EFFECTS OF PSYCHOTROPIC DRUGS ON HYPEREMOTIONALITY OF RATS WITH BILATERAL ABLATIONS OF THE OLFACTORY BULBS AND OLFACTORY TUBERCLES

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Abstract-The effects of psychotropic drugs on hyperemotionality of the rat with lesions in the olfactory system, including the olfactory bulbs and olfactory tubercles (O.B.-O.T. rat), were investigated, and compared with the neurotoxic effects of these drugs measured on rotarod performance of the mice with bilateral olfactory bulb ablations (O.B. mice). Chlorpromazine, reserpine and meprobamate inhibited the hyperemotionality at doses close to their neurotoxic dose. Pentobarbital showed only a slight effect on the hyperemotionality at subhypnotic doses. Chlordiazepoxide, diazepam and haloperidol markedly inhibited the hyperemotionality at lower doses without causing neurotoxicity. Imipramine and amitriptyline selectively inhibited mouse-killing behavior (muricide) of the O.B.-O.T. rat without affecting the other hyperemotional responses to various stimuli, thus differing from tranquilizers. The mode of action of these drugs in the O.B.-O.T. rat was essentially the same as observed in either the O.B. and the septal rats. For evaluating the effects of psychotropic drugs, the O.B.-O.T. rats are superior to the O.B. and septal rats, as they share both offensive aggression of the O.B. rat and hyperreactivity of the septal rat, and furthermore they exhibited muricide in a much higher incidence soon after the brain lesions.

For evaluating the taming effect of psychoactive agents, a number of investigators have attempted to utilize aggressive behavior induced by various experimental procedures in laboratory animals. Well known models of aggressive behavior used in drug tests are fighting behavior of mice induced by either electric foot shock (1) or long-term isolation (2), hyperemotionality of the rat with lesions either of the septum (septal rat) (3–9) or of the olfactory bulbs (O.B. rat) (8–17), aggressiveness of some spontaneous killer rats (18, 19) and of long-term isolation rats (7, 20, 21) and so on. These models of aggressiveness have their respective characteristics and advantages in their usefulness for drug evaluations.

In the course of studies attempting to clarify neural mechanisms for the development of hyperemotionality following bilateral ablations of the olfactory bulbs in rats, the present authors found in a previous study (22) that lesions in the olfactory system, including the olfactory bulbs and olfactory tubercles caused a more marked hyperemotionality and muricide in a much higher incidence than those in either the septal or O.B. rats. It has also been shown that this aggressiveness developed soon after brain lesioning and remained unchanged at least for a month. It is therefore worthwhile to investigate the responses to

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drugs in rats with such lesions in the olfactory system, and make a comparison with those in the septal and O.B. rats.

The authors, in the present study, attempted to investigate the effects of various psychotropic drugs on hyperemotionality of the rats with such olfactory lesions, in the hope of evaluating the relevance of utilizing this model of aggression for drug screening.

**MATERIALS AND METHODS**

**Subjects:** Thirty-eight male Wistar-King A strain rats, weighing 200-250 g at the beginning of experiments, and forty-two male dd-K strain mice, weighing 20-25 g, were used. After inducing brain lesions, the rats were individually housed in cages with a wire mesh wall, while the mice were housed in plastic cages in a group of 5 to 6. The animals were given food and water ad libitum.

**Surgery:** Brain lesioning in the olfactory system of rats or mice was performed by the same procedures as described in previous studies (22, 23). Rats were anesthetized with sodium pentobarbital 40 mg/kg i.p. and placed on a stereotaxic instrument. The olfactory bulbs were exposed by trepanation of the skull just above the bulbs and were bilaterally removed by suction pump, thereafter the ablations were further enlarged approx. 6 mm posterior to the rostrum of the frontal cortex, 2 mm lateral to the midline and 1 mm deep from the ventral surface of the brain. The olfactory bulbs, anterior olfactory nuclei, medial olfactory tracts and olfactory tubercles are included bilaterally in such lesions. Such rats correspond to group IV in a previous study (22), and are referred to the O.B.-O.T. rats in the present experiments.

Mice were subjected to bilateral ablations of the olfactory bulbs by the same surgical technique used for rats but were not placed on a stereotaxic instrument (O.B. mice).

Penicillin 150,000 units was injected s.c. into rats and 30,000 units were given to mice, after surgery. Extent of the brain lesions was verified histologically at the conclusion of experiments by means of the frozen-tissue and hematoxin-eosine staining technique.

**Experimental procedures:** The measurement of hyperemotionality was carried out by scoring the responses of rats to 1) a mouse (attacking, biting and/or killing behavior), 2) a rod presented in front of the snout (attacking and/or biting responses), 3) light tapping on the back (jumping and/or startle responses), 4) pinching the tail with forceps (flight or escape) and 5) handling or capture (squeak), as described in the previous studies (8, 9, 22). The response in each item was graded as follows; 0: no reaction; 1: slight, 2: moderate, 3: marked and 4: extreme response.

Ten days after brain lesioning, the O.B.-O.T. rats which showed definite muricide within 3 min after a mouse had been placed into the cage and more than 100% increase in total emotionality score, were selected and used for the drug tests. Hyperemotionality, of the O.B.-O.T. rats was determined before, and 30, 60, 120, 180 and 240 min after the drug administration. When hyperemotionality was decreased by more than 50% of the pre-drug score, the taming effect was regarded as positive.

Neurotoxicity of drugs was determined by measuring the effects on rotarod perform-
ance of the O.B. mice according to the method of Dunham & Miya (24). The O.B. mice were placed on a slowly revolving wooden rod (3.5 mm in diameter, 12 rpm) at the time of peak effect. When the animals fell down from the rotarod more than once during a 2 min test period, neurotoxicity was defined as positive.

Groups of 5 rats or mice were used during the period of 10 to 60 days after surgery.

Drugs: The drugs used in this experiments were chlorpromazine hydrochloride, haloperidol, reserpine, meprobamate, chlordiazepoxide hydrochloride, diazepam, sodium pentobarbital, imipramine hydrochloride and amitriptyline hydrochloride. Drugs were dissolved in 0.9% saline, except for diazepam, haloperidol and meprobamate which were suspended in 0.5% CMC solution, and were injected i.p.

Statistical analyses: The ED50's of the taming effect and the 50% neurotoxic doses (NTD50s) were calculated by using the Weil's method (25), and intergroup comparisons of mean values were calculated according to the Student's t-test.

RESULTS

1. Effects of major tranquilizers

Chlorpromazine at doses of 5-10 mg/kg i.p. showed marked sedation and muscle relaxation 30-60 min after injection in the O.B.-O.T. rats. Haloperidol 1-2 mg/kg i.p. and reserpine 2-5 mg/kg i.p. exerted sedation and moderate catalepsy, without causing ataxia and muscle relaxation. Accompanying these behavioral changes, hyperemotionality score of the O.B.-O.T. rats was significantly decreased by chlorpromazine 5-10 mg/kg i.p., haloperidol 1-2 mg/kg i.p. and reserpine 2-5 mg/kg i.p. The time of peak effect was 1 hr for chlorpromazine, 2-3 hr for haloperidol and 4-5 hr for reserpine after administration (Fig. 3). The effects lasted for approx. 5-6 hr after chlorpromazine and over 6 hr

| Drug and dose (mg/kg i.p.) | A mouse | A rod | Jump | Flight | Squeak | Total |
|---------------------------|---------|-------|------|--------|--------|-------|
| Control (Saline)          | 4.0±0.0 | 3.4±0.2 | 3.6±0.4 | 2.8±0.5 | 1.2±0.2 | 15.0±0.7 |
| Chlorpromazine 2           | 2.0±0.6* | 3.4±0.2 | 3.4±0.2 | 2.6±0.4 | 1.4±0.5 | 12.8±0.9 |
| 5                         | 1.4±0.2** | 1.8±0.4** | 3.2±0.4 | 1.6±0.2 | 1.6±0.4 | 9.6±0.9** |
| 10                        | 1.0±0.3** | 1.4±0.2** | 2.0±0.3* | 0.8±0.4 | 7.2±1.1** |
| Control (Saline)          | 3.6±0.4 | 3.4±0.4 | 3.4±0.4 | 3.4±0.4 | 1.4±0.4 | 15.2±1.4 |
| Haloperidol 0.5           | 2.8±0.6 | 3.2±0.5 | 2.2±0.4 | 3.0±0.3 | 1.6±0.2 | 12.8±1.1 |
| 1                         | 1.8±0.6* | 1.8±0.6 | 1.4±0.2** | 2.4±0.5 | 1.8±0.4 | 9.2±0.5** |
| 2                         | 1.8±0.6* | 1.6±0.6* | 1.0±0.0** | 2.2±0.6 | 2.8±0.2* | 8.6±1.2** |
| Control (Saline)          | 3.6±0.4 | 3.2±0.5 | 2.8±0.4 | 3.4±0.4 | 1.2±0.2 | 14.2±1.0 |
| Reserpine 1               | 3.6±0.2 | 3.2±0.5 | 2.4±0.2 | 3.2±0.4 | 1.4±0.2 | 13.8±0.5 |
| 2                         | 3.2±0.4 | 1.6±0.2* | 2.4±0.2 | 2.4±0.5 | 1.0±0.0 | 9.8±0.7** |
| 5                         | 2.8±0.2 | 1.2±0.2** | 1.4±0.2* | 2.2±0.5 | 1.0±0.0 | 7.8±0.7** |

Each value represents mean±S.E. of the emotionality scores (N=5).

*p<0.05, **<0.01 : Significantly different from control.
after haloperidol and reserpine. The effects of these drugs at the peak time on each item of the hyperemotional responses to various stimuli are shown in Table 1. All items of the responses to given stimuli except for the squeak response, were similarly inhibited by these drugs.

| Drug and dose (mg/kg i.p.) | Attack (A mouse) | Attack (A rod) | Jump | Flight | Squeak | Total |
|----------------------------|-----------------|----------------|------|--------|--------|-------|
| Control (Saline) 10         | 3.8±0.2         | 2.8±0.7        | 1.4±0.2** | 2.0±0.5* | 1.2±0.2** | 0.4±0.2* | 7.4±1.2** |
| Chlordiazepoxide 5          | 2.4±0.7         | 3.2±0.4        | 2.4±0.2  | 2.0±0.4 | 0.6±0.2 | 10.6±1.7 |
| 10                         | 2.8±0.7         | 2.8±0.5        | 2.4±0.2  | 2.0±0.3* | 0.6±0.2 | 10.6±1.7 |
| 20                         | 1.4±0.2**       | 2.0±0.5*       | 1.2±0.2**| 0.4±0.2*| 4.0±0.2**| 7.4±1.2**|

Each value represents mean±S.E. of the emotionality scores (N=5).

* p<0.05, ** p<0.01: Significantly different from control.

**Fig. 1. Effects of diazepam on hyperemotional responses to given stimuli in the O.B.-O.T. rats.**
2. Effects of minor tranquilizers

The O.B.-O.T. rats showed marked sedation and ataxia with muscle relaxation following i.p. administration of diazepam 5-10 mg/kg, chlordiazepoxide 20 mg/kg and meprobamate 100-200 mg/kg. The hyperemotionality of the O.B.-O.T. rats was also markedly reduced by these drugs. The time of peak effect of these drugs was approx. 1 hr after administration. The scores in each test item of emotionality were similarly decreased as is shown in Table 2. In order to visualize such a pattern of drug action, the effect of diazepam on each emotional response is illustrated as a histogram in Fig. 1. This figure clearly indicates that diazepam inhibits all 5 emotional responses to the same degree and thus causes a marked decrease in total emotionality score.

3. Effect of pentobarbital

Pentobarbital, at a dose of 20 mg/kg i.p., caused a loss of righting reflex lasting for 10 to 30 min in 3 out of 5 rats, and produced marked inhibition of hyperemotionality of the O.B.-O.T. rats. The rats, however, recovered their normal righting reflex 1 hr after administration, though a staggering gait remained. The taming effect of pentobarbital in the O.B.-O.T. rats was very slight at subhypnotic doses of 10-15 mg/kg (Table 3).

Table 3. Effect of pentobarbital on the hyperemotional responses to given stimuli in the O.B.-O.T. rats.

| Drug and dose (mg/kg i.p.) | Attack | Jump | Flight | Squeak | Total |
|---------------------------|--------|------|--------|--------|-------|
|                           | A mouse| A rod|        |        |       |
| Control (Saline)          | 3.8±0.2| 3.4±0.4| 3.2±0.5| 3.0±0.3| 1.4±0.2| 14.8±1.1|
| Pentobarbital 10           | 3.0±0.6| 3.2±0.5| 3.0±0.4| 3.8±0.3| 1.0±0.0| 13.2±1.2|
| 15                        | 2.4±0.5*| 3.0±0.6| 2.6±0.6| 2.6±0.2| 1.0±1.0| 11.6±1.1|
| 20                        | 1.4±0.7**| 1.4±0.6*| 1.6±0.7| 1.4±0.5| 0.6±0.2| 6.4±2.2**|

Each value represents mean±S.E. of the emotionality scores (N=5). * p<0.05, ** p<0.01: Significantly different from control.

4. Effects of antidepressants

The administration of imipramine and amitriptyline even at a dose of 40 mg/kg i.p. produced only slight changes in total emotionality score of the O.B.-O.T. rats as shown.

Table 4. Effects of antidepressants on the hyperemotional responses to given stimuli in the O.B.-O.T. rats.

| Drug and dose (mg/kg i.p.) | Attack | Jump | Flight | Squeak | Total |
|---------------------------|--------|------|--------|--------|-------|
|                           | A mouse| A rod|        |        |       |
| Control (Saline)          | 4.0±0  | 3.8±0.2| 3.2±0.5| 2.8±0.6| 1.0±0 | 14.8±0.7|
| Imipramine 20             | 1.6±0.4**| 3.4±0.2| 3.2±0.6| 2.8±0.5| 1.6±0.2| 12.6±1.5|
| 40                        | 1.8±0.6**| 2.6±0.4*| 3.0±0.3| 3.2±0.4| 2.2±0.4*| 12.8±1.2|
| Control (Saline)          | 3.8±0.2| 3.6±0.2| 3.2±0.4| 3.4±0.4| 1.0±0 | 15.0±0.9|
| Amitriptyline 20          | 1.2±0.2**| 2.6±0.5| 3.0±0.5| 3.4±0.4| 1.4±0.2| 11.6±1.4|
| 40                        | 1.4±0.2**| 2.8±0.6| 2.6±0.6| 3.0±0.4| 1.0±0 | 10.8±1.4|

Each value represents mean±S.E. of the emotionality scores (N=5). * p<0.05, ** p<0.01: Significantly different from control.
in Table 4, although a slight respiratory depression was observed. It was observed that both imipramine and amitriptyline significantly inhibited the attack and killing response to a mouse, without affecting the other emotional responses to given stimuli in the O.B.-

![Graph](image)

**AMITRIPTYLINE**
- : control
- : 20 mg/kg i.p.
- : 40 mg/kg i.p.

**Fig. 2.** Effects of amitriptyline on hyperemotional responses to given stimuli in the O.B.-O.T. rats (N=5).

![Graph](image)

**Fig. 3.** Time course of effects of various psychotropic drugs on the hyperemotional responses to given stimuli in the O.B.-O.T. (N=5).
- : total emotionality score.
- : control (saline), ▲-▲ : chlorpromazine 10 mg/kg i.p., ×—× : reserpine 5 mg/kg i.p., ○—○ : diazepam 10 mg/kg i.p., ∧—∧ : pentobarbital 15 mg/kg i.p., ●—● : imipramine 40 mg/kg i.p.
O.T. rats (Table 4). Such a pattern of the effect of amitriptyline is clearly shown as a histogram in Fig. 2. The squeak response was rather increased by imipramine, while it was not altered by amitriptyline. The time course of the effects of chlorpromazine, reserpine, diazepam, pentobarbital and imipramine on hyperemotionality of the O.B.-O.T. rats is illustrated in Fig. 3.

The ratio of 50% neurotoxic doses (NTD50s) to the taming ED50's for all drugs is summarized in Table 5. The ED50's of the taming effect were calculated from decreases in the total emotionality score of the O.B.-O.T. rats. Haloperidol, chlordiazepoxide and diazepam inhibited the hyperemotionality of the O.B.-O.T. rats at significantly smaller doses than neurotoxic doses. The taming ED50's of chlorpromazine, reserpine, meprobamate and pentobarbital were close to the neurotoxic doses. Imipramine and amitriptyline were ineffective on the hyperemotionality as far as measured on the basis of total emotionality score.

**DISCUSSION**

In the present investigation, anti-aggressive effects of psychotropic drugs were examined in the O.B.-O.T. rat. Drug effects were tested on the score in each item of the hyperemotional responses to various stimuli, and the ED50's were calculated from decreases in total emotionality scores.

In order to determine specificity of selectivity of the drug effect, it is necessary to compare the dose inducing anti-aggressive effect with that causing behavioral toxicity or neurotoxicity, such as muscular weakness, motor incoordination etc. For practical purposes, impairment of rotarod performance has been rather commonly employed as an index for neurotoxicity of psychotropic drugs, although the relevance has not theoretically been verified (5, 7, 21).

In the present study, therefore, the effects of drugs on rotarod performance in mice were examined to determine the neurotoxic dose (NTD) and the ratio of the anti-aggressive
ED50 to the NTD50 was calculated for each drug (Table 5). In the present study, the mice with bilateral ablations of the olfactory bulbs (O.B. mice) were used to measure the neurotoxic effects of drugs. The O.B. mice were regarded as being more suitable than intact animals for this experiment, as the susceptibility to convulsion induced by electroshock as well as by certain CNS stimulants was found to be markedly changed following bilateral ablations of the olfactory bulbs of mice (23).

Major tranquilizers, such as chlorpromazine, haloperidol and reserpine markedly reduced hyperemotionality of the O.B.-O.T. rat and the emotionality scores in each test item, except the squeak response, were similarly decreased at relatively lower doses. However, the anti-aggressive ED50's of major tranquilizers were not significantly different from the NTD50s, with the exception of haloperidol. This may indicate that the anti-aggressive effect of these drugs is not so specific.

Chlordiazepoxide and diazepam inhibited the hyperemotionality at doses significantly lower than the neurotoxic dose. This may indicate that the taming effect of these drugs is relatively specific. Meprobamate showed the same effect on the hyperemotionality, only at doses close to the neurotoxic dose. These minor tranquilizers similarly reduced the emotionality scores in all items. Pentobarbital showed only a slight effect on the hyperemotionality at subhypnotic doses, and the difference between the anti-aggressive ED50 and the NTD50 was very little.

Imipramine and amitriptyline caused a slight inhibition of the hyperemotionality of the O.B.-O.T. rat even at larger doses, when measured from the total emotionality score. However, these drugs selectively suppressed the attack and killing response to a mouse, without affecting other responses to given stimuli. Horovitz et al. (20) and Sofia (7, 21) also reported that the muricide of the rat induced by long-term isolation was selectively blocked by antidepressants, antihistaminics and certain CNS stimulants at doses much lower than the neurotoxic doses. Such a selective antimuricidal effect of antidepressants was observed as well by the authors in the O.B. rats (9).

These effects of various psychotropic drugs on the hyperemotionality of the O.B.-O.T. rats were quite similar to those in both O.B. rats and septal rats. The O.B.-O.T. rat would therefore be just as useful for evaluating the effect of psychotropic drugs, as are the septal and O.B. rats.

As described in a previous study (22), the O.B.-O.T. rat shares both offensive aggression which is characteristic to the O.B. rat and hyperreactivity which is characteristic of the septal rat (8), and the total emotionality score of the O.B.-O.T. rat is thus generally higher than that of either the O.B. or the septal rat. The O.B.-O.T. rats may be superior to the septal rats from the fact that they demonstrate muricide which is rarely observed in the latter. This display of behavior is useful in detecting the selective inhibitory effect of antidepressant drugs. The O.B.-O.T. rats may also be superior to the O.B. rats in that they show more marked hyperreactivity than the latter. This makes the test of taming effect of tranquilizers much easier. Furthermore, the O.B.-O.T. rat develops hyperemotionality soon after brain lesioning and exhibits a much higher incidence of muricide than...
the O.B. rat. It is therefore concluded that the O.B.-O.T. rat has many advantages over the septal and O.B. rats for utilization in evaluating psychotropic drug effects.

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REFERENCES

1) Tedeschi, R.E., Tedeschi, D.H., Mucha, A., Cook, L., Mattis, P. and Fellows, E.J.: J. Pharmacol. exp. Ther. 125, 28 (1959)
2) Yen, C.Y., Stanger, L. and Millman, N.: Arch. int. Pharmacodyn. Thér. 123, 179 (1959)
3) Azuma, N.: Folia pharmacol. japon. 60, 259 (1964) (in Japanese)
4) Brady, J.V. and Nauta, W.J.H.: J. comp. physiol. Psychol. 46, 339 (1953)
5) Horovitz, Z.P., Furgiuele, A.R., Brannick, L.J., Burke, J.C. and Craver, B.N.: Nature 200, 369 (1963)
6) King, F.A. and Meyer, P.M.: Science 128, 655 (1958)
7) Sofia, R.D.: Life Sci. 8, 705 (1969)
8) Ueki, S., Nurimoto, S. and Ogawa, N.: Folia psychiat. neurol. japon. 26, 227 (1972)
9) Ueki, S., Nurimoto, S. and Ogawa, N.: Folia psychiat. neurol. japon. 26, 245 (1972)
10) Douglas, R.L., Isaacson, R.L. and Moss, R.L.: Physiol. Behav. 4, 379 (1969)
11) Kumada, N., Hitomi, M. and Kumada, S.: Japan. J. Pharmacol. 17, 659 (1967)
12) Mallick, J.B.: Physiol. Behav. 5, 679 (1970)
13) Mallick, J.B., Sofia, R.D. and Goldberg, M.E.: Arch. int. Pharmacodyn. Thér. 181, 459 (1969)
14) Myer, J.S.: J. comp. physiol. Psychol. 58, 112 (1964)
15) Ueki, S. and Sugano, H.: Abst. 23rd Internat. Congress of Physiol. Sci. 457 (1965), Tokyo
16) Vergnes, M. and Karli, P.: Compt. Rend. Soc. Biol. 157, 1061 (1963)
17) Watson, J.B.: Psychol. Rev. Monogr. Suppl., No. 33, 43 (1907)
18) Karli, P.: Behavior (Leiden) 10, 81 (1956)
19) Karli, P. and Vergnes, M.: Compt. Rend. Soc. Biol. 159, 754 (1965)
20) Horovitz, Z.P., Piala, J.J., High, J.P., Burke, J.C. and Leaf, R.C.: Int. J. Neuropharmacol. 5, 405 (1966)
21) Sofia, R.D.: Life Sci. 8, 1201 (1969)
22) Nurimoto, S., Ogawa, N. and Ueki, S.: Japan. J. Pharmacol. 24, 175 (1974)
23) Araki, Y. and Ueki, S.: Japan. J. Pharmacol. 22, 447 (1972)
24) Dunham, N.W. and Miyata, T.S.: J. Am. pharm. Ass. (Sci. Edn) 46, 208 (1957)
25) Weil, C.S.: J. biometric Soc. 8, 249 (1952)