fact that very low concentrations of tetrodotoxin are enough to block the sodium conductance increase makes us think that these sites are sparsely distributed on the membrane surface. It is known that guanidine can pass through the squid nerve membrane and produce the action potential under certain conditions as sodium does (*13*), and that the tetrodotoxin molecule has a guanidinium group (*12*). It appears possible that the guanidinium of tetrodotoxin becomes lodged in the gate of the sodium channel on the surface of the nerve membrane, thereby blocking the movement of sodium ions as suggested for saxitoxin (*14*).

The situation might be quite different for lipid-soluble blocking agents such as procaine and alcohols which can diffuse into the phospholipid layer of the nerve membrane, thereby requiring much higher concentrations to become effective in blocking excitability (*1, 10, 15*).

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References and Notes

1. R. E. Taylor, *Am. J. Physiol.* **196**, 1071 (1959); A. M. Shanes, W. H. Freygang, H. Grundfest, E. Amatniek, *J. Gen. Physiol.* **42**, 793 (1959); M. P. Blaustein and D. E. Goldman, *ibid.* **49**, 1043 (1966).
2. T. Narahashi, T. Deguchi, N. Urakawa, Y. Ohkubo, *Am. J. Physiol.* **198**, 934 (1960); S. Nakajima, S. Iwasaki, K. Obata, *J. Gen. Physiol.* **46**, 97 (1962).
3. T. Narahashi, J. W. Moore, W. R. Scott, *J. Gen. Physiol.* **47**, 965 (1964).
4. Y. Nakamura, S. Nakajima, H. Grundfest, *ibid.* **48**, 985 (1965).
5. M. Takata, J. W. Moore, C. Y. Kao, F. A. Fuhrman, *ibid.* **49**, 977 (1966).
6. P. F. Baker, A. L. Hodgkin, T. I. Shaw, *Nature* **190**, 885 (1961); T. Oikawa, C. S. Spyropoulos, I. Tasaki, T. Teorell, *Acta Physiol. Scand.* **52**, 195 (1961).
7. T. Narahashi, *J. Physiol.* **169**, 91 (1963); ———, *J. Gen. Physiol.* **48** (No. 5, part 2), 19 (1965); P. F. Baker, A. L. Hodgkin, T. I. Shaw, *J. Physiol.* **164**, 355 (1962); I. Tasaki, A. Watanabe, T. Takenaka, *Proc. Natl. Acad. Sci. U.S.* **48**, 1177 (1962); I. Tasaki and T. Takenaka, *ibid.* **52**, 804 (1964); E. Rojas, *ibid.* **53**, 306 (1965).
8. J. W. Moore, *J. Gen. Physiol.* **48** (No. 5, part 2), 11 (1965).
9. P. F. Baker, A. L. Hodgkin, T. I. Shaw, *J. Physiol.* **164**, 330 (1962); W. K. Chandler and A. L. Hodgkin, *ibid.* **181**, 594 (1965); A. L. Hodgkin and B. Katz, *ibid.* **108**, 37 (1949).
10. J. W. Moore, W. Ulbricht, M. Takata, *J. Gen. Physiol.* **48**, 279 (1964).
11. J. W. Moore, T. Narahashi, W. Ulbricht, *J. Physiol.* **172**, 163 (1964).
12. H. S. Mosher, F. A. Fuhrman, H. D. Buchwald, H. G. Fischer, *Science* **144**, 1100 (1964).
13. I. Tasaki, I. Singer, A. Watanabe, *Proc. Natl. Acad. Sci. U.S.* **54**, 763 (1965).
14. C. Y. Kao and A. Nishiyama, *J. Physiol.* **180**, 50 (1965).
15. C. M. Armstrong and L. Binstock, *J. Gen. Physiol.* **48**, 265 (1964); T. Narahashi, *J. Cell. Comp. Physiol.* **64**, 73 (1964).
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1 June 1966

Corticosteroid Responses to Limbic Stimulation in Man: Localization of Stimulus Sites

Abstract. *Corticosteroids in human plasma and urine increase after amygdala stimulation, and plasma corticosteroids decrease after hippocampus stimulation. Five subjects underwent unilateral temporal lobectomy, and histopathologic localization of electrode sites was attempted. Localization was successful for six sites: three in basolateral amygdala and three in hippocampus.*

The effects of electrical stimulation of limbic structures upon plasma corticosteroid levels in man have been reported (*1*). Four patients with severe temporal-lobe epilepsy had chronic implantations of electrodes in these areas as part of a study to localize seizure focuses prior to attempts at control by surgical removal of one anterior temporal lobe (*2*). Three of the patients underwent stimulation of the amygdala, one patient bilaterally, and in each instance the 17-hydroxycorticosteroid level in plasma rose significantly within the 1st hour after stimulation. Three of the patients underwent stimulation of the hippocampus, one patient bilaterally, and in each instance 17-hydroxycorticosteroid level in plasma rose significantly within the 1st hour after stimulation. Maximal responses occurred after 5 to 30 minutes in the two patients who were sampled several times during the hour.

The location of each electrode was ascertained at the time of implantation by x-ray measurements and stereotaxic-atlas verification. Marker lesions were made before explantation. Since the first report, all four patients have undergone unilateral temporal lobectomy, and exact histopathologic localization of the electrodes has been attempted (Fig. 1)—successfully for all electrode tracts examined except for two in the fourth patient; the sites verified are listed in Table 1.

Also since the first report a fifth patient has undergone stimulation of both amygdalas, in alternating fashion, urinary 17 - hydroxycorticosteroids [Porter-Silber chromagens (*3*)] being measured hourly, both on a control day with mock stimulation and following the stimulus train. For this patient, stimulus parameters were bidirectional square-wave pulses (10 volts, ten per second, 1-msec duration) for the first 20 seconds of each minute. The left and right amygdalas were stimulated

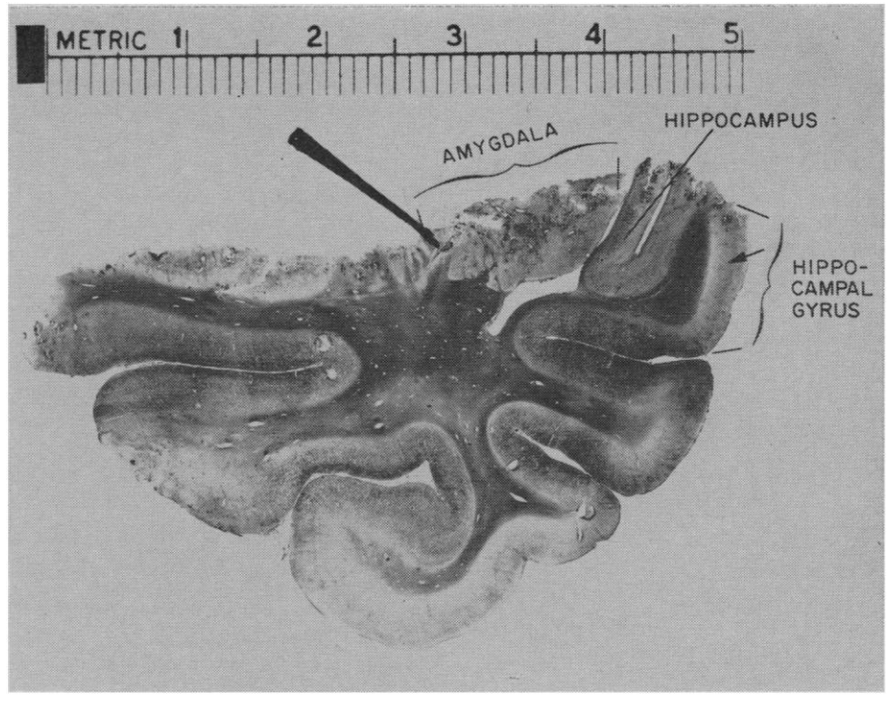

Fig. 1. Section of right temporal lobe, showing marker lesion (electrode tip) in basolateral amygdala. Lesion is the darker area at the immediate right of the pointer tip; Weil stain.

Table 1. Plasma corticoid responses to limbic stimulation.

Patient	Location of stimulation	17-Hydroxy-corticoids in plasma: maximum change from control amount (%)	Time
1	R-CA 2 hippocampus (histopath.)	−28	1-hour specimen only
2	R-basolateral amygdala (histopath.)	+12	1-hour specimen only
3	R-hippocampus (stereotaxic)	−88	30-minute specimen
3	L-subiculum hippocampus (histopath.)	−90	15-minute specimen
3	R-basolateral amygdala (stereotaxic)	+360	15-minute specimen
3	L-basolateral amygdala (histopath.)	+232	15-minute specimen
4	Anterior to L-amygdala (histopath.*)	+415	30-minute specimen
4	L-anterior hippocampus (histopath.*)	−18	15-minute specimen
4	L-CA 1 hippocampus (histopath.)	−100	15- and 30-minute specimens

* Unverified.

15 times each, in alternate minutes, for a total stimulus period of 30 minutes. The hourly urinary 17-hydroxycorticosteroids for both control and stimulation days are shown in Fig. 2; corticosteroid excretion was 454-percent higher in the 1st-hour urine on the stimulation day than on the control day. Histopathologic study of this patient's left temporal lobe after surgical excision showed the electrode tip to have been located in the basolateral amygdala.

It was suggested (*1*) that the findings then reported were consistent with observations by others of increased corticosteroid levels in the adrenal venous effluents of cats after stimulation in the anteromesial amygdala (*4*); stimulation in the basolateral amygdala resulted in decreased corticosteroid release. From our current histopathologic data we can state that stimulation of the basolateral amygdala in humans increases corticosteroid levels in plasma (patients 2, 3, 5); this response is probably not confined to the basolateral area, but may result from more diffuse stimulation of other areas in the region of the amygdala (patient 4). This finding is somewhat at variance with the reported experiments on cats (*4*), wherein different rates of corticosteroid release followed stimulation of anteromesial and basolateral amygdala.

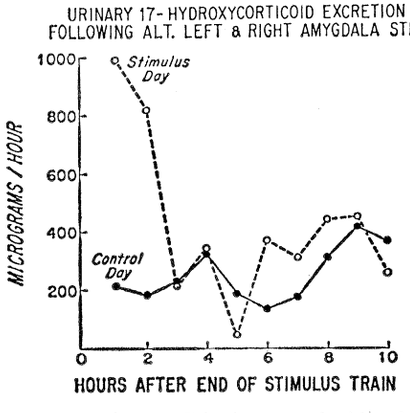

Fig. 2. Urinary 17-hydroxycorticoids after amygdala stimulation in patient 5.

Recent data on the "limbic system–midbrain circuit," of which the amygdala-hypothalamus connections are a part, suggest that the functional state of the hypothalamus, as manifested by endocrine phenomena, reflects activation or inhibition of neural mechanisms of this circuit, mechanisms which are "diversified and potentially of reciprocal sign," consistent with the structural heterogeneity of this circuit (*5*). Our data from studies of man indicate that stimulation in the region of the amygdala—and basolateral amygdala stimulation in particular—leads to increases in corticosteroids in plasma and urine, and that hippocampal stimulation leads to decreases in corticosteroids in plasma.

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References and Notes

1. A. J. Mandell, L. F. Chapman, R. W. Rand, R. D. Walter, *Science* **139**, 1212 (1963).
2. P. H. Crandall, R. D. Walter, R. W. Rand, *J. Neurosurg.* **20**, 827 (1963).
3. R. H. Silber and C. C. Porter, *J. Biol. Chem.* **210**, 923 (1954).
4. M. A. Slusher and J. E. Hyde, *Endocrinology* **69**, 1080 (1961).
5. W. H. J. Nauta, in *Advances in Neuroendocrinology*, A. V. Nalbandov, Ed. (Univ. of Illinois, Urbana, 1963), p. 19.
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23 May 1966

Pressure Regulation in the Middle Ear Cavity of Sea Lions: A Possible Mechanism

Abstract. *The mucosa lining the cavity of the middle ear of sea lions contains a complex network of venous channels and sinuses. During dives the pressure within the middle ear may be equalized with that in the external auditory meatus either by the distention or depression of the mucosa due to the presence or absence of blood in the sinuses.*

If the pressure of the air in the middle ears of mammals is unequal to that of the environment, the tympanic membrane suffers a decreased sensitivity to the reception of sound vibrations (*1*). More important is the fact that severe injury can occur if the difference between these pressures exceeds a certain limit (*2*). Sea lions have a mechanism which allows them to adapt to extreme changes of pressure in their middle ear cavities.

Two Steller sea lions (*Eumetopias jubata*) and the heads of two California sea lions (*Zalophus californianus*) were embalmed with 10 percent formalin; their vascular systems were injected with colored latex. One of the Steller sea lions was injected through the posterior vena cava; all of the other specimens were injected through the external jugular vein. The middle ears were grossly dissected, and microscopic sections of the muscles and mucosa were prepared.

The portion of the temporal bone forming the ventral aspect of the middle ear cavity in sea lions is a flat, uninflated, relatively thick bone compared to that of terrestrial mammals. Numerous foramena perforate the bone and carry many veins into the middle ear cavity to the mucosa. The basic morphology of the inside of the cavity is similar to that of the typical mammalian middle ear. The Eustachian tube runs from the anterio-ventral aspect of the middle ear to the nasal cavity. The tensor tympani and stapedius muscles are both present. The ratio of the area of the tympanic membrane to that of the footplate of the stapes approaches 1:1 instead of 21:1 which is their ratio in man (*3*). The stapes is also correspondingly larger relative to the malleus than it is in most terrestrial mammals.

The mucous membrane lining the