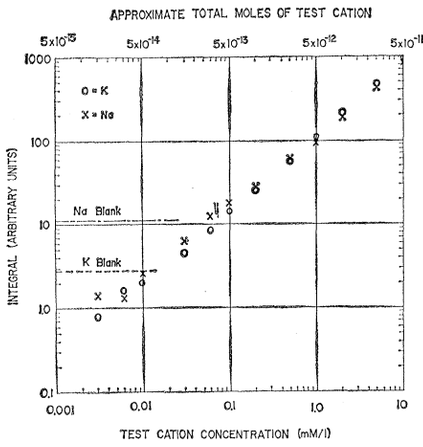

Fig. 3. Integrator responses (corrected for blank values) to various sodium and potassium contents of samples. Values in the upper scale are based on the estimated (within 20 percent) volume of the pipette used.

reduces interaction between sodium and potassium. Samples are diluted at least 20-fold with this diluent. Presence of sodium and potassium as impurities in the diluent can limit the sensitivity of the method. Figure 3 is a plot of the integral (in arbitrary units) of the sodium and potassium light over a four-decade range, showing results that can be obtained; the value for the blank was subtracted from each point. The volume of the pipette used for this run was estimated from its dimensions and was not determined precisely, so that the sample size shown on the top scale is approximate. Each point is the mean of readings for three sample portions; the spread between readings was less than 2 percent of the mean.

The analytic routine consists of several steps. Drops of tissue-extract fluid or tubule fluid diluted 20-fold, together with drops of standard composition, are placed under oil in the sample trough. Portions of the samples are transferred to the iridium wire, the chamber is closed, and atmospheric gases are purged from the chamber by the flowing helium. After purging for 2 minutes, the glow discharge between the iridium and ring electrodes is initiated by application of the rf field. Concurrently with the start of the glow, the iridium wire is heated electrically, and the sample is volatilized into the glow region. The light emitted is measured with the phototube integrator systems, the outputs of which are shown by the meter. After the meter readings are recorded, the glow and iridium heating currents are turned on again for 5 seconds to remove any sample residue. Each operational cycle requires about 3 minutes.

We made several samples of "artificial kidney-tubule fluid" and tested them for sodium content. Table 1 lists the compositions of the samples and the amount of sodium found in each; the potassium-sensing portion of the system was not used during this test. Portions of the diluted solutions were transferred to the iridium wire, and each solution was run three times. Agreement between the true and "found" values is within 3.5 percent.

To test the usefulness of the helium-glow photometer for determining sodium and potassium contents of tissue, five samples of kidney tissue were extracted with nitric acid. The extracts were approximately neutralized with NH_4OH, and a portion of each was analyzed for sodium and potassium on a Baird flame photometer. Another portion was diluted and tested with the helium-glow photometer as in the previous experiment. Table 2 compares the results by the two methods, which differed by not more than 8.4 percent; the difference was generally less than 5 percent.

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References and Notes

1. P. A. Bott, *Anal. Biochem.* **1**, 17 (1960); P. Müller, *Exp. Cell Res. Suppl.* **5**, 118 (1958); J. A. Ramsey, R. H. J. Brown, S. W. H. W. Falloon, *J. Exp. Biol.* **30**, 1 (1953).
2. R. L. Bowman, Trans. Ann. Conf. Elect. Tech. Biol. Med. 13th, paper 8-4 (1960).
3. D. J. Prager, R. L. Bowman, G. G. Vurek, *Science* **147**, 606 (1965).
4. During development of the helium-glow photometer it was observed that sodium light was emitted from platinum electrodes after the electrodes were heated to incandescence; the effect was more pronounced if a sodium-free sample portion containing potassium was placed on the wire. This retention effect is also observed with tungsten, tantalum, and molybdenum. Pure iridium does not show the effect significantly.
5. Dow Corning, RTV 732.
6. Westinghouse type-794 Odorout.
7. Liberty Mirror Div., Libbey-Owens-Ford Glass Co., Brackenridge, Pa., No. 90-600.
8. Thin Films, Inc., Cambridge, Mass. Sodium filter: center wavelength, 5895 Å; peak transmission, 52 percent; 1-percent transmission bandwidth, 30 Å. Potassium filter: center wavelength, 7658 Å; peak transmission, 46 percent; 1-percent transmission bandwidth, 75 Å.
9. Philbrick Researches, Inc., Dedham, Mass. Type SP656.
10. A. P. I. Instrument Co., Chesterland, Ohio; mirror scale, 1-percent tracking accuracy.
11. We thank John Dirks and Maurice Abramow for assistance with the "tubule fluid" and the kidney-tissue extracts, and Judith Driver for help with the analyses.

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Brain Catecholamines: Relation to the Defense Reaction Evoked by Amygdaloid Stimulation in Cat

Abstract. *Electrical stimulation of the amygdala of cats, evoking a defense reaction, was associated with a reduction in the noradrenalin content of the brain and adrenal glands but not in the dopamine content of the brain. When stimulation resulted in quieting or sleep, catecholamine concentrations were unaffected. Changes in brain noradrenalin, therefore, appear related to production of the defense response and not to nonspecific amygdalofugal pathway excitation.*

In a previous study (*1*) we showed that the production of a defense reaction (*2*) or "sham rage" in the cat by electrical stimulation of the amygdaloid nucleus results in changes in catecholamines of the brain and adrenal glands. Both the noradrenalin (NA) content of the telencephalon, brainstem, and adrenals, and the adrenalin (A) content of the adrenals are significantly reduced while the dopamine (DA) content of the cerebrum is unaffected. It is not known, however, whether the chemical changes are specifically related to the induction of excited behavior by the brain stimulus or are the result of nonspecific activation of amygdalofugal pathways. In the study described here, we attempted to delineate the relationship between the chemical and behavioral consequences of amygdaloid stimulation. Changes in the NA and DA content of the brain and the NA and A content of the adrenals were compared in cats in which the stimulus produced rage and cats in which the stimulus resulted in quieting, sedation, or sleep.

The physiological and chemical methods are described in detail elsewhere (*1*, *3*). We studied 22 adult cats of both sexes; some had been included in our previous study (*1*). Three cats served as nonoperated controls. Under Nembutal anesthesia the other 19 animals had single stainless-steel Hess electrodes, insulated except for 2 mm at the tips, placed stereotaxically in the brain and fixed to the skull with dental cement. A screw in the calvarium was the indifferent (anodal) electrode. In 17 of the cats, electrodes were implanted in the right amygdaloid region. Six of these cats were not stimulated electrically and thus served as operated

controls. Electrodes were placed in the septum in the diagonal band of Broca in two cats. Two to five days after recovery from anesthesia, when the animals appeared behaviorally normal, each cat was placed individually in an observation box connected through a stimulus isolation unit to a Grass stimulator and was electrically stimulated. The stimulus pulse was a square wave of 1.0- to 1.5-msec duration delivered in trains of 3-minute duration at a frequency of 6 to 8 cy/sec and with an output voltage of 3 to 12 volts. The voltage was set at the threshold intensity for the first somatic, autonomic, or behavioral change observed. Four to five minutes elapsed between periods of stimulation. The trial period lasted for 3 hours and the stimulated animals, like the controls, were killed by decapitation after being anesthetized with choloroform. The brains of the animals were removed promptly and divided into: (i) brainstem (medulla, pons, mesencephalon, and diencephalon); (ii) left hemisphere, including basal ganglia; and (iii) right hemisphere. The right hemisphere was fixed in 10 percent formalin, sectioned, and stained with cresyl blue for confirmation of the electrode position. The chemical methods were those of Bertler *et al.* (*4*).

No changes occurred in the catecholamine content of the brain or adrenal glands of the control animals (Table 1). In six cats, stimulation of the amygdala at sites in both the basolateral and corticomedial nuclear areas resulted in the production of an outburst of excited behavior characterized by hissing, snarling, clawing, mydriasis, tachypnea, piloerection, arching of the back, and intermittent, wild, attacking movement (Table 1, amygdala stimulation and rage). As reported earlier (*1*), this pattern was not usually present at the beginning of the 3-hour period of stimulation but became apparent within the 1st hour of stimulation and increased in intensity until, at the end of the session, fragments of the behavior persisted interictally. In these animals, the NA content of the brain and adrenal glands and the A content of the adrenals decreased significantly without a change occurring in the DA content of the brain. In five cats (Table 1, amygdala stimulation and no rage) with electrodes in the basolateral nuclei, the stimulation initially resulted in alerting, mydriasis, ipsilateral facial twitching, and head turning, indicating activation of amygdalofugal projections. Within the 1st hour, however, these cats became quiet, purred, and groomed themselves or even fell asleep during the stimulus train, awakening at its termination. Toward the end of the stimulus period, only the facial movements persisted. In these animals, the catecholamine content of the brain and adrenal glands did not change. The two animals with septal electrodes (Table 1, septum stimulation and no rage) showed alerting responses during the first few trials but later also showed quiet behavior. They also failed to show changes in brain or adrenal NA or A.

Table 1. Changes in mean catecholamine concentration of cat brain and adrenal gland associated with electrical stimulation of the amygdaloid nucleus. Abbreviations: stim, stimulation; NS, not significant.

Group	No. of cats	Mean catecholamine content				
		Telencephalon (ng/g)		Brainstem (ng/g)	Adrenal gland (μg/g)	
		NA	DA	NA	NA	A
Control animals						
Nonoperated	3	229	323	345	583	350
Operated	6	207 ± 19	348 ± 29	332 ± 8.9	717 ± 91	392 ± 33
Stimulated animals						
Amygdala stim; rage	6	98 ± 13*	428 ± 35 (NS)	235 ± 25†	277 ± 23*	222 ± 58‡
Amygdala stim; no rage or sedation	5	196 ± 14 (NS)	323 ± 16 (NS)	315 ± 20 (NS)	770 ± 88 (NS)	407 ± 57 (NS)
Septum stim; no rage or sedation	2	182 (NS)	336 (NS)	284 (NS)	680 (NS)	400 (NS)

* Difference from nonstimulated controls highly significant, $p < .001$. † Difference from nonstimulated controls significant, $p < .01$. ‡ Difference from nonstimulated (operated) controls probably significant, $p < .05$.

This study indicates that with electrical stimulation of the amygdala in the cat, the depletion of brain NA and adrenal NA and A will occur only when the stimulus results in excited behavior of the defense reaction. Stimulation of the amygdala or septum producing quieting or sleep does not alter the concentration of these catecholamines. Hence, we conclude that the chemical change was not the result of behaviorally nonspecific excitation of amygdalofugal pathways.

That the DA concentration did not decrease during the production of rage by amygdaloid stimulation suggests some specificity of noradrenergic neurons in the mediation of this behavior. The histochemical fluorescence technique has demonstrated that the catecholamine depletion which is associated with the defense reaction elicited from the amygdala affects practically all noradrenergic terminals in the forebrain and parts of the hypothalamus (*5*). This depletion should probably be regarded as the end result of vigorous stimulation of the noradrenergic apparatus in these regions.

In the cat, qualitatively similar changes in the catecholamine content of the brain and adrenal gland have been produced when the defense reaction is elicited by electrical stimulation of the lateral hypothalamus (*6*) and when a comparable behavioral state is induced by the administration of morphine (*3*). These observations, taken in conjunction with the present results, suggests that central adrenergic activity may be a common denominator in excited behavior.

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References and Notes

1. L.-M. Gunne and D. J. Reis, *Life Sci.* **11**, 804 (1963).
2. S. M. Hilton and A. W. Zbrozyna, *J. Physiol.* **165**, 160 (1963).
3. L.-M. Gunne, *Acta Physiol. Scand.* **58**, suppl. 204 (1963).
4. A. Bertler, A. Carlsson, E. Rosengren, *ibid.* **44**, 273 (1958).
5. K. Fuxe and L.-M. Gunne, *ibid.* **62**, 493 (1964).
6. L.-M. Gunne and T. Lewander, paper presented at the Symposium on Mechanics of Release of Biogenic Amines. Stockholm, 1965, in press.
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