Pharmacology Biochemistry & Behavior, Vol. 33, pp. 545–548. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Pretest β-Endorphin and Epinephrine, But Not Oxotremorine, Reverse Retrograde Interference of a Conditioned Emotional Response in Mice

IVAN IZQUIERDO,* N. RAMSEY BARCIK† AND JORGE D. BRIONI†

**Centro de Memoria, Departamento de Bioquimica, Instituto de Biociencias U.F.R.G.S., 90050 Porto Alegre, RS, Brazil*
†Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717

Received 12 August 1988

IZQUIERDO, I., N. RAMSEY BARCIK AND J. D. BRIONI. *Pretest β-endorphin and epinephrine, but not oxotremorine, reverse retrograde interference of a conditioned emotional response in mice.* PHARMACOL BIOCHEM BEHAV **33**(3) 545–548, 1989.—CD-1 mice were trained in a classically conditioned emotional response paradigm and tested 24 hr later. Exposure to an open field 0 or 1, but not 3 hr after training retroactively interfered with retention of the conditioned emotional response. The retroactive interference was counteracted by the pretest IP administration of β-endorphin (0.05 μg/mouse) or epinephrine (1 μg/mouse), but not by that of oxotremorine (5 μg/mouse). The three drugs were able to enhance retention test performance in animals not exposed to the open field after training. In view of evidence in the literature that β-endorphin and epinephrine are released during training in an aversive task like this, it seems likely that these two agents were able to overcome the effect of retroactive interference by reinstating neurohumoral attributes of the conditioned emotional task at the time of testing.

Retroactive interference Pretest treatments β-Endorphin Epinephrine Oxotremorine

INFORMATION interpolated after a learning experience or event may alter retention of that experience or event. This is called retroactive interference and was first described for human verbal learning (17). Retroactive interference has been recently studied in mice (3), rats (14, 16, 18, 19), and humans (9). It was found to be time dependent (9, 16, 18, 19), which makes it different from extinction (9, 17–19), and confirms Muller and Pilzecker's (17) early descriptions of this phenomenon. In addition, posttraining facilitation by vasopressin, epinephrine or ACTH makes inhibitory avoidance learning in rats resistant to extinction, but not to retroactive interference (14). This has led to the suggestion that retroactive interference, unlike extinction (14,18), and contrary to early suggestions (17), does not consist of the weakening of a previously acquired trace, but rather of the addition of negative information to it so as to make it less retrievable or less accessible to retrieval (14, 18, 19). Consistent with this interpretation, retroactive interference was found to require the recording of the interfering task: Treatments that make animals unable to retain that additional information, such as diazepam, inhibit its interfering effect (4, 13, 18, 20). Retroactive interference was also found to be task dependent: In mice and rats, various forms of habituation [to an open field (14, 16, 19), to a Y maze (4), to a tone (14,18)] presented 20 to 120 min after inhibitory or active avoidance training interfere with retention of the avoidance tasks, but the opposite does not happen (18). It would seem that the interfering task must be less stressful or alerting than the main task in order to be effective (4, 13, 18, 19).

The present study investigates interference with retention of a conditioned emotional response (2, 3, 7) by the posttraining exposure to an open field, in mice, and the influence thereupon of *pretest* β-endorphin, epinephrine, or oxotremorine administration. These treatments are known to enhance the retention test performance of a variety of aversive tasks in rats or mice, and to reverse the amnesia caused by a variety of treatments, such as ethanol, diazepam, electroconvulsive shock, and others (3, 6, 8, 11–14).

METHOD

Two hundred CD-1 mice (median weight, 27 g; Charles River) were used; 38 in a pilot study (Table 1) and 162 in the main experiments shown in Figs. 1 and 2.

The task used was a classically conditioned emotional response paradigm (2, 3, 7), with a training-test interval of 24 hr. The paradigm was based on the previous studies by Hunt and Brady (7) and Kameyama and Nagasaka (15), who showed that footshock training in a given environment produces a conditioned suppression of activity in that environment in a test session carried out 1 or more days later.

Training and testing were performed in a dark room. The training procedure was as follows (2,3). Animals were placed in

one of the arms of a trough-shaped Y maze with their head pointing opposite to the door. The door of the arm was kept closed, which turned it into a compartment 13.5 cm high, 15.5 cm long, and 11.5 cm wide at the top and 2.5 cm wide at the base. Starting after 15 sec, a 5-sec, 1-kHz tone was presented 20 times, at 20-sec intervals. In the no-footshock groups, the tone was presented alone. In the footshock-trained groups,the last 2 sec of each tone overlapped with a 0.75-mA scrambled footshock delivered to the floor of the closed compartment. The training session lasted 400 sec.

In the test session, the animals were placed again in one of the arms of the maze with the head pointing opposite to the door, but the doors of the three arms were kept open, and the number of entries into the arms was counted. The test session lasted 120 sec. In the pilot study shown in Table 1, testing was carried out either without the tone, or with the tone continuously on starting 3 sec after the animals were placed in the apparatus. In the main study, whose results are shown in Figs. 1 and 2, the tone was on during the test session.

In the pilot study, two groups, one trained with and the other without footshock (n = 10 per group), were tested with the tone; and two other groups were similarly trained (N = 9 per group) and tested without the tone.

In the main study, in which all animals were tested with the tone, 7 groups (N = 6 per group) were trained with no footshock and 10 groups (N = 12 per group) were trained with the footshock. Three of the no-footshock groups, and 6 of the footshock-trained were exposed, after training, during 10 min, to a brightly-illuminated 95-cm diameter circular open field with a 19-cm high wall made of styrofoam, painted yellow. The animals were placed in this apparatus immediately after training (0 hr), 1 hr, or 3 hr after training in the Y maze, and explored it thoroughly during the time they were in it. Before and after exposure to the open field the mice were kept in their own home plastic cages where they were housed in groups of 6. Animals not exposed to the open field were kept in their home cages throughout the training-test interval.

Six min prior to testing, the mice received an IP injection of saline, oxotremorine sesquifumarate (5 μg/mouse), epinephrine HCl (1 μg/mouse), or camel β-endorphin (0.05 μg/mouse). All drugs were dissolved in saline and injection volume was 0.1 ml/mouse in all cases.

Statistical analysis was by one-way ANOVAs followed by a Newman-Keuls test.

RESULTS

Results of the pilot study are shown in Table 1. Regardless of the presence of the tone in the test session, footshock-trained animals made less entries in the test than the animals trained with no footshock. This ruled out mere habituation or dishabituation effects of the training session on responsiveness to the tone in the test session and was evidence of conditioned suppression (15) or emotional (2, 3, 7) learning. However, the difference in the number of entries between footshock-trained and no-footshock groups was slightly but significantly larger when the tone was on in the test session. This suggested that there was a small but detectable classically conditioning component in the footshock-trained groups caused by the tone-footshock pairing, which is properly revealed only when animals are tested in the presence of the tone. Accordingly, and in order not to lose this component, the main study (Figs. 1 and 2) was carried out with the tone on during the test session.

A one-way ANOVA applied to all groups in Figs. 1 and 2, regardless of whether they were trained with or without footshocks, showed a significant groups effect, $F(16,145) = 41.63$, $p < 0.0001$.

TABLE 1

EFFECT OF TONES ON TEST SESSION PERFORMANCE

Training Condition	N	Test Condition	Mean ± SEM Entries in Test Session
No footshock	10	Tone on	12.3 ± 0.4
Footshock-trained	10	Tone on	4.2 ± 0.5*
No footshock	9	Tone off	10.4 ± 0.6
Footshock-trained	9	Tone off	5.2 ± 0.6*

*Significant difference from no-footshock group submitted to the same test condition (tone on or off) at $p<0.01$ level in the Newman-Keuls test. The mean ± SEM of individual differences between test session performance of footshock-trained animals and the mean of the corresponding no-footshock group was 8.1 ± 0.6 in the animals tested with the tone, and 5.2 ± 0.7 in the animals tested without the tone. The difference between these two means was significant at a $p<0.01$ level in a t-test.

Mean ± SEM entries in the arms of a Y-maze in a 2-min test session carried out 24 hr after a training session that consisted of 20 tones (no-footshock groups), or of 20 tone-footshock classical conditioning trials (footshock-trained groups), presented every 20 sec in one of the closed arms of the maze. Animals were tested either with the tone on starting 3 sec after being placed in the maze, or without the tone.

Results obtained in the control animals trained with no footshock are shown in Fig. 1. Differences among these groups were not significant, $F(6,35) = 0.23$, $p = 0.95$. Therefore, exposure to the open field 0, 1 or 3 hr after training with no footshock, and pretest β-endorphin, oxotremorine or epinephrine administration had no effect on mere exploratory activity in the Y maze in animals previously presumably habituated to the tone one day before.

Results obtained in the footshock-trained animals are shown in Fig. 2. Differences among groups in performance of the conditioned emotional response [i.e., the suppression of exploratory activity in the test session (2, 3, 7, 15)] were significant, $F(9,108) = 36.19$, $p<0.0001$. Exposure to the open field 0 or 1 hr, but not 3 hr after training caused a significant reduction of retention test performance of the conditioned emotional behavior. Actually, performance in the test session of the animals submitted to the open field 0 hr after training was not significantly different from that of its own no-footshock control ($p>0.2$); test performance of all other footshock-trained groups, including the one exposed to the open field 1 hr after training, was significantly lower than that of any of the no-footshock groups at a $p<0.01$ level in a Newman-Keuls test. The retroactive interference caused by the immediate posttraining exposure to the open field was reversed by the pretest administration of β-endorphin or epinephrine, but not by that of oxotremorine. In control animals, all three drugs significantly enhanced retention test performance, in confirmation of previous results obtained in this (2,3) or in other tasks, in rats and mice (5, 10–14).

DISCUSSION

Footshock-trained control animals performed less entries in the test session than the no-footshock controls. The difference in the number of entries between footshock-trained and no-footshock groups was larger in animals tested with the tone, which suggests several things. First, that the influence of the footshock in the training session was not just due to changes in habituation to the tone. Second, that footshock (or tone-footshock) training induces conditioned emotional (or conditioned suppression) learning (2, 3, 7, 15). Third, that there is a small but detectable classical conditioning component (2,3) that is best observed when the

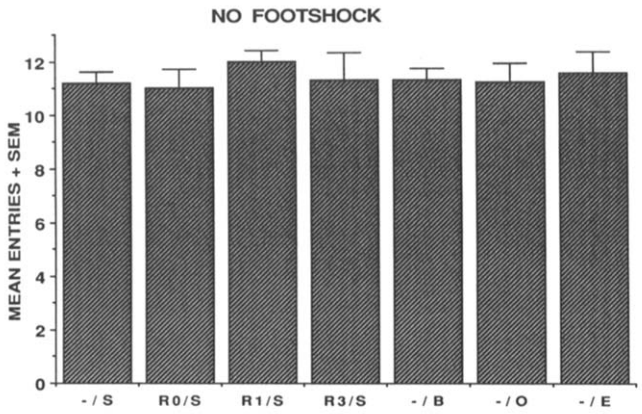

FIG. 1. Mean+SEM number of entries into the arms of a Y maze in a test session carried out 24 hr after a training session that consisted of 20 five-sec tones presented every 20 sec in one of the closed arms of the maze (training with no footshock). In this and the following figure, the first symbol in the legend to the abscissae indicates the posttraining treatment (−/: no treatment; R0/, R1/, R3/, exposure to an open field 0, 1, or 3 hr after training, respectively); and the second symbol indicates the pretest treatment (S: saline; B: β-endorphin, 0.05 μg/mouse; O: oxotremorine, 5 μg/mouse; E: epinephrine, 1 μg/mouse). Differences in test session performance among groups were not significant either in a one-way ANOVA or in a Newman-Keuls test.

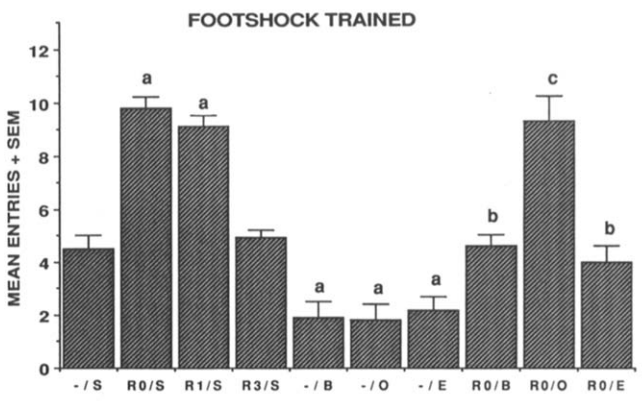

FIG. 2. Mean + SEM number of entries into the arms of a Y maze in a test session carried out 24 hr after a training session that consisted of 20 tone-footshock classical conditioning trials presented every 20 sec in one of the closed arms of the maze. Except for the R0/S group, the number of entries was in all cases lower than that of any of the no-footshock groups in Fig. 1 ($p<0.01$ in a Newman-Keuls test), which means that they showed retention of the conditioned emotional response [i.e., suppression of exploratory activity in the test session (1, 2, 6)]. Exposure to the open field 0 or 1 hr (R0/S, R1/S) but not 3 hr (R3/S) after training caused a significant impairment of retention in the animals that received saline prior to testing (a: $p<0.01$ in a Newman-Keuls test). Pretest β-endorphin (−/B), oxotremorine (−/O) and epinephrine (−/E) (same doses as in Fig. 1) significantly enhanced retention test performance in animals not exposed to the open field after training (a: $p<0.01$ in a Newman-Keuls test). In the animals exposed to the open field immediately after training (R0), pretest β-endorphin (R0/B) and epinephrine (R0/E), but not oxotremorine (R0/O), attenuated the retrograde interference caused by that exposure and carried memory scores to levels similar to those of the control group (b: significant difference from R0/S gorup, $p<0.01$ level; c: difference not significant from R0/S group, $p>0.2$, but significant from −/S group, at $p<0.01$ level in a Newman-Keuls test).

animals are tested with the tone.

Exposure to an open field caused an increase of the number of entries in the test session in the footshock-trained animals, but not in the no-footshock groups. Assuming that footshock-trained animals effectively learned a conditioned emotional response (2, 3, 7), the effect of exposure to the open field can be interpreted as due to retrograde interference (14, 16–19). The time dependency of the effect was similar to that described in rats for retrograde interference caused by similar or by other forms of habituation on previously acquired inhibitory or active avoidance behaviors (16, 18, 19). It is unlikely that the effect of the open field seen in the present experiment was due to an influence on prior habituation to the tone, or of extinction of the conditioned emotional response because of the cognitive contrast between the main and the interfering task (a small, dark compartment versus an ample, brightly-illuminated space). Posttraining exposure to the open field had no effect on the number of entries performed by control animals trained without footshocks, and reasons to think that footshock training did induce conditioned emotional learning (2, 3, 7) were given above.

The present findings are in agreement with previous evidence on the lack of a need of any cognitive relevance between interfering and main tasks (13, 14, 18, 19), and suggests that the reasons for the interference should perhaps be sought elsewhere, such as in the novelty of the interfering task (16), or in the fact that interfering tasks are usually, and perhaps necessarily, less stressful or alerting than those whose memory they interfere with (18,19).

In a previous paper (14) it was found that posttraining epinephrine, vasopressin and ACTH did not affect retroactive interference but were able to antagonize extinction. Here we find that pretest β-endorphin or epinephrine administration are, and pretest oxotremorine is not, able to reverse retroactive interference at the doses used. The three treatments are known to enhance retention test performance of a variety of tasks (2, 3, 8, 10–14), and in fact they did so in the present case, in animals not exposed to the interfering task, and to a similar extent (Fig. 2). The fact that, at doses equipotent to enhance retention test performance in control animals, two of the drugs were, and the other was not, able to partially overcome the effect of retrograde interference is difficult to interpret, and the present findings provide no direct answer to this question.

β-Endorphin and epinephrine are believed to enhance retrieval by reinstating neurohumoral attributes of the task at the time of testing (10–14, 21). Brain β-endorphin is released during a very wide variety of training procedures and peripheral epinephrine is released during alerting or aversive trainings (12,13). On account of the prior release of β-endorphin by the main task, which induces a depletion that takes over 3 hr to recover, the interfering task would not be expected to release brain β-endorphin (18). On account of the fact that exposure to the open field is less stressful than footshock training (it was painless to begin with), it is certainly likely to release less epinephrine than the latter [see (13)]. Thus, it is possible that β-endorphin and epinephrine were able to counteract retroactive interference by reinstating neurohumoral attributes of the main task rather than of the interfering task at the time of testing (10, 13, 21). It may be noted that epinephrine also releases brain β-endorphin, which may partly explain its pretest memory enhancing effect (10, 12, 13) and/or its reversal of retrograde interference effects.

On the other hand, there is no evidence that the pretest enhancing effect of oxotremorine represents a reinstatement of a neurohumoral condition prevalent at the time of training. It is assumed that cholinergic systems are necessary for learning and/or consolidation (1,6), but there is no evidence that training is accompanied by a hypersecretion of acetylcholine as it is, instead,

accompanied or followed by a hypersecretion of brain β-endorphin and peripheral epinephrine (6, 12, 21). The available evidence suggests instead that pretest oxotremorine facilitates retrieval independently of acquisition or postacquisitional variables (2,3). Therefore, oxotremorine enhances retention test performance by a mechanism apparently not involving a reinstatement of neurohumoral conditions present during training; which may be the reason why it did not reverse retrograde interference effects. Clearly, further investigation of this point is desirable.

The present findings suggest that an investigation of other drugs capable of reversing retroactive interference by a pretest action may be interesting, and could be of potential clinical interest (9,13).

ACKNOWLEDGEMENTS

Supported by USPHS grant MH12526 and by a grant from the Office of Naval Research (No. N00014-87-k-0518) to James L. McGaugh and by FINEP (Brazil) grant No. 42.88.0273.00 to Ivan Izquierdo. The authors are indebted to Prof. James L. McGaugh for his advice, encouragement, and valuable comments, as well as for laboratory facilities.

REFERENCES

1. Bartus, R. T.; Dean, R. L.; Pontecorvo, M. J.; Flicker, C. The cholinergic hypothesis: A historical overview, current perspective, and future directions. Ann. NY Acad. Sci. 444:332–358; 1985.
2. Brioni, J. D.; Izquierdo, I. Retention enhancement by pre-test β-endorphin and oxotremorine and its reversal by scopolamine. Behav. Neural Biol. 50:251–254; 1988.
3. Brioni, J. D.; McGaugh, J. L.; Izquierdo, I. Amnesia induced by short-term treatment with ethanol: Reversal by pretest oxotremorine administration. Pharmacol. Biochem. Behav. 33:27–29; 1989.
4. Cahill, L.; Brioni, J. D.; Izquierdo, I. Retrograde memory enhancement by diazepam: Its relation to anterograde amnesia, and some clinical implications. Psychopharmacology (Berlin) 90:554–556; 1986.
5. de Wied, D.; Bohus, B.; van Ree, J. M.; Urban, I. Behavioral and electrophysiological effects of peptides related to lipotropin (β-LPH). J. Pharmacol. Exp. Ther. 204:570–580; 1978.
6. Flood, J. F.; Smith, G. E.; Cherkin, A. Memory enhancement: Supraadditive effect of subcutaneous cholinergic drug combinations in mice. Psychopharmacology (Berlin) 86:61–67; 1985.
7. Hunt, H. F.; Brady, J. V. Some effects of electro-convulsive shock on a conditioned emotional response ("anxiety"). J. Comp. Physiol. Psychol. 44:88–98; 1951.
8. Izquierdo, I.; Cardoso Ferreira, M. B. Diazepam prevents post-training drug effects related to state dependency, but not post-training memory facilitation by epinephrine. Behav. Neural Biol. 51:73–79; 1989.
9. Izquierdo, I.; Chaves, M. L. F. The effect of a non-factual post-training negative comment on the recall of verbal information. J. Psychiatry Res. 22:165–170; 1988.
10. Izquierdo, I.; Dias, R. D. Influence on memory of posttraining and pre-test injections of ACTH, vasopressin, epinephrine and β-endorphin, and their interaction with naloxone. Psychoneuroendocrinology 10:165–172; 1985.
11. Izquierdo, I.; Dalmaz, C.; Dias, R. D.; Godoy, M. C. G. Independent memory enhancement by posttraining and pre-test administration of ACTH, vasopressin and epinephrine. Behav. Neurosci., in press; 1988.
12. Izquierdo, I.; McGaugh, J. L. Retention impairment by post-training epinephrine: Role of state dependency and of endogenous opioid mechanisms. Behav. Neurosci. 101:778–781; 1987.
13. Izquierdo, I.; Netto, C. A.; Dalmaz, C.; Chaves, M. L. F.; Pereira, M. E.; Siegfried, B. Construction and reconstruction of memories. Braz. J. Med. Biol. Res. 21:9–25; 1988.
14. Izquierdo, I.; Pereira, M. E. Posttraining memory facilitation prevents extinction but not retroactive interference. Behav. Neural Biol. 51:108–113; 1989.
15. Kameyama, T.; Nagasaka, M. Effects of apomorphine and diazepam on a quickly learned conditioned suppression in rats. Pharmacol. Biochem. Behav. 17:59–63; 1982.
16. Meere, M.; Borrell, J. Psychoneurogenic stress influences upon retention of an inhibitory avoidance response in the rat. Behav. Neural Biol. 49:1–11; 1988.
17. Muller, G. E.; Pilzecker, A. Experimentelle Beitrage zur Lehre vom Gedachtniss. Zeitschr. Psychol. 1:1–288; 1900.
18. Netto, C. A.; Dias, R. D.; Izquierdo, I. Interaction between consecutive learnings: Inhibitory avoidance and habituation. Behav. Neural Biol. 44:515–520; 1985.
19. Pereira, M. E.; Dalmaz, C.; Rosat, R.; Izquierdo, I. Diazepam blocks the interfering effect of posttraining behavioral manipulations on retention of a shuttle avoidance task. Psychopharmacology (Berlin) 94:402–404; 1988.
20. Pereira, M. E.; Rosat, R.; Huang, C. H.; Godoy, M. C. G.; Izquierdo, I. Effect of additional training on the retention of shuttle avoidance behavior in rats, and the influence of diazepam thereupon. Behav. Neurosci. 103:201–205; 1989.
21. Zornetzer, S. F. Neurotransmitter modulation and memory: A new neuropharmacological phrenology? In: Lipton, M. A.; DiMascio, A.; Killam, K. F., eds. Psychopharmacology: A generation of progress 1967–1977. New York: Raven Press; 1978:638–647.