mean ± standard error) and 2970 ± 932 ng of oxytocin.

The results reported here are in accord with the concept that both supraoptic and paraventricular nuclei are major sources of both vasopressin and oxytocin in humans. In addition, the results agree with previous findings in the rat (*3*) that both hormones are present in additional hypothalamic areas but not generally throughout the brain. The hormone-containing areas may be part of the neurosecretion system supplying the posterior pituitary or may serve some other brain function (*7*). Hormone in hypothalamic areas could be contained in axons of passage through nuclear areas, be synthesized in multiple hypothalamic nuclei, or be taken up in these areas after being synthesized elsewhere. There are two reasons for believing that I measured immunoreactive vasopressin and oxytocin in human brain tissue rather than a general artifact of human postmortem brain tissue: the hormones were found in some tissue samples but not others, and immunoreactivity in serial dilutions of tissue samples was identical with that obtained with synthetic hormone.

There was no direct correlation between the hormone concentration and the time interval between death and freezing of the brain tissue. Hormone concentration in areas of two brains obtained 3 and 5.5 hours after death were not consistently different from those in two brains obtained at 12 hours after death. Although the results in Table 1 are qualitatively meaningful in delineating hormone-containing areas, the quantitative relationships are suspect because of possible postmortem degeneration. For instance, median eminence had a lesser concentration of vasopressin than either supraoptic or paraventricular nuclei in contrast to previous findings in the rat where median eminence had the highest concentration of any hypothalamic area (*3*).

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Morphine Tolerance: Is There Evidence for a Conditioning Model?

Siegel reports that a Pavlovian interpretation can account for tolerance to the analgesia produced by small doses of morphine (*1*). He shows that animals repeatedly exposed to morphine paired with one environment and test situation show less of an analgesic response to morphine when tested in the presence of those same cues than when tested in the presence of different cues. Thus, he concludes that the presence of stimuli reliably associated with systemic morphine administration is crucial to the development of tolerance to the analgesic effects of morphine. We believe that Siegel's experiments are inconclusive.

Siegel did not distinguish adequately between Pavlovian contingencies and the possibly independent process of behavioral tolerance. The well-documented phenomenon of behavioral tolerance, extensively studied by the "hot plate" test (*2–4*), refers to the fact that powerful interactions can occur between the administration of drugs and the test situations used to evaluate drug effects. Thus, prior experience in the test apparatus is a significant determinant of the amount of tolerance produced by certain drug regimens. For example, animals repeatedly injected with morphine and tested on the hot plate show a greater reduction in analgesia than animals injected with equivalent doses of morphine but tested only once on the hot plate at the end of the injection regimen.

Siegel argues that his work has extended previous findings "by demonstrating that the display of tolerance is specific to the environment in which the drug has been administered, and that 'morphine tolerant' rats, when assessed for the effects of the narcotic in an environment other than that in which they became tolerant, evidence a relatively nontolerant response" (*1*). Yet, in that experiment he did not distinguish exposure to the environmental cues associated with morphine administration from exposure to the test procedures used to evaluate morphine analgesia. That is, when rats were tested in a novel environment to determine whether they became relatively nontolerant, they were also tested with the analgesiometric device with which they had no prior experience, thereby also preventing any manifestation of behavioral tolerance to the test situation. Similarly, when rats were tested in the same environment in which they had previously received the drug in order to determine if they were relatively more tolerant, they were also tested with the analgesiometric device with which they had prior experience. This procedure maximized the chances of observing behavioral tolerance. Therefore, Siegel's experimental design did not distinguish between differences in analgesia attributable either to changes in general environmental cues or to the presence or absence of prior experience with the test apparatus (that is, behavioral tolerance). The experiment shows only that behavioral tolerance to the analgesic effect of morphine can develop after repeated testing with the paw pressure analgesiometer as well as with the hot plate.

In order to provide support for the role of Pavlovian contingencies in the development of behavioral tolerance, one should show that identifiable conditioned stimuli contribute to the reduced analgesia resulting from repeated pairings of morphine administration with the same test situation. One should, for example, show that, in the same animals repeatedly tested on the hot plate, the presentation of one environmental cue associated with repeated morphine administrations [that is, a conditioned stimulus or (CS) (+)] results in a greater decrement in morphine analgesia than the presentation of a different environmental cue associated with repeated saline administra-

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