

Midbrain Hemisection: Effect on Cortical Acetylcholine in the Cat

Abstract. *A midbrain hemisection in a midpontine pretrigeminal preparation of the cat causes an asymmetry in the electroencephalogram with a marked increase in the acetylcholine content of the cortex from the synchronized hemisphere. It is suggested that the increase in acetylcholine is related to the decrease of nervous activity caused by the hemisection.*

It has been demonstrated that an increase in the total amount of acetylcholine in the rat brain occurs during spontaneous and drug-induced sleep (1). The purpose of the experiments described here was to investigate the acetylcholine content of the cerebral cortex and the caudate nucleus during the synchrony and asynchrony of the electroencephalogram (EEG) induced by sections of the brain stem in the cat.

In such an animal a transection of the pons at the level or immediately in front of the trigeminal rootlets is followed by an EEG pattern characterized by low voltage, fast rhythms which are considered typical of the waking state ("midpontine pretrigeminal preparation," 2). In this preparation the hemisection of the midbrain at the superior collicular level produces a striking asymmetry of the EEG. The hemisphere overlying the hemisection shows an EEG with slow waves and spikes typical of sleep, while the other hemisphere shows a waking EEG (3). This preparation therefore offers the possibility of comparing in the same animal the acetylcholine content of two symmetrical cortical areas with different EEG patterns.

In our experiments the midpontine transection was made in adult cats under ether anesthesia by means of a stereotactically oriented spatula. The anesthesia was then discontinued and the cats resumed spontaneous respiration. The blood pressure was normal (120 to 140 mm-Hg). In seven cats a midbrain hemisection was subsequently made by the same procedure. The level of the sections was confirmed macroscopically at the end of the experiments. Cats in which brain hemorrhages or edema occurred were discarded. The EEG was recorded on a Battaglia Rangoni inkwriter by means of four unipolar electrodes screwed into the skull, in the frontal and occipital areas of both sides. The cats were killed by exsanguination about 2 hours after the end of the anesthesia. Samples of about 500 mg were excised from

frontal, parietal, and occipital areas of both hemispheres. The stereotaxic apparatus was used to obtain symmetrical samples of the same depth. The brain was then removed and both caudate nuclei were isolated. The samples were rapidly chilled and weighed. Total acetylcholine was extracted by the method of Smallman (4). The acetylcholine content of the extracts was estimated on the isolated eserinated rectus abdominis muscle of the frog.

Table 1 shows the acetylcholine content of the cortical areas and the caudate nuclei of the midpontine pretrigeminal preparation. These cats showed the described (2) diffusely activated EEG pattern typical of the waking state. It appears that there are marked differences between the brain acetylcho-

line content of the seven cats but there are only small differences between the content of symmetrical areas of the same cat. The acetylcholine content of the caudate nuclei is higher than that of the cortex; a similar difference has been reported for the cholinesterase activity (5). The cats in which a midbrain hemisection was combined with the midpontine transection showed the long-lasting asymmetry of the EEG previously described (3). The acetylcholine content of the brains of these cats is reported in Table 2. It appears that there is a marked difference between the acetylcholine content of symmetrical areas of the cortex. In fact, the acetylcholine content of the "synchronized, sleeping" cortex overlying the hemisection is always much larger than that of the "awake, desynchronized" cortex of the opposite side. On the other hand, there is only a small difference between the caudate nuclei.

The increase of the cortical acetylcholine induced by midbrain hemisection is reduced or abolished by an arousing drug. After intravenous injection of DL-Dopa [β -(3,4-dihydroxyphenyl) L-

Table 1. Acetylcholine content (nanograms of the chloride per 100 mg fresh tissue) of the cerebral cortex and caudate nucleus in midpontine pretrigeminal preparations.

Cat No.	Frontal area		Parietal area		Occipital area		Caudate nucleus	
	Left	Right	Left	Right	Left	Right	Left	Right
1	52.7	67.3			61.6	62.2	255.0	262.0
2	61.2	67.8	101.3	87.9	83.7	66.0	516.1	429.9
3	72.2	77.9	50.2	59.6				
4					123.8	123.4	357.5	581.8
5	102.8	107.0	133.5	148.5	161.0	178.6	284.3	377.7
6	153.8	154.2	78.4	89.9	175.4	160.5	302.7	293.7
7	59.8	55.0					267.3	264.5
	6%		Average difference between the two sides 13.5%		8.4%		21.3%	

Table 2. Acetylcholine content (nanograms of the chloride per 100 mg fresh tissue) of the cerebral cortex and caudate nucleus in midpontine pretrigeminal preparation from cats with midbrain hemisection.

Cat No.	Frontal area		Parietal area		Occipital area		Caudate nucleus	
	Control	Hemi- sected	Control	Hemi- sected	Control	Hemi- sected	Control	Hemi- sected
1	109.2	160.1	87.2	207.6	72.0	189.0	510.2	546.2
2	71.6	194.9	96.8	187.2	65.8	231.8	363.6	413.1
3	50.6	174.4	103.8	147.6	57.2	121.2	290.1	381.7
4	165.9	188.8	91.5	195.3	103.2	230.7	328.1	372.6
5	89.6	141.8	77.9	167.0	73.5	115.0	520.0	560.0
Mean $\pm$	93.3 $\pm$	172.0 $\pm$	91.4 $\pm$	180.9 $\pm$	74.3 $\pm$	177.5 $\pm$	402.4 $\pm$	454.7 $\pm$
S.E.	20.0	9.8	3.7	10.6	7.7	25.5	47.0	40.3
	$p < .01$		$p < .001$		$p < .01$		Nonsignificant	
Average increase	73.9%		98.3%		138.9%		13.0%	
	After injection of DL-Dopa							
6	104.0	75.4	75.6	119.3	75.0	93.9	367.7	390.2
7	89.9	92.4	75.0	88.6	81.8	70.1	353.3	375.0

alanine] (25 to 35 mg/kg) in two pretrigeminal preparations from cats with midbrain hemisection, the EEG asymmetry was replaced by a bilateral symmetrical activation and the acetylcholine content of the two sides showed only small differences (Table 2). The arousing effect of DL-Dopa has been attributed to a stimulating action on the reticular activating system (6). The last experiment as well as preliminary assays of the cholinesterase activity of the cortex (7) seem to rule out the possibility that the increase of cortical acetylcholine following the hemisection is due to vascular damage or to a decrease of acetylcholinesterase activity.

Our results suggest that the amount of cortical acetylcholine in the cortex may be related to the degree of activity of the reticular activating system. The EEG activated pattern of the pretrigeminal preparation is maintained by a tonic barrage of impulses arising in the rostral portion of the pons (2). The midbrain hemisection would produce ipsilateral sleep by the decrease of the number of activating impulses impinging upon the cerebral cortex (3). Several data support the suggestion that part of the fibers ascending to the cortex are cholinergic (8). It is conceivable that the resting state of ascending fibers during the EEG synchronization leads to accumulation of acetylcholine in their endings or in the cortical neurons or in both.

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9. Supported by a grant from the Consiglio Nazionale delle Ricerche, Rome, Italy.
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17 March 1964

Method for Discriminative Avoidance Training

Abstract. Experiments were performed in which the typical procedure for obtaining discriminated avoidance behavior with rats in a lever-pressing apparatus was compared with a new procedure which allowed only avoidance but not escape from the shock. When either escape or avoidance is possible, the rats make a majority of escape responses, but where escape is impossible avoidance behavior is rapidly established.

Discriminative avoidance procedures are used extensively in studies of the effect of drugs, brain stimulation, and other variables of interest to the medical and behavioral sciences. In such a procedure an animal is trained to perform a response in the presence of a stimulus which signals the occurrence of a noxious stimulus, such as shock, strong light, or intense sound. The response, which usually consists of pressing a lever or of running from one side of an alley to the other, terminates the signal and prevents the advent of such noxious stimulation. However, the reported difficulty of establishing a discriminative avoidance habit with rats in a lever-pressing apparatus (1) severely limits the use of this much favored experimental situation and raises several problems of a theoretical nature. This report describes a procedure in which these difficulties were overcome and which resulted in the acquisition of the required behavior.

The apparatus consisted of a metal chamber fitted with a grid floor which could be electrified. A lever, requiring a downward pressure of 10 g, was fixed at one end of the chamber, and a 6-volt lamp was placed at ceiling height immediately above the lever. Electric shock (0.6 ma) was supplied by a constant current source and was delivered through a scrambling unit which successively reversed the polarity of each rod of the grid floor, thereby making it impossible for the animal to avoid shock by locating a safe standing place.

The main feature of the procedure was that the warning signal, once presented, continued until a press on the lever was made. During presentation of the signal the noxious stimulus—shock—was given at regular intervals but did not last long enough to give the

animal an opportunity of escaping from it. In the two experiments reported here the interval between the onset of the signal and the first shock was 7.5 seconds. The interval between successive shocks in the presence of the signal was varied so as to occur, on the average, every 13 seconds. A total of 13 2-hour sessions was given, with intervals of 22 hours between each session. In the first experiment, rats were exposed to the procedure described above. In the second experiment a procedure was adopted which is commonly claimed to yield negative results.

In experiment 1, three male hooded rats, about 120 days old, were used. In each of the 13 2-hour sessions the following procedure was used. Shortly after the subject was placed in the chamber a signal (light) was presented, and unless the lever was pressed within 7.5 seconds of signal onset the first of a series of brief shocks (0.2 seconds) was delivered. These shocks occurred at irregular intervals, on the average once every 13 seconds. As soon as the lever was pressed the signal terminated and the shock series was suspended. After a variable in-

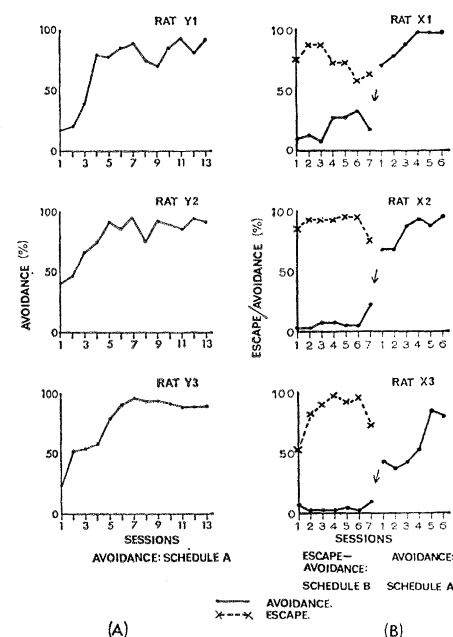


Fig. 1. (A) avoidance (percentage) as a function of the sessions conducted according to a procedure which allowed only avoidance but not escape. (B) Escape and avoidance (percentages) as functions of sessions conducted according to a procedure which allowed escape as well as avoidance. The procedure was changed after seven sessions to that used for (A).