3. E. F. MacNichol, Jr., in *Molecular Structure and Functional Activity of Nerve Cells*, R. G. Grenell and L. J. Mullins, Eds. (American Institute of Biological Sciences, Washington, D.C., 1956), pp. 34–62.
4. T. Tomita, R. Kichuchi, I. Tanaka, in *Electrical Activity of Single Cells*, Y. Katsuki, Ed. (Shoin, Tokyo, 1960), pp. 11–23.
5. T. Tomita and W. H. Miller, unpublished.
6. M. E. Behrens and V. J. Wulff, *Federation Proc.* **23**, 517 (1964).
7. K. Frank and M. G. F. Fuortes, *J. Physiol.* **134**, 451 (1956).
8. H. Grundfest, *Ann. N.Y. Acad. Sci.* **94**, 405 (1961).
9. M. G. F. Fuortes, *J. Physiol.* **148**, 14 (1959).
10. S. Yeandle, *Am. J. Ophthalmol.* **46** (No. 3, pt. 2), 82 (1958); M. G. F. Fuortes and S. Yeandle, *J. Gen. Physiol.* **47**, 443 (1964).

* Postdoctoral fellow of the National Institutes of Health. Permanent address: Institut de Physiologie, Université de Genève, Senère, Switzerland.

21 January 1965

Amino Acids Released from the Cerebral Cortex in Relation to Its State of Activation

Abstract. *The rate of release of γ-aminobutyric acid from the perforated pial surface of the cerebral cortex in the cat showed systematic variations with the state of activation or "arousal" of the electrocorticogram. In animals subjected to midbrain coagulation and showing constant spindle patterns characteristic of sleep, the rate of release was three times greater (about 2 micrograms per hour per square centimeter) than in animals showing a largely "aroused" electrocorticogram, which had sections of the upper cervical cord or were neuraxially intact but had been given local anesthesia. The rate of release of glutamic acid was lower in the "sleeping" animals than in "aroused" animals, while no such differences were found for glutamine and aspartic acid. Such studies may lead to a better understanding of chemical mechanisms involved in the control of states of consciousness by the brain stem.*

During recent years it has become apparent that "activation" of the cortex in the "arousal" reaction is manifested by both excitatory and inhibitory effects upon individual cortical neurones and that the synchronization of the electroencephalogram in states of impaired consciousness may be due in part to active inhibitory processes of brain stem origin (*1*). It may be presumed that inhibitory effects are mediated by some chemical substances liberated from synaptic terminals of a different set of cortical afferent fibers from those responsible for the excitatory effects, believed to be mediated by acetylcholine (*2*).

As a preliminary test of this hypothesis we have measured the release of γ-aminobutyric acid (γ-ABA), a candidate for the role of an inhibitory transmitter substance in the brain (*3*), and of glutamic acid, glutamine, and aspartic acid from the cerebral cortex of cats. Measurements were made both before and after the brain stem was transected; such transection is known to cause changes in the electrocorticogram (ECG) similar to those which occur during (synchronized) sleep or barbiturate narcosis. Glutamic acid, glutamine, aspartic acid, and γ-ABA are closely related metabolically. Glutamic acid is known to have an excitatory action on cortical neurones (*4*), while aspartic acid and glutamine serve as controls for (or indicators of) indirect effects such as changes in cerebral circulation.

A plastic chamber (*5*) was sealed into the skull. The pia-arachnoid membrane over the 1 cm^2 of the midsuprasylvian gyrus covered by the chamber was punctured in six to eight avascular points. The trachea was cannulated for artificial respiration and, when called for, transection or coagulation of the brain stem was carried out with suitable instruments under stereotaxic control. The fifth nerve nucleus and occipital nerves, and all points subjected to pressure by the stereotaxic frame, were injected with Zylocaine. The animal was then paralyzed with Flaxedril and allowed to recover from the surgical procedures and the general anesthesia for at least an hour. (Further injections of the local anesthetic were made from time to time during the experiment. A tendency to natural sleep was taken as assurance that the animal was free from pain.) The ECG was monitored by silver-ball electrodes resting on the cortex within the chamber.

Cats were prepared in five different ways: (i) neuraxially intact with desynchronized "aroused" ECG throughout most of the collection period (sometimes maintained so by mild sensory stimulation); (ii) "encephale isolé," upper cervical cord transections, which also showed aroused ECG patterns; (iii) "cerveau isolé," upper midbrain transections, with ECG showing constant spindle pattern characteristic of sleep; (iv) hemisection of upper midbrain with an "aroused" pattern in the ECG from the side opposite to the section in response to mild sensory stimuli and a constant sleep pattern over the ipsilateral cortex; and (v) hemicoagulation of the mesial brain stem at the mesodiencephalic junction, with ECG patterns the same as in (iv).

The chambers were irrigated with warm saline solution until the perfusing fluid was clear. Then each chamber was filled with 1 ml of warm saline solution which was allowed to remain for 15 minutes with gentle stirring and was then withdrawn by suction and frozen. Similar samples were often taken simultaneously from chambers on the two hemispheres, and in some experiments two chambers were placed over each hemisphere, making four in all, to increase the cortical area from which samples were taken.

The fluids obtained from the chambers were lyophilized and the residues were extracted with a mixture of acetone and HCl. Each extract was evaporated to dryness at reduced pressure and the residue taken up in water. The solution was passed through a column of Dowex 50 (H^+ form) and the amino acids were eluted with ammonia solution. The eluate was evaporated to

Table 1. Release of amino acids from the surface of the cerebral cortex. (γ-ABA, γ-aminobutyric acid; *P* indicates significance of difference of the mean from the mean for neuraxially intact animals; ns, difference not significant.)

Preparation	ECG pattern	No. of samples	Amino acid released ($\mu g\ hr^{-1}\ cm^{-2}$)			
			Glutamic	γ-ABA	Glutamine	Aspartic
Neuraxially intact	Aroused	8	9.2 ± 0.8*	0.7 ± 0.2	1.0 ± 0.6	2.1 ± 1.0
Cervical section	Aroused	6	8.6 ± 0.2	0.7 ± 0.25	1.1 ± 0.15	1.8 ± 0.4
Midbrain section	Sleep	13	5.7 ± 1.0	2.0 ± 0.7	1.1 ± 0.3	1.7 ± 0.4
			$P < 0.01$	$P < 0.01$	ns	ns
Left midcollicular hemisection						
Right hemisphere	Aroused	2	7.2, 7.0†	1.1, 0.8	2.0, 2.0	2.5, 2.0
Left hemisphere	Sleep	2	5.0, 4.5	2.6	2.2, 2.2	2.5, 2.0

* Mean plus or minus standard deviation. † Individual results for the two preparations.

dryness at reduced pressure and dissolved in water. Glutamic acid, glutamine, aspartic acid, and γ-ABA were determined by two-dimensional chromatography according to Gaitonde (*6*). In some cases γ-ABA was determined also by the enzymatic method of Scott and Jakoby (*7*).

Gamma-aminobutyric and other amino acids were released from the surface of the individual cerebral cortex at a constant rate for 3 to 4 hours, once the resting state of the animal had become stabilized—as judged by a constant pattern of the ECG. The amounts obtained simultaneously from the left and right hemispheres were approximately the same, unless asymmetrical lesions had been made in the brain stem. There was, however, considerable variability from animal to animal.

In neuraxially intact or "encephale isolé" preparations with relatively few "sleep" spindles appearing in the ECG, the rate of γ-ABA release was about 0.7 μg hour^{-1} cm^{-2}, as shown in Table 1. In the animals with midbrain transections and constant sleep patterns in the ECG the rate of γ-ABA release was about three times as great. In two animals, midcollicular hemisections were made so that the rates of release of amino acids from an activated and a sleeping hemisphere could be determined simultaneously in the same animal, thus the possible effect of general factors such as changes in blood pressure were controlled. The rate of γ-ABA released from the "sleeping" hemisphere was higher than that from the intact side. The rate of glutamic acid release was higher in the intact "waking" hemisphere, with no difference apparent for glutamine and aspartic acid.

In two animals, (not included in Table 2) with unilateral mesial coagulation of the brain stem at the mesodiencephalic junction, stimulating electrodes were placed in the midbrain reticular formation on the opposite side. The ECG on the side of the lesion showed constant "sleep" spindles and did not react to electrical stimulation of the reticular formation below. The same stimulation (5-second trains of 100 per second, 1-msec pulses of 5-volt peak intensity administered periodically at 2- to 5-minute intervals) maintained a constant desynchronized arousal pattern in the ECG on the side of the intact brain stem. In these animals no γ-ABA could be detected in the perfusate from this activated side, while γ-ABA was released at rates of about 1 to 2 μg hour^{-1} cm^{-2} from the side of the lesion.

The relatively constant rate of release of glutamine and aspartic acid in different preparations (Table 1) makes it unlikely that the differences in γ-ABA release might be due to circulatory changes or factors other than the effects of the brain stem lesions upon the afferent supply to the cerebral cortex. It seems that the major portion of the ascending systems of neurones which liberate γ-ABA must lie in the mesial portions of the brain stem in close association with those systems regulating the spontaneous electrical activity of the cortex.

Sensory cortical evoked potentials and responses to direct electrical stimulation are rapidly depressed and changed in form by the topical application of γ-ABA (*8*), while spontaneous slow waves and rhythmic spindle waves are enhanced. Topical γ-ABA produces a marked increase in the slow-wave component of an experimental spike and wave complex which can be produced by rhythmic electrical stimulation of the mesial thalamus; this wave is associated with inhibition of cortical neuronal activity (*9*). Iontophoretically applied γ-ABA through multibarrelled microelectrodes has also been shown to cause rapid arrest of spontaneous firing in individual cortical cells (*10*).

Our studies show that free γ-ABA is released from the cerebral cortex in amounts (probably in much larger amounts than could be detected by the methods used in our experiments) which bear a definite relation to its functional activity. They suggest the possibility that γ-ABA or some related substance may be of physiological importance as a mediator of inhibitory controls of brain activity.

That a significantly greater amount of glutamic acid was found in the activated (aroused) cortex with intact brain stem is consistent with the known excitatory action of this substance (*4*); this may mean that factors in addition to acetylcholine may have to be sought for a full explanation of the excitatory aspects of the control exerted upon the activity of the cortex by the brain stem in different states of vigilance.

H. H. JASPER
R. T. KHAN
K. A. C. ELLIOTT
Montreal Neurological Institute
and *Department of Biochemistry,*
McGill University, Montreal, Canada

References and Notes

1. E. V. Evarts, in *The Nature of Sleep* (Little, Brown, Boston, 1961), p. 171; O. Creutzfeldt and R. Jung, *ibid.*, p. 131; G. Moruzzi and H. W. Magoun, *Electroencephalog. Clin. Neurophysiol.* **1**, 455 (1949).
2. F. Bremer, *Compt. Rend. Soc. Biol.* **122**, 464 (1936); J. C. Szerb, *Can. J. Physiol. Pharmacol.* **42**, 303 (1964); J. F. Mitchell, *J. Physiol.* **165**, 98 (1963); K. Krnjevic and J. W. Phillis, *ibid.* **166**, 296 (1963).
3. K. A. C. Elliott and H. H. Jasper, *Physiol. Rev.* **39**, 383 (1959); K. A. C. Elliott, *Brit. Med. Bull.* **21**, 70 (1965); E. Roberts, Ed., *Inhibition in the Nervous System and Gamma-aminobutyric Acid* (Pergamon, Oxford, 1960).
4. K. Krnjevic, M. Randic, D. W. Straughan, *Nature* **201**, 1294 (1964).
5. K. A. C. Elliott and H. H. Jasper, *Am. J. Physiol.* **157**, 122 (1944); J. F. Mitchell, *J. Physiol.* **165**, 98 (1963).
6. M. K. Gaitonde, *J. Neurochem.* **8**, 234 (1961).
7. E. M. Scott and W. B. Jakoby, *J. Biol. Chem.* **234**, 932 (1959).
8. K. Iwama and H. H. Jasper, *J. Physiol.* **138**, 365 (1957).
9. D. A. Pollen and P. G. Sie, *Electroencephalog. Clin. Neurophysiol.* **17**, 154 (1964); D. A. Pollen, *ibid.*, p. 398.
10. D. R. Curtis, *Pharmacol. Rev.* **15**, 333 (1963).
11. Supported by the Committee on Psychobiology of the United States, the Medical Research Council of Canada, and the Donner Canadian Foundation. We are grateful to Dr. L. S. Wolfe for advice and help.

25 January 1965

Relative Radiosensitivities of Woody and Herbaceous Spermatophytes

Abstract. *The sensitivities of several woody and herbaceous species to single acute exposures to cobalt-60 gamma rays have been determined. Within each group the sensitivity of each species is largely determined by its average interphase chromosome volume (interphase nuclear volume divided by chromosome number) of shoot apical meristem cells. On the basis of the calculated amounts of energy absorbed (in kiloelectron volts) per interphase chromosome at an exposure necessary to produce a given biological effect, woody species were approximately twice as sensitive as herbaceous species.*

In herbaceous plants there is a high correlation between exposures of x- or γ-rays required to produce a lethal effect and the average interphase chromosome volume of the shoot apical meristem cells (*1, 2*). The correlation is much better than the previously reported correlation between radiosensitivity and nuclear volume (*3*).

Using the same techniques, we at-