Pharmacology Biochemistry & Behavior, Vol. 29, pp. 681–686. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Effect of Olfactory Bulbectomy and Chronic Amitryptiline Treatment in Rats. ^{3}H-Imipramine Binding and Behavioral Analysis by Swimming and Open Field Tests

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Received 9 February 1987

STOCKERT, M., J. SERRA AND E. DE ROBERTIS. *Effect of olfactory bulbectomy and chronic amitryptiline treatment in rats. ^{3}H-Imipramine binding and behavioral analysis by swimming and open field tests.* PHARMACOL BIOCHEM BEHAV **29**(4) 681–686, 1988.—An 'animal model' of depression, based on bulbectomy, followed by chronic treatment with amitryptiline was used in rats. In the synaptosomal membranes of the cerebral cortex plus hippocampus, the number of binding sites for ^{3}H-imipramine increased significantly when bulbectomy was associated with the antidepressant. In the bulbectomized rats the tendency was toward a decrease in binding. The treatment with 0.2% Triton X-100 of the membranes revealed a large increase in postsynaptic sites in the bulbectomized treated rats. The behavioral parameters analyzed by the swimming with a water wheel and the open field test revealed a series of differences in the various groups of rats, with respect to handling, bulbectomy and antidepressant treatment. Handling resulted in an increase in swimming time in controls, while bulbectomy reduced this parameter. In both the swimming and open fields tests, chronic bulbectomy reduces the motility of the rat. In control rats chronic amitryptiline increases locomotion and exploratory activity, a behavioral effect that is even more prominent in bulbectomized treated rats.

Animal depression ^{3}H-Imipramine binding Swimming test Open field test Olfactory Bulbectomy Amitryptiline

THE many neuroanatomical connections of the olfactory bulb with the limbic system suggest that, physiologically, it may influence emotional aspects of behavior. In the rat, bilateral bulbectomy produces a characteristic syndrome which includes hyperphagia, decreased REM sleep, altered thermoregulation, as well as a slower reaction toward aversive stimuli and lower learning capacity [4]. This syndrome has been considered as a model of animal depression particularly because, as occurs in human depression, it can be reversed by a chronic treatment with antidepressants such as amitryptiline and mianserin. Because of these properties the model has been successfully used for screening drugs having antidepressant activity [3, 13, 22]. Among the several neurochemical alterations produced by bulbectomy are a reduction in cholinergic and opioid receptors [8], and an increase in adrenoceptors in certain brain regions [9]. Regarding the binding of the antidepressant ^{3}H-imipramine to neural membranes, an increase in the number of sites has been reported in the midbrain, together with a decrease in the pons and hippocampus [10].

To obtain information about the possible site of the action of antidepressants, previous work from our laboratory has concentrated on the localization of ^{3}H-imipramine binding in relation to the synaptic region. Using a low concentration of Triton X-100, which dissolves the presynaptic membrane, whilst leaving intact the postsynatic membrane [5], we observed that ^{3}H-imipramine binding sites were localized in both synaptic membranes [19]. This finding was confirmed by the use of the neurotoxin 5–7 dihydroxytryptamine, that selectively destroys serotoninergic axons and nerve endings, leaving intact the postsynaptic sites [20]. These observations were suggestive that imipramine not only has a presynaptic role on the uptake of serotonin, but has also a postsynaptic action.

In the present work we have used bilateral bulbectomy in the rat, as well as chronic treatment with amitryptiline, and we have analyzed the effects produced on ^{3}H-imipramine binding to pre- and postsynaptic membranes and on certain behavioral parameters. In the first case we studied the changes in ^{3}H-imipramine binding in synaptosomal membranes of the cerebral cortex. In the second we used open field activity [11] and forced swimming with a water wheel, a test of 'biological despair' originally proposed for mice [18]. Several behavioral changes referring to exploratory activity,

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latency, rearing and grooming, as well as in swimming behavior were observed. In addition, a net increase in the number of postsynaptic ^{3}H-imipramine binding sites was found in bulbectomized rats after chronic amitryptiline treatment.

METHOD

Experimental Procedures

A total of 70 female Wistar rats of 200–250 g were used. Of these 35 were anesthetized with pentothal (32 mg/kg); the skull was exposed and a hole of 2 mm was perforated bilaterally 7 mm anterior to the bregma, using a dental type of trephine. The olfactory peduncles were transected with a microknife and the olfactory bulbs were destroyed mechanically. The bulbectomized rats were divided into 3 groups: B, B_A and B_S. B are bulbectomized rats without further treatment; B_A were injected daily IP with 12 mg/kg amitryptiline for 30 days, B_S received injections of saline for the same period of time. Both treatments were started one day after surgery.

Control groups were 15 sham operated rats without bulbectomy, 7 rats injected with the antidepressant (C_A); 6 rats injected with saline (C_S); and 14 rats control were sacrificed without any treatment (C). The C and B groups represented rats that had no handling during the time of the experiment, while all the other groups were submitted to handling, (i.e., weighing and injections) a variable that could be analyzed, both in the biochemical and behavioral experiments. All rats were sacrificed by decaptitation, 32 days after the initiation of the experiments.

Subcellular Fractionation

The brains were removed and the cerebral cortices and hippocampi of the various groups of rats were immediately dissected on ice. The tissue was homogenized in 0.32 M sucrose at 10% (w/v) using a glass homogenizer with rather loose teflon pestle. To obtain the primary fractions (nuclear and crude mitochondrial), the synaptosomes and synaptosomal membranes, we used the procedures previously described [19]. The synaptosomal membranes, resuspended in distilled water pH 7.0, were centrifuged at 100,000×g for 10 min. The pellet was resuspended in a buffer containing 50 mM Tris base, 120 mM Na Cl, 5 mM KCl and adjusted with HCl to pH 7.2. In this suspension the protein concentration was determined with the Folin reagent [14].

The membrane suspension was divided into two aliquots: one in which the ^{3}H-imipramine binding was determined directly, the other in which the membranes were treated with 0.2% Triton X-100 and were washed in distilled water and centrifuged as above, to eliminate the detergent. These membranes were resuspended again in the buffer for a new protein assay.

Binding of ^{3}H-Imipramine

The binding of ^{3}H-imipramine (50 Ci/mmol, NEN) to control and Triton X-100 treated membranes was carried out by centrifugation. The incubation was done in conic plastic tubes of 1.7 ml, in 500 μl of buffer using for each assay 0.1 mg protein and ligand concentrations between 0.7 to 12 nM. The tubes were incubated at 0°C for 60 min and the nonspecific binding, which represented 25–30% of the total, was calculated in the presence of 300 μM nortryptiline. After centrifugation at 13,000 rpm for 10 min the supernatants were discarded, the pellets were washed superficially with cold buffer and dissolved with 100 μl of Protosol overnight. After addition of 1.6 ml of scintillation fluid, the tubes were put into vials and counted in a Tracor Spectrometer. All assays were done by duplicate and at least 3–4 determination were done for each point in the saturation curve.

TABLE 1

SPECIFIC ^{3}H-IMIPRAMINE BINDING TO SYNAPTOSOMAL MEMBRANES OF CEREBRAL CORTEX AND HIPPOCAMPUS BEFORE AND AFTER TRITON X-100 TREATMENT

Groups	Specific Activity	Percent Binding
C	2.76 ± 0.8	100
C_T	1.79 ± 0.2	64
C_A	2.24 ± 0.3	100
C_{AT}	1.30 ± 0.4	58
B_S	2.17 ± 0.9	100
B_{ST}	1.14 ± 0.8	52
B_A	4.60 ± 0.6	100
B_{AT}	3.69 ± 0.4	82

Specific activity, in pmol/mg protein, represents the Bmax of the high affinity site of ^{3}H-imipramine binding before and after 0.2% Triton X-100. The K_Ds were in all cases of about 3 nM. The values are the mean ± SEM of 3 to 4 independent experiments done by duplicate. Comparison between the groups gave the following statistical differences: C vs. B_A, $p<0.01$; B_A vs. B_S, $p<0.01$; B_A vs. B_{AT}, $p<0.05$; C_T vs. B_{AT}, $p<0.001$ (Student's t-test).

Swimming Test

The apparatus used was a water tank of Plexiglas with a water wheel in the center, similar to that described by Nomura *et al.* [18] for mice, but of larger dimensions (60×22×60 cm). The wheel of 7 cm in diameter offered a resistance of 20 g/200 g rat, which could be adjusted to fit the weight of the animal. The water, maintained at 25°C, was put into the tank to a height of 25 cm, with the paddle at the level of the water surface. Rats were brought to the room of the experiment one hour before the test. The rat was dropped into the tank and the time spent in swimming and the number of rotations were measured during 4 min (see Table 2). The swimming test was carried out between 10 a.m. to 4 p.m. on a randomized population of the various groups and was done 72–96 hr before the rats were sacrificed. The injection of the drug or vehicle were continued 3 to 4 hr after the test.

Open Field Test

The open field test was carried out on a square white surface of 60×75 cm divided into 20 squares of 15 cm and surrounded by a wall of 30 cm. The surface was illuminated indirectly with a diffuse light of low intensity. The rats were brought to the room one hour before the test and they stayed without external noise and a constant background noise of low intensity. The tests were carried out between 10 a.m. and 4 p.m. Each rat, taken at random between the groups, was placed in corner of the open field and two observers counted the number of squares run, the latency (i.e., the time spent immobile), the number of rearings, groomings and defecations. The test lasted 10 min and was done 24–48 hr be-

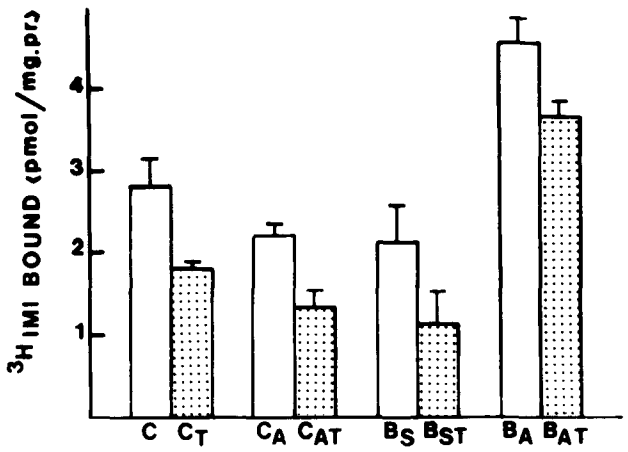

FIG. 1. Histograms representing the ^{3}H-imipramine (^{3}H-IMI) binding, in pmol/mg protein (Bmax), to synaptosomal membranes of cerebral cortex plus hippocampus. The bars marked T correspond to membranes treated with 0.2% Triton X-100. The results are the mean±SEM of 3–4 independent experiments done by duplicate. (Statistics of the data in Table 1.)

fore sacrifice. The administration of the drug or saline was continued 2–4 hr after the test. The floor of the open field was thoroughly cleaned after each test.

Statistics

The results of the ^{3}H-imipramine binding were analyzed using an EBDA program for one type of binding sites and adapted to an IBM PC computer [15]. The statistical significance was determined by the Student's *t* test. The results of the swimming and open field tests were statistically treated using a variance analysis (ANOVA).

RESULTS

^{3}H-Imipramine Binding to Synaptosomal Membranes

The binding of ^{3}H-imipramine to control and Triton X-100 treated synaptosomal membranes was done at a range of ligand concentration that only detects the high affinity site. In control membranes this site has a K_D of 3 nM and a Bmax of 2.76±0.8 pmol/mg protein (Table 1). Using higher ligand concentrations, we previously demonstrated the additional presence of a low affinity site with a K_D of 99 nM and Bmax of 14.2 pmol/mg protein [19].

Table 1 and the histograms of Fig. 1 show the results of ^{3}H-imipramine binding in the various groups of experimental rats. In each case they correspond to the Bmax both before and after the treatment of the synaptosomal membranes with 0.2% Triton X-100. Since the results of all the controls (C, C_S and the sham operated rats) did not show differences in ^{3}H-imipramine binding they were pooled together. In all groups the loss of protein by the detergent was about 30%.

In the Triton X-100 treated control membranes (C_T) there was a loss of 36% of ^{3}H-imipramine binding sites. In the control rats treated with antidepressant (C_A) there was a reduction in ^{3}H-imipramine binding, however, the results were not statistically significant. In the bulbectomized rats (B) and in those in which the bulbectomy was followed by saline (B_S) there was no difference in binding, although in both groups there was a small reduction in ^{3}H-imipramine binding with respect to control values. The most striking change was,

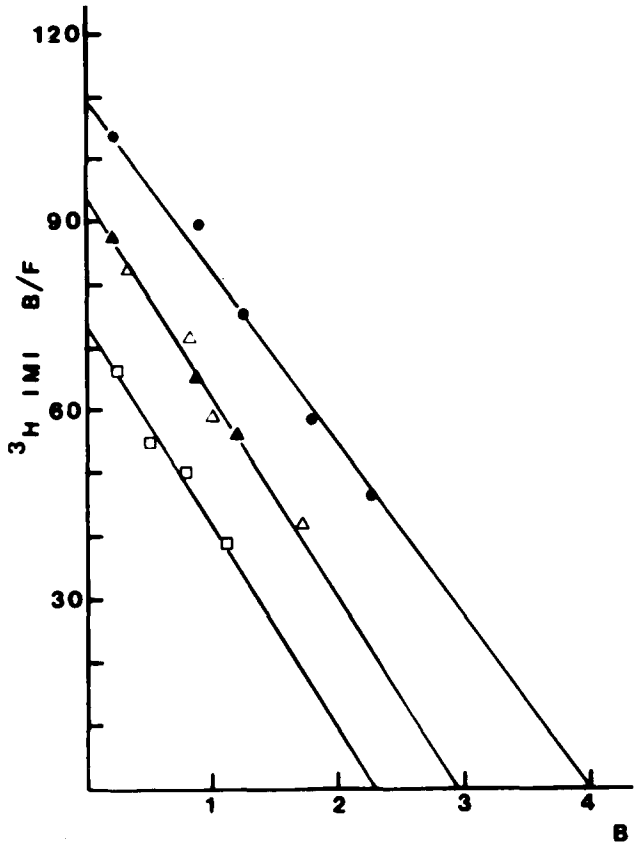

FIG. 2. Scatchard plots of selected saturations of experiments with ^{3}H-imipramine binding to synaptosomal membranes of the various experimental groups: △, C group; ▲, C_S group; ●, B_A group; □, B_S group. Observe the increase in Bmax in B_A and the decrease in B_S with respect to controls. Bmax for C 2.78 pmol/mg protein, correlation coefficient, Cr 0.97; for B_S 4 pmol/mg protein Cr 0.96; for B_S 2.3 pmol/mg protein, Cr 0.94.

however, the considerable difference in ^{3}H-imipramine binding in the group of bulbectomized rats treated with amitryptiline. In group B_A there is a large increase in ^{3}H-imipramine binding sites both in the synaptosomal membranes and in the remaining postsynaptic membranes after Triton X-100. Comparing C and B_A there is a 65% increase ($p<0.01$) in binding and between C_T and B_{AT} a 106% increase ($p<0.001$). The effect of the antidepressant treatment is even more evident when we compare these membranes with those of controls with the drug (C_A) or with bulbectomized rats with saline (B_S). An important point is that the Triton X-100 treatment of B_A produces a reduction of only 18% of binding sites compared with 36% in controls indicating an increase of postsynaptic binding sites in the bulbectomized treated rats.

Figure 2 shows Scatchard plots of the binding in bulbectomized rats (B_S), controls (C), controls with saline (C_S) and bulbectomized plus antidepressant (B_A). There are differences in Bmax between the groups without significant changes in K_D which is about 3 nM for all groups. It is also evident that the values of C and C_S coincide on the same line. The Hill plots obtained by the EBDA program gave values between 0.75 and 0.98 for the various experimental groups.

Swimming and Open Field Test

Table 2 shows the data obtained with the swimming test in

TABLE 2

BEHAVIORAL DATA OBTAINED WITH THE SWIMMING TEST IN THE VARIOUS GROUPS OF RATS

	Population Groups					
Variables	C	C_S	C_A	B	B_S	B_A
Turns	21	26	52	48	40	65
Swimming	105	200	71	98	83	65

Each number represents the mean of the values obtained for each group of 7 rats, regarding the number of turns and time of swimming in sec. In all cases the time of the experiment was of 4 min.

TABLE 3

BEHAVIORAL DATA OBTAINED WITH THE OPEN FIELD TEST IN THE VARIOUS GROUPS OF RATS

	Population Groups					
Variables	C	C_S	C_A	B	B_S	B_A
Squares	87	104	148	30	55	116
Latency	298	403	259	489	397	186
Rearing	51	34	37	33	27	46
Grooming	5	12	5	8	29	21

Each number represents the mean of the values obtained for each group of 7 rats and for each variable assayed in 10 min. Squares represent number of squares crossed; latency is expressed in sec, rearing and grooming, as numbers occurring in the time of the experiment.

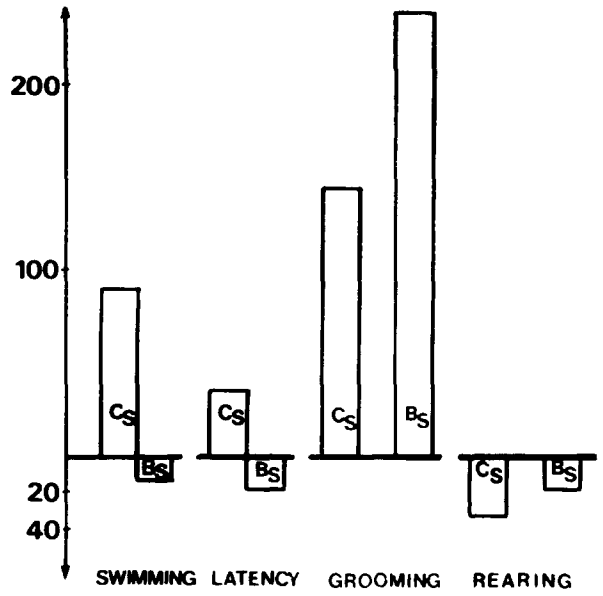

FIG. 3. Histograms representing the effect of handling in the various groups of rats. The results are expressed as percent increase or decrease with respect to groups C and B (without handling) taken as a basal line for each variable (for example, C_S is compared to C and B_S to B). Only statistically significant pairs ($p<0.01$ and $p<0.05$) are shown in Figs. 3, 4 and 5.

the various groups of rats. The number of turns of the wheel and the time spent in swimming, two inversely related parameters are recorded.

Table 3 shows the number of squares crossed, as an indication of locomotion activity. Latency corresponds to the time in which the rats remain immobile, generally near the walls of the open field [11]. Rearing corresponds to the number of times in which the rats stand and assume an exploratory attitude. It is interesting to mention that population B_A had a special behavior not found in all other groups. Put in the open field these rats had a kind of vocalization with the emission of 2 to 6 acute noises.

The comparative analysis of the different groups led to a complex set of pairs. For example, in the case of latency we compared populations C with C_S, C_S with C_A, B with B_S, B_S with B_A, C_A with B_A, C_S with B_S and C with B. Of a total of 84 comparable pairs, that were analyzed by ANOVA for statistical significance, 40 showed differences better than 1%, 7 better than 5% and 37 had no significant differences.

In Figs. 3, 4 and 5 we express, as percent variation, the pairs of rat populations that showed significant differences in the two behavioral tests. In them, to facilitate the interpretation we have selected respectively the effects of handling of the rats, of bulbectomy and of treatment with amitryptiline.

The effect of handling on the behavior of the rat is shown in Fig. 3, in this case the results are referred to groups C and B taken as the basal line. It may be observed that in C_S there is an increase in swimming time (90%), in latency (35%) and in grooming (144%), with respect to C and a decrease in rearing (−32%). On the other hand, in B_S there is a slight decrease in swimming time (−14%), in latency (−19%) and in rearing (−18%) with respect to B, while there is a large increase in grooming (238%).

The effect of bulbectomy on the behavior of the rat is shown in Fig. 4, in this case populations C, C_S and C_A were taken as the basal line. It may be observed that bulbectomy, produces a decrease in locomotion (−63%) and a corresponding increase in latency (55%); in other words, bulbectomized rats tend to show less displacement in the open field and stay longer time immobile. In bulbectomized rats submitted to handling (B_S) there is also a decrease in locomotion (−47%) and in swimming time (−59%), while they show an increase in the number of turns (52%). In the bulbectomized rats, treated with antidepressant (B_A), there is a smaller decrease in locomotion (−21%) and a decrease in latency time (−28%).

Figure 5 shows the statistically significant changes observed with the treatment with amitryptiline. In this case the basal line corresponds to populations C_S and B_S. In the swimming test it may be observed that the antidepressant produces an increase of 66% in the number of turns in controls rats (C_A) and of 69% in the bulbectomized rats (B_A), as compared with the respective controls. The inverse results are observed when considering the swimming time, there is a decrease of −64% in C_A and of −21% in B_A. The antidepressant treatment increases the locomotion in C_A by 42% and in B_A by a striking 112%. This change is accompanied by a reverse effect on the latency time (−36% in C_A and −53% in B_A). The grooming is also decreased in these two populations (−56% in C_A and −37% in B_A) and the rearing is increased only in population B_A (69%). Regarding the defecations, no differences were observed in the number of boluses.

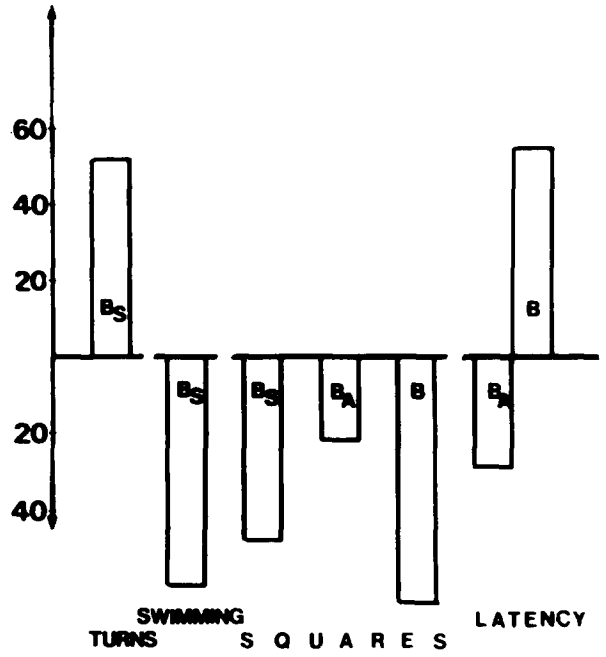

FIG. 4. Histograms representing the effect of bulbectomy in the various groups of rats. The results are expressed as percent increase or decrease with respect to C_S, C_A, and C taken as a basal line for each variable (for example, B_S is compared to C_S, B_A to C_A and B to C).

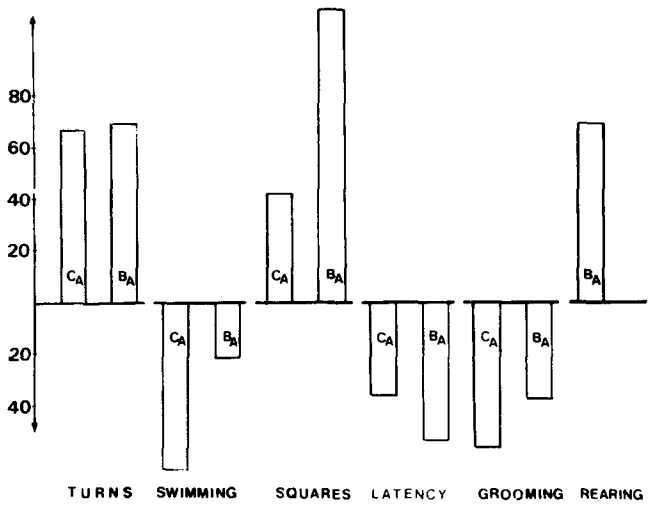

FIG. 5. Histograms representing the effect of the antidepressant treatment. The results are expressed as percent increase or decrease with respect to C_S and B_S taken as a basal line for each variable (for example, C_A is compared to C_S, B_A to B_S).

DISCUSSION

In the present work we have considered bulbectomy as an animal model of depression (reviews in [10,24]) and we have studied the effect of the chronic administration of amitryptiline on ^{3}H-imipramine binding to synaptosomal membranes and on certain behavioral tests. In addition, we have taken into consideration the effect of handling of the rat, as opposed to conditions in which naive controls or bulbectomized rats without any other treatment, were studied. This parameter is important to be considered since handling has marked neurochemical effects related to stress, particularly on the GABAergic system [2].

Interpretation of the biochemical and behavioral results is a very complex task specially if one tries to establish a correlation between ^{3}H-imipramine binding and the behavioral parameters analyzed. In attempting to present the data more clearly we have taken into consideration the three variables: handling, bulbectomy and antidepressant treatment that are illustrated in Figs. 3, 4 and 5.

Handling effect. Regarding the ^{3}H-imipramine binding we did not observe differences between the naive (C), the saline injected (C_S) and the sham operated rats and also between groups B and B_S. However, there was a different behavior of the rats that were naive, or bulbectomized 30 days before, when compared to those submitted to handling every day, by the injection of saline (Fig. 3). The action of handling on control rats resulted in an increase in swimming time compared to the naive rats; on the other hand, in the bulbectomized rats this behavioral parameter was reduced. Both in C_S and B_S, the effect of handling was also evident in the grooming.

Bulbectomy effect. After bulbectomy the number of ^{3}H-imipramine binding sites were reduced without change in affinity (Fig. 2); however, because of the dispersion of the values, the results were not statistically significant compared to the control group (Table 1). In spite of this, our observations tend to suppor the finding that ^{3}H-imipramine binding sites are reduced in the hippocampus of bulbectomized rats [16].

The behavioral effects of bulbectomy are observed not only in the B and B_S rats but also in those treated with the antidepressant (B_A). In the swimming and open field tests, chronic bulbectomy reduces the motility of the rat; while amitryptiline improves the situation by increasing the activity (Fig. 4). This finding agrees with that of Marks *et al.* [16] who, using an activity wheel, observed a decrease in motility after bulbectomy.

Antidepressant effect. When bulbectomy was associated with chronic amitryptiline there was a large increase in ^{3}H-imipramine binding sites in synaptosomal membranes (Fig. 1, Table 1), without changes in affinity (Fig. 2). These findings suggest that bulbectomized rats react differently than controls to the antidepressant and that an up regulation of ^{3}H-imipramine binding is produced when the two experimental effects are associated.

Early work from this laboratory had demonstrated that low concentrations of Triton X-100 (01–02%) produced the dissolution of the presynaptic membrane, with maintenance of the postsynaptic one. This finding was corroborated using electronmicroscopy, enzyme markers and binding studies [5], and led to a series of investigations about the localization of several central synaptic receptors (see [19]). Here we confirm that 0.2% Triton X-100 produces a reduction of ^{3}H-imipramine binding sites, not only in control membranes, but also in membranes of bulbectomized rats, or of rats treated with the antidepressant (Table 1). Most striking is however the finding that, in bulbectomized rats treated with amitryptiline, the reduction in ^{3}H-imipramine binding by Triton X-100 is significantly lower, suggesting an increase in the number of postsynaptic sites. The up regulation of

^{3}H-imipramine binding by amitryptiline, here observed, lends support to the interpretation of Aprison *et al.* [1], Fleisher *et al.* [6], Nagayama *et al.* [17] and of Hingtgen *et al.* [7] who postulated that the effect on depression of amitryptiline and other antidepressants, could be based on a postsynaptic action rather than on the presynaptic effect on serotonin uptake.

In relation to the behavioral effects of the antidepressants confirming previous findings of Katz and Hersh [11] and Vogel *et al.* [23], we observed that the chronic treatment with amitryptiline increases locomotion and exploratory activity. This effect is even more effective in the bulbectomized rats. Here the B_A group shows a more than 100% increase in squares crossed as compared to B_S (Fig. 5).

Of the behavioral data obtained, the only finding that seems to be correlated with the biochemical results is in the case of the bulbectomized rats treated with antidepressant. One is tempted to relate the increase in postsynaptic ^{3}H-imipramine binding sites with the improvement in locomotion and exploratory activity of these 'depressed' rats. However, we have to consider that the antidepressant treatment produces many other neurotransmitter and neuroreceptor changes at the synaptic junction and thus the effect may be very complex (see [21]). Studies along these lines seem worthwhile to further elucidate the molecular mechanism of depression and the action of antidepressants.

ACKNOWLEDGEMENTS

This work was supported by a grant from the CONICET of Argentina. We express our gratitude to Dr. C. Baratti for his advice on the behavioral experiments and for critically reading the manuscript.

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