Brit. J. Pharmacol. (1961), **17**, 473–478.

EFFECT OF CHLORPROMAZINE ON SEPTAL HYPERACTIVITY IN THE RAT

BY

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(*Received August* 21, 1961)

The effect of chlorpromazine on the overt emotional activity of a hybrid strain of rats has been measured using an altered "emotionality rating scale." It has been found that 8 mg/kg of chlorpromazine intraperitoneally reduced the activity of rats with the septal nuclei destroyed, normal rats and control rats to the same emotional rating. This effect has been related to the postulated interrelationship of the septal area and the amygdaloid complex of nuclei. It seemed that impulses traversing the septal nuclei might be re-routed, so that destruction of the septal area need not necessarily cause total abolition of "septal-function."

It has been suggested by Olds & Travis (1960) that chlorpromazine can inhibit a forebrain system, while leaving intact the functions of a midbrain system. This is compatible with the hypothesis that chlorpromazine activity is localized within the limbic lobe.

A marked increase in emotional behaviour of animals after experimental ablation of the septal region of the forebrain has been reported (Brady & Nauta, 1953; King, 1958; King & Meyer, 1958). This hyper-emotionality is manifested by violent attack or flight reactions in response to previously neutral or innocuous stimuli. There is extreme muscular tension to a degree rarely, if ever, noted in the normal animal. Therefore, it is possible that the septal nuclei exert an important function in the "physiological tranquillization" of rats. Destruction of the septal nuclei results in the excited animal because the septum normally counterbalances a stimulatory area.

Chlorpromazine has been shown to cause an activity shift in the opposite direction to that produced by septal destruction (Hunt, 1957). This author showed that, following chlorpromazine administration, both septal operated and unoperated rats were depressed.

A study of the activity of chlorpromazine in rats with surgically destroyed septal nuclei has been made with a view to testing the hypothesis that the septal area "counterbalances" a stimulatory area of the brain. To locate the site of actions of chlorpromazine the extent of the effect of the drug on animals which were septally hyperactive was observed, and compared with the effect of chlorpromazine on normal rats and on control animals which had had a "sham" operation.

METHODS

Animals. Lashley strain hooded rats were bred with Sprague-Dawley albino rats, and the second generation of this hybrid used. Preliminary investigations showed that these hybrid animals had greater physical endurance, being able to swim twice as long as their albino forebears. They were more resistant than the albinos to post-operative infection, to barbiturate sedation and were more docile than the Lashley strain. Animals with albino physical characteristics were discarded and only those animals with the typical hooded features were used.

Thirty-six young adult rats (90 to 120 days old) of each sex were divided into three groups composed of (*a*) 24 which had septal destruction, (*b*) 24 unoperated controls and (*c*) 24 receiving a sham operation.

The three groups of animals were rated as to their activity, and were then given 8 mg/kg of chlorpromazine hydrochloride intraperitoneally on each of the test days. The activity rating of each of the animals was again determined 30 min later. Each control rat was tested for activity rating for a total of 10 days. Those undergoing surgery were given 3 days of pre-operative and 7 days of post-operative testing.

Previous investigations here had shown that the amount of chlorpromazine used (8 mg/kg) and the time between administration and rating (30 min) were optimal.

Activity ratings. An activity rating scale of six components was used to evaluate the behaviour of each animal. It had been adapted with certain minor modifications from one designed by Brady & Nauta (1953), and later modified by King (1958). The term "activity rating" has been substituted for "emotionality rating," for the present authors believed that activity was being measured as a direct result of emotionality, rather than emotionality itself.

The six components of the rating scale were (*a*) reaction to object presentation (a probe presented close to the snout), (*b*) response to a light tap on the back with the probe, (*c*) resistance to capture (slowly approaching the animal and grasping it firmly but not roughly), (*d*) resistance to handling, including a gross evaluation of muscle tone, (*e*) vocalization during the stages of capture and handling, and (*f*) urination and defaecation in response to capture and handling.

Each of the preceding components of the scale was evaluated on a six-point scale with scores from 0 to 5. An overall score was assigned to each animal by totalling the six scores. Each animal had the potential of attaining any score between 0 and 30. The activity ratings reported are average values from each group of 24 rats.

The result of trial runs made to train the observer were in accord with those previously obtained by King (1958) and King & Meyer (1958).

Surgical procedure. Veterinary pentobarbitone sodium (1 part) and 10% ethyl alcohol (8.5 parts) were mixed and general anaesthesia induced by injecting intraperitoneally 0.01 ml. of the mixture for each 1 g of body weight. Atropine sulphate (1 mg/100 g) was administered intraperitoneally to prevent excessive secretion.

After incising the skin of the dorsal surface of the head, the tissue overlying the skull was gently removed. A single trephine hole was drilled over the points at which the lesion was to be made. The co-ordinates used were 2.5 mm anterior to the bregma, 0.5 mm bilateral from the midline, and 6.38 mm beneath the surface of the cerebral hemispheres. Bilateral electrolytic lesions of the septal region were placed by means of a stereotaxic instrument (Baltimore Instrument Co.). A stainless steel electrode insulated except for 0.75 mm at the tip was passed into the brain, and a direct current of 2 mA passed for 20 sec. The circuit was completed by rectal cathode. The wound was then closed by suture with surgical silk, and 30,000 u. of procaine penicillin administered intramuscularly.

The "sham" operation was identical in every respect to the actual surgery performed, but no current was passed through the electrode inserted into the brain after the "sham" operation.

Histological procedure. At the conclusion of the testing period, the entire brains of one-third of the rats selected at random from each group were fixed in formalin initially by injecting a 10% solution intracardially, and then immersing the brains in 37% formalin for at least 24 hr. The brains were then removed from formalin, washed in running water for 24 hr, and dehydrated with successively increasing concentrations of alcohol. The brains were then cleared with xylene, embedded, mounted, sectioned at 10 μ, and stained with cresylecht violet or thionin. Every tenth section was placed on a slide and the focus and extent of the electrolytic lesion estimated.

Statistical analysis. The results obtained were analysed statistically using a design designated as a Type I " Mixed " Design (Lindquist, 1953). Since the F-ratios for variance between groups, within groups, and interaction were significant, the *t* test for " critical difference," d (Lindquist, 1953), was applied for indication of differences between means.

RESULTS

Activity ratings. The normal activity rating for these rats was 7.89 (7.36 to 8.71). After chlorpromazine (8 mg/kg) the activity rating of normal animals decreased to 2.99 (2.21 to 3.86). In animals with the septum destroyed, the activity rating rose sharply to 20 (15 to 28) on post-operative day 1. This extreme activity gradually decreased until, on post-operative day 7, and for approximately six weeks thereafter, the activity rating was 9.01 (8.24 to 10.86), slightly but significantly above the normal value of 7.89. The mean septal activity for the seven-day post-operative testing period was 14.68 (10.86 to 16.11). On the first post-operative and on all subsequent days of rating, the activity of the septal animals was reduced to 3.03 (1.57 to 4.00) by chlorpromazine.

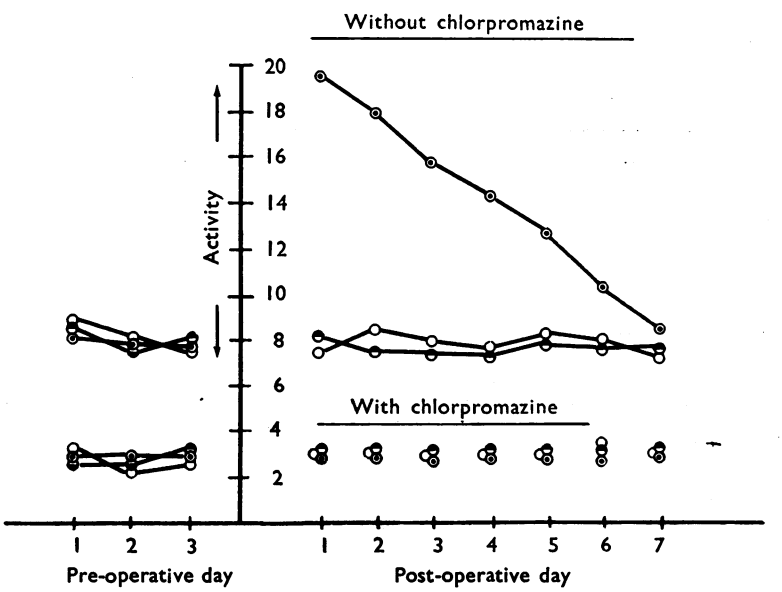

Fig. 1. Mean daily activity of rats before and after septal operation. Septally-operated rats ○——○; sham-operated rats ◓——◓; and normal rats ○——○. Ordinate: activity in arbitrary units. Abscissa: time in days before and after the operation. Upper three curves refer to untreated animals; lower three curves to chlorpromazine treated animals.

The "sham" operated rats, normal rating 7.93 (7.14 to 9.00), when treated with chlorpromazine, whether pre-operatively or post-operatively, had their activity ratings reduced to 3.05 (2.58 to 3.57). It was striking that the activity ratings of all animals, whether operated or not, were reduced to a value of about 3 by the administration of 8 mg/kg of chlorpromazine. These results are summarized in Fig. 1.

Statistical evaluation. The results were examined statistically for significant differences beyond the 0.01 level of confidence. There was a significant difference between the septally-operated group, the control operated and normal groups before treatment with chlorpromazine ; there was no significant difference between operated control and normal groups, and no significant difference between any of the groups after being treated with chlorpromazine.

TABLE 1

SUMMARY OF A LINDQUIST TYPE I "MIXED" DESIGN ANALYSIS OF VARIANCE OF THE ACTIVITY OF NORMAL, OPERATED CONTROL AND SEPTAL HYPER-ACTIVE ANIMALS

* Significant at the 1% level of confidence

Source	df	Sums of squares	Mean square (variance)
Between rats	71	368·79	
B	2	367·29	183·65*
Error (b)	69	1·50	0·02
Within rats	72	2,292·72	
A	1	1,835·77	1,835·77*
AB	2	366·18	183·09*
Error (w)	69	90·67	1·31
Total	143	2,661·51	

As the significant F-ratio demonstrated significant difference between groups, within groups, and interaction (Table 1), the t test for "critical difference" d was applied for indication of difference among means. The formula by which d is calculated is:

$$d = t\sqrt{\frac{2MS\,(v)}{n}}$$

where the "critical difference" is determined by multiplying t at a certain level of confidence and degrees of freedom by the square root of twice the mean square variance, divided by n, the number of subjects.

Histological studies. Microscopic examination of the brains of rats in the group in which the septum had been destroyed surgically showed extensive damage to the septal region. Massive damage was sustained by the precommissural portion of the septum, and heavy damage sustained by the supracommissural portion. In many instances the post-commissural portion was undamaged, leaving intact the post-commissural fornix system. The anterior commissure was always undamaged.

DISCUSSION

Our results confirm the findings of Brady & Nauta (1953), King (1958) and King & Meyer (1958) that septal destruction causes an increase in affective behaviour

and emotionality, and are in agreement with the conclusions of Hunt (1957) that chlorpromazine depresses the activity of normal rats and those with damage in the septal region.

If it is assumed that the septal area functions as a " quiescent " area or is related to the function of calming, it is of interest to inquire into the basic anatomical design of the septal area and to seek the system with which it is in functional balance.

The septal area is thought to have as its function, or one of its functions, a type of depression usually termed emotional depression. With destruction of the septal nuclei, there is an immediate and violent increase in activity, possibly due to a mechanism in the rat which parallels emotionality in the human. With the passage of time, a release phenomenon seems to occur, and the animal gradually returns to a near-normal state. Our results do not support the view that there is any localization of depressive function within the septal nuclei even though the nuclei exist as a discrete, accurately localized histological entity.

The rats used did not return to a completely normal state of activity during the period of observation up to six weeks, so it is highly probable that the septal nuclei do play some, but not a very important, role in " emotional quiescence." Our results suggest that the septal nuclei are a " bridge " over which emotional impulses normally pass. With destruction of this " bridge," the impulses are gradually re-routed until a near normal state is achieved.

The drastic increase in activity after destruction of the septal nuclei could be due to a functional imbalance in the mechanism of the animal's emotionality. It would seem, then, that there is a discrete area, or groups of areas, which are concerned with the function of stimulation of the animal. In recent years many workers have postulated that the amygdaloid complex of nuclei is most likely to be the area that is in functional balance with the septal nuclei (Brady, Schreiner, Geller & Kling, 1954 ; Karli, 1956 ; Schreiner & Kling, 1953 ; Woods, 1956 ; King & Meyer, 1958). This being so it follows that the former area is highly stimulatory in function, and that its ablation would cause profound depression, an effect found by several authors (Brady *et al.*, 1954 ; Karli, 1956 ; Schreiner & Kling, 1953 ; Woods, 1956). Depression of the amygdaloid by chlorpromazine should then be an effective means to combat septal hyperactivity and could explain the lack of significant difference among all the groups treated with chlorpromazine.

By depressing the amygdaloid complex to a constant degree in all animals, a certain percentage of its stimulatory function would be disrupted. In all animals, this disturbance of function would be constant. In the normal and operated control animals, the depressant portion of the emotional cycle is functioning normally, so that the animals exhibit a rating level of about 8. The balance is absent in those whose septa are destroyed with the resulting rating, 1 day post-operative, of about 20. Both levels of emotional activity are presumably being caused by constant stimulation from the amygdaloid complex. The explanation for septal hyperactivity is simply a lack of functional balance. With depression of the amygdaloid complex in all animals, to a constant degree, emotional activity will drop to a constant level, for stimulatory activity is being equally suppressed in all animals.

This work also suggests that there is a possibility of a base line to which emotional activity may be lowered, and it is of interest to speculate whether the dose of 8 mg/kg of chlorpromazine intraperitoneally giving an activity rating of about 3 brought the rats to this base line of activity. This dose has been shown by Rothwacks (1958) to be the most active dose of chlorpromazine within the range from 2 mg/kg to 11 mg/kg. For this reason the present authors assumed that 8 mg/kg was the maximal effective dose, and would presumably depress the activity of the amygdaloid complex maximally.

The work reported here was undertaken by Jacob R. Raitt in partial fulfilment of the requirements for the Ph.D. degree of the Ohio State University during the tenure of a post-doctoral Fellowship MF-10,438 of the National Institutes of Health, United States Public Health Service.

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