LOCUS COERULEUS LESIONS AND AVOIDANCE BEHAVIOR IN RATS

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Abstract. Bilateral electrolytic lesions of the locus coeruleus were performed in rats. In comparison with controls, lesioned rats showed decreased acquisition of two-way avoidance response, less intertrial responses, longer latency of avoidance responses on the first day of training, and needed more trials to reach the extinction criterion. It is suggested that locus coeruleus, contrary to the ventral noradrenergic bundle, facilitates avoidance behavior in rats.

INTRODUCTION

In recent years, outstanding interest has been directed toward the possible role of brain noradrenergic (NA) neurons in conditioned behavior and self stimulation mechanisms (2, 4, 15, 24, 25). According to the Stein and Wise theory of two opposite systems (26, 27), catecholamines such as dopamine (DA) and NA are involved in a reward system, while acetylcholine and serotonin (5-HT) are involved in punishment system.

Noradrenergic neurons form at least two main fiber systems. The dorsal bundle (DB) originates mainly in the area corresponding to the nucleus locus coeruleus (LC), and the ventral bundle (VB) originates more heterogenously from NA cell groups in the ventral tegmentum (5).

The DB projects to the cerebral, hippocampal and cerebellar cortical areas as well as to the amygdala, thalamic and hypothalamic regions. The VB innervates a variety of basal brain structures associated with hypothalamus and limbic system (5, 16).

It has been suggested that Na released from neurons belonging to the LC is critically involved in the learning processes (10). According to Crow and co-workers (3, 4) NA released in the cortex stimulates synaptic processes which are necessary for initiating the transmission from a short- to a long-term memory system. This hypothesis is supported by some papers reporting impairment of acquisition of a food rewarded runway response after electrolytic lesion to the LC (2, 11, 23).

However, some observations seem to argue against the role of the LC and DB in learning mechanisms. It was reported that animals with chemical or electrolytic lesions to the LC or the DB, when showing an impairment in some types of conditioned responses, can still learn a wide variety of other conditioned tasks (18, 20, 21). Moreover, lesions to the DB produced significant resistance to extinction i.e., perseveration in responding to no longer reinforced stimuli (18–20). On the basis of similar results, Gray (8) suggested that DB is involved in the coding of "frustration" produced by omission of an expected reward. This hypothesis is supported by findings that omission of reward is correlated with the occurrence of hippocampal theta rhythm at the frequency of 7.7 Hz, and that lesions to the DB raise the threshold for this frequency (6, 7). Another explanation for the resistance to extinction in DB lesioned animals was proposed by Mason et al. (19, 21). This explanation is based on the observation that the number of stimuli connected with learned responses during acquisition training may determine the rate of extinction (17). In the brain there are mechanisms which select irrelevant stimuli and are responsible for habituation (17). Mason et al. (19, 21) suggested that DB may play a role in these processes and that lesions of this structure result in resistance to extinction due to an increase in the number of stimuli that the animal has sampled during training and have become attached to reward.

The present study proposed to determine the effect of bilateral lesions to the LC on avoidance acquisition, extinction and retention. The role of this structure in avoidance behavior is unclear and some contradictory results have been reported (11, 23). Recently we found (13) that electrolytic lesion of the second main NA system in the brain, the VB, surprisingly facilitated two-way avoidance acquisition in rats. We emphasized that brain NA neurons are not functionally homogenous and supposed that VB seems to play an opposite role to the DB in behavioral processes (9).

METHODS

Subjects. Twenty seven naive male Wistar rats, weighing 190–200 g at the start of the experiment, were used. Rats were housed in groups of 3–4 in macrolon cages (20 × 24 × 43 cm) with food and water freely available. The holding room was kept constant at 20 ∓ 2°C and a 12 h reversed light/dark cycle was maintained (light 7 : 00 p.m. to 7 : 00 a.m.). Then rats were randomly divided into 2 groups: 15 for LC lesions and 12 for sham operated control.

Surgical and histological procedures. Electrolytic lesions were performed under chloral hydrate anaesthesia (400 mg/kg i.p.) using a stereotaxic Stoelting instrument and stainless steel electrode (0.25 mm in diameter insulated except for the tip). The coordinates for LC were: P 1.6 mm, L 1.1 mm and H 2.5 mm above the interaural line (according to König and Klippel, 14). Lesions were made by passing d.c. current of 1.0 mA for 5 s. Operated control subjects (sham lesioned) were treated in the same manner, except that the electrode was not lowered into the brain. The lesions were made one week before the experiment started. At the end of the experiment the animals were killed by decapitation, their brain stems were placed in 10% formalin solution, and 15 μm sections were stained with haematoxylin and eosin. The placement and size of lesions were checked microscopically without knowledge of individual behavioral results.

Apparatus. Avoidance testing was performed in a shuttle box consisting of two identical compartments (24 × 24 × 76 cm) separated by a 8 cm high hurdle. The grid floor consisted of stainless steel rods 2.0 mm in diameter, spaced 1.5 cm apart. Illumination was provided by 25 W bulbs centered 30 cm above each compartment. An electric shock of approximately 2.0 mA was delivered through the grid floor of the compartments.

Behavioral procedure. On the first day of two-way avoidance training the animals were habituated to the shuttle box apparatus for 5 min. The conditioned stimulus (CS) was a light of 5 s duration, and the unconditioned stimulus was the electric footshock. The CS–UCS interval was 5 s, the intertrial interval averaged 30 s. Rats received 10 trials daily during 10 consecutive days or until the criterion 9 avoidance responses in two consecutive days was reached. When the avoidance criterion was established, extinction testing began, during which the CS was not reinforced. Initially the animals were subjected to 6 CS presentations without any possibility of making avoidance responses and than spontaneous extinction of avoidance was observed until the extinction criterion of 1 avoidance response on 10 trials was obtained. During

the next 2–3 days, rats were retrained to the previous avoidance criterion. Two weeks after the last retraining session, subjects that reached the retraining criterion level received a retention test. During avoidance conditioning and retention the following parameters were recorded: the number of avoidance responses for each session, the number of non-punished intertrial crossings between both compartments, and the latencies of avoidance and escape responses.

Statistical analysis. The latency data were analyzed by the Kolmogorov-Smirnov test. The remaining data were analyzed using the Mann and Whitney two tailed test.

RESULTS

Location of lesions. Histological examination showed that lesions bilaterally involved the LC. Usually lesions also partially destroyed the mesencephalic root nucleus of trigeminal nerve (Fig. 1). In 5 rats, lesions were not accurately positioned in the LC and these animals were excluded from the analysis of the results.
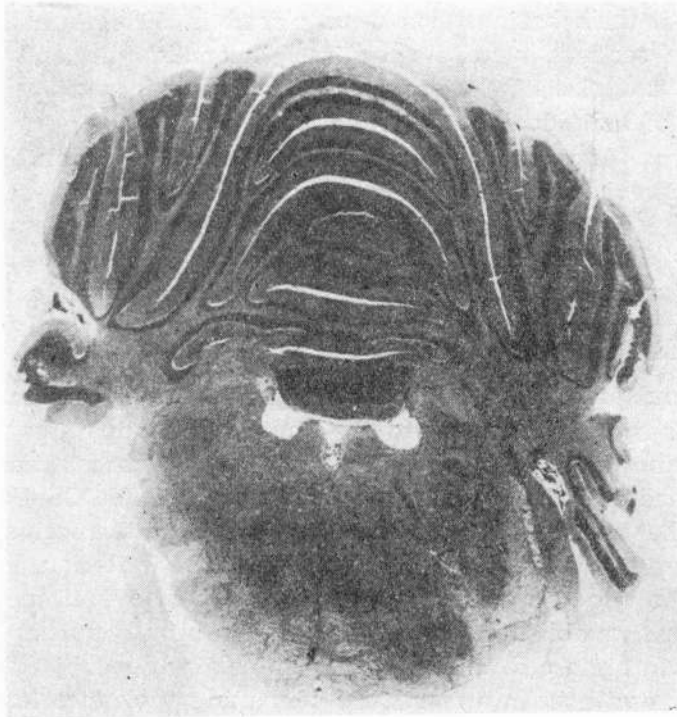

Fig. 1. Frontal section showing typical bilateral lesion involving the locus coeruleus.

Two-way avoidance. Lesions involving the LC significantly decreased the rate of acquisition of this task during first 5 days of training, (1st day: $U = 90.0$, $P = 0,05$; 2nd day: $U = 77.0$, $P = 0.005$; 3rd day: $U = 84.0$, $P < 0.025$; 4th day: $U = 78.0$, $P < 0.01$, 5th day: $U = 90.0$, $P = 0.05$; 6th day: $U = 91.0$, $P > 0.05$; 7th day: $U = 99.0$, $P > 0.05$). The number of intertrial responses was smaller in LC lesioned animals thant in control group, but the difference was not significant (Fig. 2).
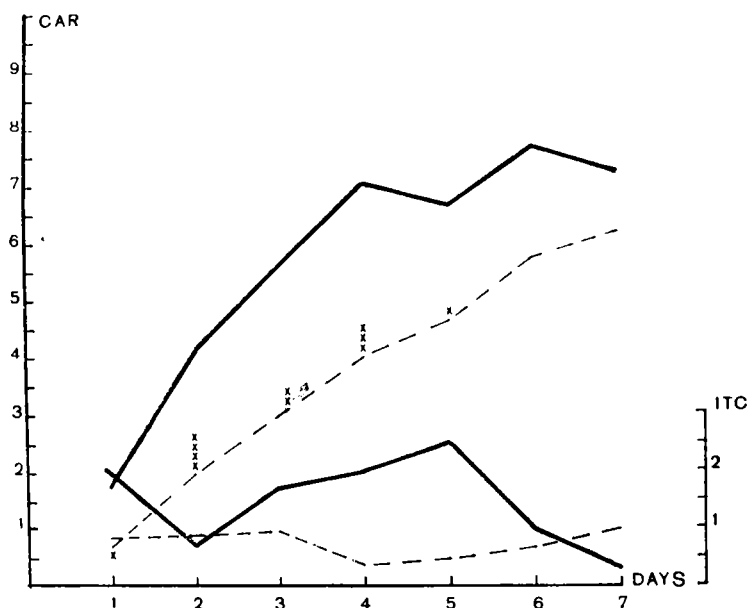

Fig. 2. Acquisition of two way conditioned avoidance response (CAR) and number of intertrial crossings (ITC) during avoidance sessions. Solid lines, sham operated rats; dashed lines, rats with lesioned locus coeruleus. Differences from sham operated rats: $x = P < 0.05$; $xx = P < 0.025$, $xxx = P < 0.01$, $xxxx = P < 0.005$. For number of rats see text.

The latencies of avoidance responses were prolonged in animals with lesioned LC. This effect was significant on the very first day of training, ($k = 1.418$ $P < 0.05$). The latency of escape reaction was also prolonged but this effect was not significant (Fig. 3).

Four rats with LC lesions and 3 sham operated control, did not obtain avoidance criterion and were rejected from futher experiment. It was left: 6 rats with lesions to the LC and 9 sham operated control animals. Rats with lesioned LC needed significantly more trials to reach the extinction criterion then sham operated animals, ($U = 64.5$ $P < 0.025$), (Fig. 4). One sham operated rat did not reach avoidance

criterion during retraining and was rejected from the retention test. There was, however, no differences in performance between both groups during retention test, ($U = 41.5$ $P > 0.05$), (Fig. 4).

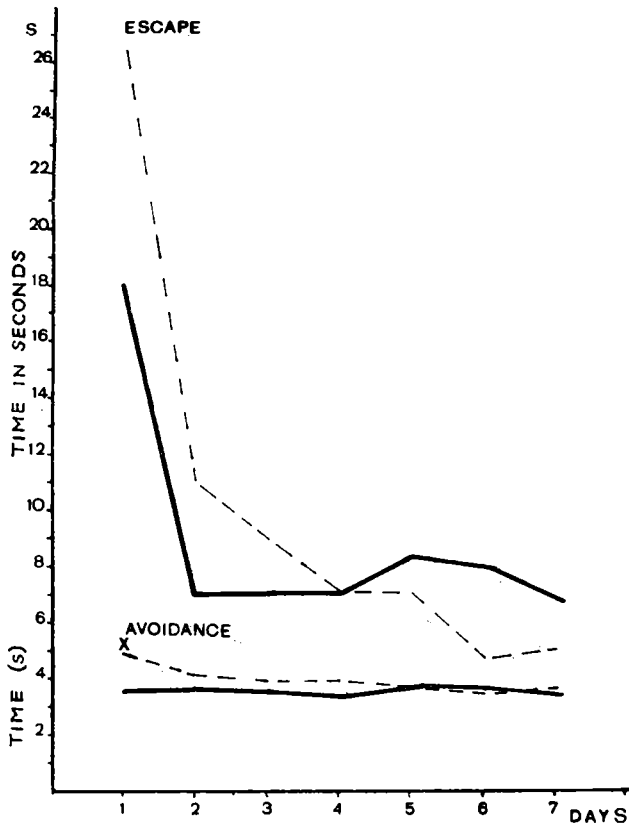Fig. 3. Latencies of avoidance and escape responses during training of two way avoidance in s. Other denotations as in Fig. 2.

DISCUSSION

Our results support the hypothesis of an involvement of the LC and DB in learning processes. The performance decrements on avoidance and appetitive tasks after electrolytic lesions to the LC have been observed by numerous investigators (2, 11, 23). There are, however, studies which do not confirm these findings (1, 19, 20). The most intriguing question arising from these conflicting data is of the essence of action of NA on the learning processes. Gray (6–8) suggested that this amine is involved in the coding of nonreward and that this mechanism is responsible for

retardation of extinction after destruction of the LC. Mason and Fibiger (19) proposed that in LC lesioned animals, the changes in affectional processes might lead to the retardation of extinction. According to these authors NA is important for the regulation of activity of brain structures selecting the incoming informations from the environment. Lesions of NA neurons should, therefore, lead to the disinhibition of these processes resulting in resistance to extinction.
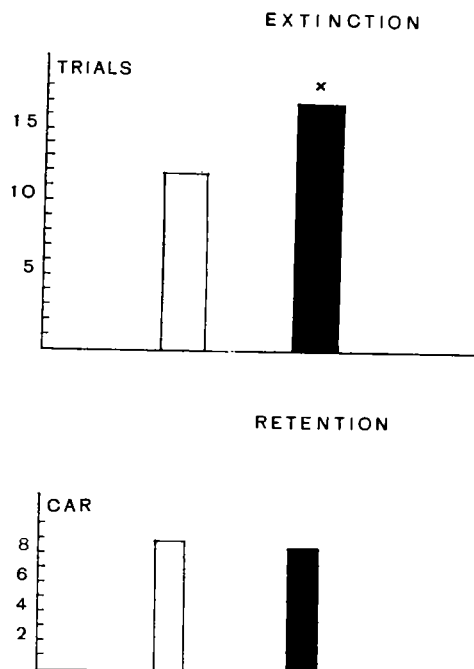

Fig. 4. Number of trials to the extinction criterion (upper) and performance of rats during the retention test (lower). White columns, sham operated rats; black columns, rats with lesioned locus coeruleus. Difference from sham operated rats: $x = P < 0.05$.

The possible involvement of NA in emotional processes may also explain some effects observed in LC lesioned animals. This problem may be discussed in term of reward and punishment theory, according to which NA neurones are involved in positive reinforcement and 5-HT neurones in punishment — i.e., negative reinforcement. It should be mentioned that the decrease in activity of NA neurones leads to increased activity in 5-HT neurones (12). This mechanism may be involved in increased fear and unpleasant feelings that may disturb avoidance acquisition and performance. The resistance to extinction may be also explained by the increase in fear after lesion of NA neurons. Such an interpretation is compatible with finding of Mason et al (21) who observed increase in "neophobia" to a number of novel tasks in rats with lesioned DB by intracerebral injections of 6-OH dopamine. Our result demonstrating that resistance to extinction following electro-

lytic lesion to the LC can be obtained in a two-way avoidance situation
has important implications for theories which explain the resistance to
extinction in terms of "frustration". It is difficult to suppose that rats
are "frustrated" when they not receive a punishment, (i.e., an electric
footshock), which they expect (as might take place in the case of positive
reinforcement). This conclusion is compatible with that of Mason and
Fibiger (19) who demonstrated that resistance to extinction following
injection of 6-OH dopamine to the DB can be demonstrated in passive
avoidance situation.

It is proper to add that recently we found that lesions of the second
NA pathway, the VB, facilitated avoidance acquisition in rats (13). Such
a result was unexpected, since up to that time lesions of the brain NA
system either reduced or failed to change the acquisition of a variety
of tasks. According to this finding, the VB seems to play an opposite
role in learning processes than the DB does. It should be therefore
concluded that NA neurones in the brain are not functionally homo-
genous.

REFERENCES

1. AMAREL, D. G. and FOSS, J. A. 1975. Locus coeruleus lesions and learning.
   Science 188: 377–378.
2. ANLEZARK, G. M. CROW, T. J. and GREENWAY, A. P. 1973. Impaired
   learning and decreased cortical norepinephrine after bilateral locus coeruleus
   lesions. Science 181: 682–684.
3. CROW, T. J. and ARBUTHNOTT, G. W. 1972. Function of catecholamine —
   containing neurons in mammalian central nervous system. Nature 238: 245–246.
4. CROW, T. J. and WENDLANDT, S. 1976. Impaired acquisition of a passive
   avoidance response after lesions induced in the locus coeruleus by 6-OH
   dopamine. Nature 259: 42–44.
5. DAHLSTRÖM, A. and FUXE, K. 1964. Evidence for the existence of mono-
   amine containing neurons in the central nervous system. I. Demonstrations
   of monoamines in the cell bodies of the brain stem neurons. Acta Physiol.
   Scand. Suppl. 232: 1–55.
6. GRAY, J. A. 1972. Effects of septal driving of the hippocampal theta rhythm
   on resistance to extinction. Physiol. Behav. 8: 481–490.
7. GRAY, J. A., McNAUGHTON, N., JAMES, D. F. and KELLY, P. H. 1975.
   Effect of minor tranquillisers on hippocampal theta rhythm mimicked by
   depletion of forebrain noradrenaline. Nature 258: 424–425.
8. GRAY, J. A. 1977. Drug effects on fear and frustration: possible limbic site
   of action of minor tranquilizers. In L. L. Iversen, S. D. Iversen and S. H. Sny-
   der (ed.), Handbook of psychopharmacology. Vol. 8, Plenum Press, New York.
9. JERLICZ, M., KOSTOWSKI, W., BIDZINSKI, A. and HAUPTMANN, M. 1978.
   Effects of lesions in the ventral noradrenergic bundle on behavior and res-

ponse to psychotropic drugs in rats. Pharmacol. Biochem. Behav. 9: 721–724.

10. KETY, S. S. 1970. The biogenic amines in the central nervous system, their possible roles in arousal, emotions and learning. *In* F. O. Schmitt (ed.), The neuroscience. Rockefeller Univ. Press, New York, p. 324–336.

11. KOOB, G. F., KELLEY, A. E. and MASON, S. T. 1978. Locus coeruleus lesions: learning and extinction. Physiol. Behav. 20: 709–716.

12. KOSTOWSKI, W. 1975. Interactions between serotonergic and catecholaminergic systems in the brain. Polish J. Pharmacol. Pharm. 27: Suppl. 15–24.

13. KOSTOWSKI, W. and PŁAŹNIK, A. 1978. Effect of lesions of the ventral noradrenergic bundle on the two-way avoidance behavior in rats. Acta Physiol. Pol. 29, 6: 509–514.

14. KÖNIG, J. T. and KLIPPEL, R. A. 1963. The rat brain: a stereotaxic atlas of the forebrain and the lower part of the brain stem. Williams and Wilkins, Baltimore.

15. LENARD, L. G. and BEER, B. 1975. Modification of avoidance behavior in 6-OH dopamine treated rats by stimulation of central noradrenergic and dopaminergic receptors. Pharmacol. Biochem. Behav. 3: 887–983.

16. LINDVALL, O. and BJÖRKLUND, A. 1974. The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta Physiol. Scand., Suppl. 412: 1–48.

17. MACKINTOSH, N. J. and HOLGATE, V. 1968. Effects of inconsistent reinforcement on reversal and nonreversal shifts. J. Exp. Psychol. 76: 154–159.

18. MASON, S. T. 1978. Parameters of the dorsal bundle extinction effect: previous extinction experience. Pharmacol. Biochem. Behav. 8: 655–659.

19. MASON, S. T. and FIBIGER, H. C. 1978. 6-OH dopamine lesion to the dorsal noradrenergic bundle alters extinction of passive avoidance. Brain Res. 152: 209–214.

20. MASON, S. T. and IVERSEN, S. D. 1977. Effects of selective forebrain noradrenaline loss on behavioral inhibition in the rat. J. Comp. Physiol. Psychol. 91: 165–173.

21. MASON, S. T. and IVERSEN, S. D. 1978. Reward, attention and the dorsal noradrenergic bundle. Brain Res. 150: 135–148.

22. MASON, S. T., ROBERTS, D. C. S. and FIBIGER, H. C. 1978. Noradrenaline and neophobia. Physiol. Behav. 21: 353–361.

23. SESSIONS, G. R., KANT, G. J. and KOBB, G. F. 1977. Locus coeruleus lesions and learning in the rat. Physiol. Behav. 17: 853–859.

24. STEIN, L. C., BELLUZZI, J. D., RITTER, S. and WISE, C. D. 1974. Self-stimulation reward pathways: norepinephrine vs. dopamine. J. Psychiatr. Res. 11: 115–119.

25. STEIN, L. C. and WISE, C. 1973. Amphetamine and noradrenergic reward pathways. *In* Frontiers in catecholamine research. Pergamon Press, p. 963.

26. STEIN, L. C. and WISE, C. D. 1974. Serotonin and behavioral inhibition. Adv. Biochem. Psychopharmacol. 11: 281–286.

27. WISE, C. D., BERGER, B. D. and STEIN, L. C. 1973. Evidence of noradrenergic reward receptors and serotonergic punishment receptors in the rat brain. Biol. Psychiatr. 6: 3–8.

28. WISE, R. A. 1978. Catecholamine theories of reward: a critical review. Brain Res. 152: 215–247.

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