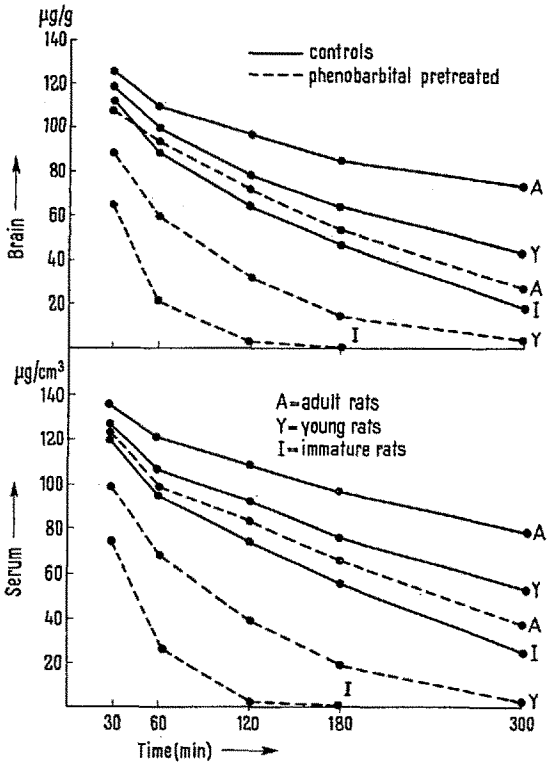
Effect of phenobarbital or phenaglycodol pretreatment on metabolism of meprobamate in relation to age of rats

Age	Pretreatment	Meprobamate concentration serum (μg/ml)	brain (μg/ml)	*In vivo* metabolism (μg/100 g/2 h)	Variation (%)	Biological half-life (min)	Variation
Adult	–	108 ± 4.1 (12)	97 ± 3.7 (12)	35		345	
Adult	Phenobarbital	84 ± 3.5 (8)	72 ± 3.5 (8)	60	+ 71	150	× 0.44
Adult	Phenaglycodol	96 ± 4.0 (8)	84 ± 3.4 (8)	49	+ 40	–	
Young	–	92 ± 3.4 (15)	79 ± 2.9 (16)	53		195	
Young	Phenobarbital	40 ± 2.1 (8)	31 ± 2.3 (8)	104	+ 96	58	× 0.30
Young	Phenaglycodol	59 ± 3.4 (8)	59 ± 3.5 (7)	75	+ 41	–	
Immature	–	77 ± 2.9 (16)	65 ± 2.7 (16)	67		130	
Immature	Phenobarbital	3 ± 0.9 (8)	4 ± 1.1 (8)	141	+110	31	× 0.24
Immature	Phenaglycodol	28 ± 2.2 (4)	21 ± 1.8 (4)	117	+ 75	–	

Meprobamate concentration was determined 2 h after the injection of meprobamate (150 mg/kg). Metabolism *in vivo* is represented by metabolized meprobamate during 2 h after the injection of meprobamate (150 mg/kg) per 100 g body weight. The numbers in brackets show number of the determination.



Influence of different ages of rats on the induction of increase of meprobamate metabolism. Meprobamate (150 mg/kg) was injected intraperitoneally at 0 time. The abscissas show serum and brain meprobamate concentration.

6.7 mg for immature rats; the biological half-life of meprobamate was 345 min in adult rats, 195 min in young rats and 130 min in immature rats.

It is also demonstrated that the induction of increased meprobamate metabolism is more remarkable in younger rats than in older rats. For example, the meprobamate metabolism in adult rats was increased 71% by the pretreatment with phenobarbital, while in young rats and in immature rats, the metabolism was increased 96% and 110% respectively, and also biological half-life of meprobamate was shortend 56% in adult rats, 70% in young rats and 76% in immature rats by the pretreatment with phenobarbital. It is not yet known why the liver of younger rats is more sensitive than that of older ones in response to the phenobarbital pretreatment. On the other hand, recently it was reported that no difference in the induction of liver tryptophanperoxydase exists in the different ages of the rats used[6].

According to our results, the regenerating liver of the adult rats was still less sensitive than the liver of young rats in the induction, therefore the factors which determine the sensitivity of liver in the induction of the enzyme by phenobarbital are not related to the age of the liver but it might be related to some humoral factors in these animals.

Riassunto. Si è osservato che il metabolismo del meprobamato è tanto più rapido quanto più giovani sono i ratti usati ed anche che l'induzione dell'aumento del metabolismo *in vivo* è tanto più intensa quanto più giovani sono i ratti usati.

R. Kato, E. Chiesara, and G. Frontino

Istituto di Farmacologia e di Terapia, Università degli Studi, Milano (Italy), July 4, 1961.

[6] R. I. Gregerman, Amer. J. Physiol. *197*, 63 (1959).

Effect of Amygdaloid Lesions on Plasma Corticosterone Response of the Albino Rat to Emotional Stress

The rich projection of the amygdaloid nucleus to the hypothalamus (Gloor[1]), suggests that this limbic structure may modulate the hypothalamic-hypophysial response to stress. Experiments undertaken to date have supported this assumption. Mason[2] found in the monkey that partial or complete bilateral amygdalectomy temporarily inhibited the plasma 17-hydroxycorticosteroid response to emotional stress (bar-pressing to avoid electric shock), delaying peak output as much as 6 h. This effect was only obtained, however, at 4 weeks following the operation. This work suggests that the amygdala normally boosts ACTH secretion in the monkey. Martin[3] et al. found high adrenal venous corticosteroid output in dogs

[1] P. Gloor, EEG Clin. Neurophysiol. *7*, 223 (1955); *7*, 243 (1955).
[2] J. W. Mason, Amer. Psychosom. Soc., Montreal, March 26 (1960); Psychosom. Med., in press.
[3] J. Martin, E. Endroczi, and G. Bata, Acta physio. Hung. *14*, 131 (1958).

with previous bilateral removal of the amygdala, suggesting an inhibitory influence of the amygdala on ACTH secretion in this species. In the rat, a recent study by KNIGGE[4] showed that amygdaloid lesions depressed the rate of plasma corticosterone response to immobilization stress, though peak output was eventually comparable to that of normal rats undergoing the same experience.

These experiments suggest that a more precise study of amygdaloid function in stress, using relatively circumscribed lesions within certain amygdaloid sub-nuclei, would provide information unobtainable from studies consisting of extensive or complete amygdalectomy.

The plan of this study was to place bilateral electrolytic lesions in the amygdala of one rat of a pair, and bilateral cortical lesions on the brain convexity in the other (control) animal, then 3 weeks later subject both rats to 15 min of immobilization[5]. Blood samples from each rat were then to be processed together after GUILLEMIN's method[6] to determine fluorometrically the plasma corticosterone level in each one.

A series of 20 male albino rats, Wistar strain, was operated on in pairs at about 90 days of age, the operation on each pair being carried out the same day, usually within an hour or two. The animals were anesthetized by Nembutal, injected intraperitoneally. A bipolar electrode was placed stereotaxically in the brain of each experimental animal at skull coordinates of 3 mm posterior to bregma, 4.5 mm lateral to the midline suture, and to a depth of just beyond 8 mm from the skull surface. The same coordinates were used for the control except that the depth from the skull was 1.5 mm. Holes in the skull were made by a dental drill. Current was applied through a Wyss high-frequency generator for 5 sec at each site, after preliminary trials in egg-white.

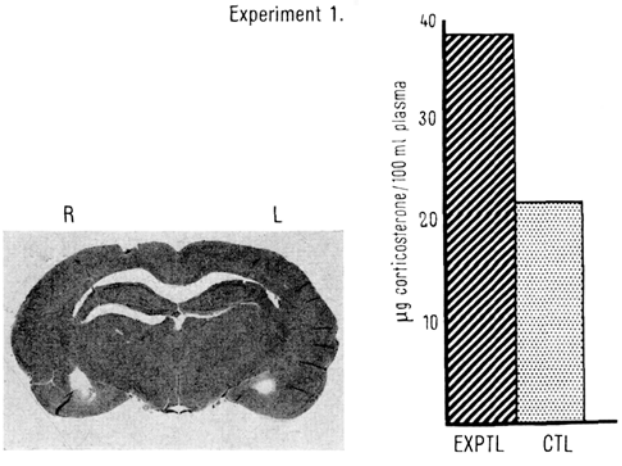
Three weeks following operation of each pair, experimental and control rats were (one at a time) bound up in adhesive tape so that hindlegs and forelegs could not be moved, but not so tightly as to interfere with breathing. Each animal was then placed on its back in a white basin and taped in this position for 15 min. It was then decapitated and blood collected in a 50 ml beaker containing a drop of heparin, care being taken to shake the beaker gently while collecting the blood from the rat's trunk.

The blood sample was then centrifuged and prepared for fluorometry[6]. Plasma from the experimental rat and plasma from the control were then processed at the same time, together with one blank (water) and three standard solutions, containing known amounts of corticosterone. A series of 10 calibration experiments earlier had yielded a recovery rate of corticosterone from plasma of 104.7%, with a standard error for the mean of 3.9%. The average plasma corticosterone level for these unstressed rats was 5.7 μg/100 ml of plasma.

Immediately on decapitation, the brain of each rat was removed and fixed in a solution of 10% formalin for 1 week. The brains were then mounted in paraffin, 10 μ sections were made through each brain at 0.5 mm intervals, and the sections were stained with hematoxylin and eosin.

All lesions mentioned here were identified by histological examination[7]. The cortical lesions in the control rats were found to be located in the superior part of the cortical convexity.

A total of 18 rats was available for immobilization, one amygdaloid lesion animal having died post-operatively. Of the nine remaining pairs, two were eliminated from the experimental results; an error in chemical procedure was made in the case of one pair, and in the case of the other, the experimental rat resorted to abdominal breathing under stress and appeared near death, unlike any other rat, although as far as could be ascertained this was not



Lesions in region of central nucleus of amygdaloid complex, found most effective in inducing increased plasma corticosterone response of rat to brief immobilization, compared to response of paired control rat with cortical lesions (not shown). See Table for details.

[4] K. M. KNIGGE, Fed. Proc. *20*, 185 (1961).

[5] C. FORTIER, in Endocrinology *49*, 782 (1951), has shown immobilization requires participation of the central nervous system to effect a pituitary-adrenal response, and thus can be termed an emotional or neurogenic stress.

[6] R. GUILLEMIN, G. W. CLAYTON, H. S. LIPSCOMB, and J. D. SMITH, J. lab. clin. Med. *53*, 830 (1959); Endocrinology *63*, 349 (1958).

[7] In the case of amygdaloid lesions, this examination was undertaken without prior knowledge of the plasma corticosterone levels resulting in each experiment.

Plasma corticosterone response to immobilization stress of paired rats with amygdaloid lesions (E) and cortical lesions (C). Lesion damage indicated for experimental animals (E) only. Parentheses indicate minimal lesion damage to structures included.

Experiment no.	Lesion damage (E only) to amygdaloid nuclei and adjacent structures		Plasma corticosterone level after stress in μg/100 ml of plasma		
	L side	R side	E	C	E – C
1	central	central (basal, lateral, putamen)	38.50	21.75	16.75
3	(cortical)	(central), optic tract	27.00	20.75	6.25
4	central, basal, (lateral)	central, basal, (medial), optic tract	22.00	29.50	– 7.50
5	central, cortical, (hippocampus)	central, putamen, (hippocampus)	25.25	20.25	5.00
6	(lateral), external capsule, (claustrum)	central, (medial, putamen, cerebral peduncle), optic tract	24.50	17.25	7.25
7	central (basal, putamen, optic tract)	central (medial, optic tract)	33.75	17.00	16.75
9		central, basal (putamen)	28.25	29.25	– 1.00

due to taping. This left a total of 14 rats, or seven pairs, for comparison.

The Table shows that five of six rats with bilateral amygdaloid lesions displayed a greater plasma corticosterone response to the stress of immobilization than their respective paired controls with cortical lesions. There was a reversal in only one case, experiment 4. A unilateral lesion of the amygdala (experiment 9) produced no appreciable difference in plasma corticosterone level. On being taped for immobilization, the amygdaloid lesion rats were in general more emotionally reactive and harder to handle than the controls.

Examination of effects of electrode placement in individual amygdaloid sub-nuclei showed that, when there was substantial bilateral damage to the central nucleus, but no bilateral damage to any other amygdaloid subnucleus, as in experiments 1, 5, and 7, the plasma corticosterone level was distinctly more elevated in the rat with bilateral amygdaloid lesions. The most clear-cut symmetrical lesions of the central nucleus were obtained in experiment 1 (Figure), where the rat with amygdaloid lesions showed an increase in plasma corticosterone following stress that was 16.75 µg/100 ml of plasma greater than the increase for the control.

These three experiments, 1, 5, and 7, showed on the average a difference of 12.83 µg of corticosterone per 100 ml of plasma in favor of the animals with amygdaloid lesions, compared to an average of 1.25 µg in favor of the rats with amygdaloid lesions in the remaining four experiments, 3, 4, 6, and 9. In experiment 4, where as noted above, a reversal occurred, there was substantial bilateral damage to the central nucleus, but there was in addition bilateral damage to the basal nucleus. This was the only instance in this series where this occurred.

These individual comparisons of histologically-determined damage in individual amygdaloid sub-nuclei with the plasma corticosterone response to emotional stress of the rat therefore suggest, in particular for experiments 1, 5, and 7, that bilateral lesions confined to the central nucleus of the amygdala result in an increased plasma corticosterone response to emotional stress in the laboratory rat. In view of the small number of animals and of the topographical scattering of the lesions among various amygdaloid sub-nuclei (Table) statistical methods cannot be justifiably applied to this series[8]. These observations suggest that under normal conditions the central nucleus in the rat amygdala may exert an inhibitory effect upon ACTH release.

Our results, taken together with previous work[2,3] suggest the possible existence of separate inhibitory and facilitatory mechanisms for the pituitary-adrenal response to stress at the amygdaloid level in the rat. Such inhibitory and facilitatory mechanisms have been found at the hypothalamic level in the dog[9] and at the mid-brain level in the rat[10].

Zusammenfassung. Im Vergleich zu Kontrolltieren mit zweiseitigen Rindenläsionen führt beidseitige Zerstörung des Nucleus centralis im Mandelkernkomplex der Ratte zu erhöhter Plasma-Corticosteron-Produktion nach kurzfristigem Stress durch entsprechende Immobilisierung.

E. W. Bovard[11] and P. Gloor

Department of Neurology and Neurosurgery, McGill University, and the Montreal Neurological Institute, Montreal (Canada), June 5, 1961.

[8] An overall comparison of plasma corticosterone response between experimental and control rats was nevertheless made. This showed an average plasma corticosterone level of 28.46 µg per 100 ml of plasma for the seven experimental rats, compared to 22.25 µg for the seven paired controls, a difference of 6.21 µg. This difference was not statistically significant. However, since this sample, for reasons given above, does not lend itself to a meaningful statistical analysis, this negative result does not invalidate the findings. For the purposes of this study, effects of lesion placements in individual sub-nuclei are more relevant, but this work should be repeated on a larger series of animals to allow for the application of statistical methods.

[9] T. Suzuki, E. B. Romanoff, W. P. Koella, and C. K. Levy, Amer. J. Physiol. *198*, 1312 (1960).

[10] M. A. Slusher and V. Critchlow, Proc. Soc. exp. Biol. Med. *101*, 497 (1959).

[11] This study was made possible by a special research fellowship to one of us, E. W. Bovard, from the United States Public Health Service, sponsored by the National Institute of Mental Health. The writers would like to acknowledge with thanks the interest and friendly cooperation of Dr. H. Jasper, Dr. Hanna Pappius, Dr. Marion K. Birmingham, and the medical and technical staff of Montreal Neurological Institute in this work. This paper was originally presented at a meeting of the Montreal Physiological Society, March 8, 1961.

Present address of Bovard: Department of Anatomy, Albert Einstein College of Medicine, New York (USA).

PRO EXPERIMENTIS

Fine Tipped Metal Microelectrodes with Glass Insulation

It is profitable to use very fine tipped metal microelectrodes, when leading off extracellularly action potentials from single nerve cells in the brain, because the finer the electrode tip, the greater is the recorded potential from a nearby cell in respect to those from units farther away.

With the electropolishing technique, it is possible to make very fine tipped microelectrodes of tungsten[1] or steel[2], which are then insulated with lacquer. It is, however, very difficult to obtain a thin, smooth lacquer layer when the electrodes have a long shaft of less than 20 µ in diameter. Moreover, the mechanical strength of the lacquer layer is unsatisfactory, and when coagulating with high frequency alternating current, the loss over the insulation (or through minute cracks?) is great. A glass insulating layer would be much more satisfactory. Indeed, Johnson and Manhof[3] drew a coat of glass around a platinum wire, but the tips of their electrodes were not thinner than the wire used and blunt. I therefore developed a method for drawing a thin layer of pyrex glass, under microscopical inspection, around an electropolished platinum wire and so obtained tip sizes of controlled, very small dimensions.

A length of platinum wire (10 or 20 µ diameter), a little longer than the desired shaft length, is soldered with silver solder to a short length of thicker copper wire to facilitate the handling (Figure 1a).

Polishing is done in the same way as with tungsten[1]: the utmost tip of the platinum wire is dipped in saturated sodium or potassium nitrite solution, and an alternating

[1] D. H. Hubel, Science *125*, 549 (1957).

[2] J. D. Green, Nature *182*, 962 (1958).

[3] M. W. Johnson and L. J. Manhoff, Science *113*, 812 (1951).