Neuroscience Letters 444 (2008) 102–105

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Behavioral differences on memory retrieval between two variants of step-through inhibitory avoidance task in mice

Mariano G. Blake*, Mariano M. Boccia, Carlos M. Baratti

Laboratorio de Neurofarmacología de los Procesos de Memoria, Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 5 May 2008
Received in revised form 1 August 2008
Accepted 6 August 2008

Keywords:
Inhibitory avoidance
Memory retrieval
Mice
Diazepam
Picrotoxin

ABSTRACT

For several decades, one-trial inhibitory avoidance (IA) tasks have been used in the study of memory processing. In the present work, the effects of diazepam (DZP) (0.5 mg/kg) and picrotoxin (PIC) (0.3 mg/kg) on memory retrieval were assessed using two variants of a step-through IA situation in CF-1 mice. In the first variant, animals get into a dark compartment from an open illuminated platform (platform), whereas in the other, from an enclosed illuminated one (box). PIC impaired retention performance in the "platform-type" IA, but not in the "box-type". DZP enhanced retention performance in both types of IA task. These results evidence critical differences between the two step-through inhibitory avoidance tasks used, that might be relevant not only for retention performance during memory retrieval, but also for the theoretical interpretations and conclusions obtained from behavioral results.

For several decades, one-trial inhibitory avoidance (IA) tasks have been used for the study of memory processing [14]. Step-through IA appears to be an adequate task because memories of IA are formed through only one learning trial, generating responses that animals show in natural conditions (avoidance) and that appear to be long lasting [18], the latter shared with other aversive learning tasks [11,15,20]. The strength of avoidance responses may exist due to fear and avoidance serve functions that are critical to the survival of animals and protect subjects against potentially environmental threats [12].

During the training session of the step-through IA task, animals are placed into a bright compartment and they are allowed to enter to a dark one. Typically, animals employ about 5–10 s to leave the illuminated compartment and enter to the dark one. Once inside, animals receive a scramble footshock. On a subsequent test, animals are placed again in the bright compartment and the latency to get into the dark one is recorded. So, during training, animals must make a response (that is, entering the dark compartment) before being shocked. Then, it is likely that the footshock acts both to condition the dark compartment as an aversive context through Pavlovian conditioning processes, and to punish the behavior of getting into the dark compartment through instrumental conditioning processes [23].

In the IA task, animals experience a pairing of previously neutral stimulus, the conditioned stimulus (both the context and entering the dark compartment) with an unconditioned stimulus (the footshock). Then, during the test animals avoid a punishment (a footshock) by inhibiting an instinctive behavior (entering a dark compartment).

More than one kind of apparatus has been designed for this task. The illuminated compartment of the IA apparatus employed by Essman and Alpern [10] had an elevated illuminated platform attached to the front centre of the entrance to the dark compartment. On the contrary, the illuminated compartment of the apparatus first described by Jarvic and Kopp [17], had a corridor, enclosed with walls, which did not allow the mice to look out of it.

CF-1 male mice (FUCAL, Buenos Aires, Argentina) were used (age, 40 days; approximate weight 30 g). They were individually identified and group-housed in stainless-steel cages (10 per cage). The mice were kept in a climatised animal room (21–23 °C) maintained on a 12 h light/dark cycle (lights on 06:00 h) with ad libitum access to dry food and tap water. Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23/96), and local regulations. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Inhibitory avoidance behavior was studied in one-trial learning, step-through type situation [2], which utilizes the natural preference of mice for a dark environment. The apparatus consisted of a dark compartment (20 cm × 20 cm × 15 cm) with a stainless-steel grid floor and a sliding door opened in its front centre communicating with an illuminated one. Two different types of illuminated

* Corresponding author at: Junín 956 5° Piso, C1113, Capital Federal, Argentina. Tel.: +54 11 4964 8266; fax: +54 11 4964 8266.
E-mail address: blakion@fmed.uba.ar (M.G. Blake).

0304-3940/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2008.08.010

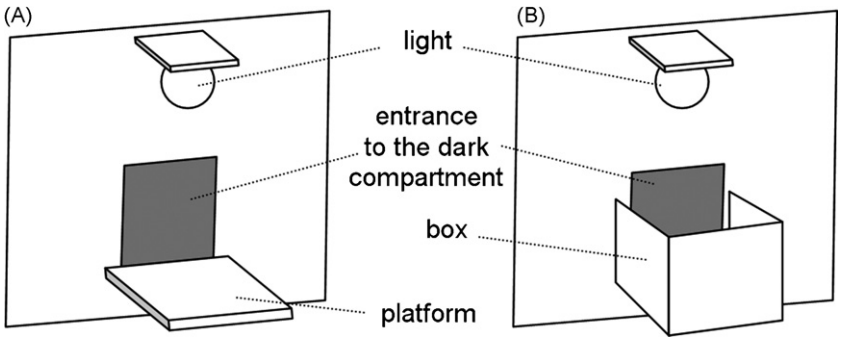

Fig. 1. Diagram showing the two types of IA apparatus. The "platform-type" (A), similar to the described by Essman and Alpern, has an elevated platform with 5 cm × 5 cm dimensions stated 100 cm elevated from the floor. The "box-type" (B), similar to the first described by Jarvic and Kopp, has a 5 cm × 5 cm box having 10 cm height white walls (the transparent roof was omitted in the diagram). More details in the text.

compartment were used. The first, from now named "platform", was similar to the described by Essman and Alpern [10], and consisted in an elevated platform with 5 cm × 5 cm dimensions, stated 100 cm elevated from the floor (Fig. 1A). The other illuminated compartment, from now named "box", was similar to the first described by Jarvic and Kopp [17], and consisted on a 5 cm × 5 cm box having 10 cm height white walls and a transparent roof (Fig. 1B). The mice were not exposed to the task before the learning trial. During training, each mouse was placed in the illuminated compartment, either on the platform or on the box, and received a footshock (0.8 mA, 50 Hz, 1 s) as it stepped into the dark compartment. Forty-eight hours later, the retention test was performed. Thus, each mouse was placed on the illuminated compartment again, and the latency to step-through (LTST) was recorded. The retention test was finished either when the mouse stepped into the dark compartment or failed to cross within 300 s. In the latter case the mouse was immediately removed and assigned a score of 300 s. In the retention test session the footshock was omitted. All the mice were tested using the same illuminated compartment used during the training session.

The drugs used were diazepam (DZP) (Lamar SA, Argentina) and picrotoxin (PIC) (BDH Ltd, Poole, England). All other chemicals and reagents were of analytical grade and obtained through local commercial sources. All the drugs were dissolved in saline solution immediately before their use and administered intraperitoneally (10 ml/kg). Controls received the same volume of saline solution. The experiments were conducted blind with respect to the drug treatments.

Diazepam is a drug that promote the binding of GABA to the $GABA_A$ receptors subtype, thereby increasing the inhibitory activity of GABA in the central nervous system (CNS). The effects of DZP are dose-dependent and virtually all of them result from its actions on the CNS [6]. Only two of its effects result from peripheral actions: coronary vasodilation (observed only after intravenous administration) and neuromuscular blockade (seen only with very high doses) [6]. Neither of these two last effects are applicable to the experiments described below.

Picrotoxin is a drug that blocks the chloride pore of the $GABA_A$ receptors subtype, thereby antagonizing the inhibitory effects of GABA in the CNS. The effects of PIC are mainly exerted on the CNS, being a powerful stimulant, but no appreciable effect is seen until convulsive doses are given [13]. In this sense, the DE_{50} for the convulsant effect of PIC in mice was found to be near 5.5 mg/kg [3].

Retention latencies during the retention test, are expressed as medians and interquartile ranges and were analyzed with the nonparametric analysis of variance of Kruskal–Wallis, and the differences between groups were estimated by individual Mann–Whitney *U*-tests (two tailed) [22]. In all cases, *p*-values less than 0.05 were considered significant.

Eight groups of 20 mice were trained in the inhibitory avoidance task. Four of these groups were trained using the platform and the other four groups using the box. Thirty minutes prior to the retention test, which was performed 48 h after training, mice received an intraperitoneal injection either of saline solution (SS), diazepam (DZP) (0.5 mg/kg), picrotoxin (PIC) (0.3 mg/kg) or DZP + PIC as a single injection.

Eight additional groups of 10 mice each were included in this experiment in order to test for unspecific effects of the drugs. The behavioral procedures and drug administrations were the same, but mice were trained without the footshock (unshocked groups).

At the end of the retention test, animals were exposed to an activity cage (LE 886 Motor Activity Cage, LSI Letica) in order to test for spontaneous motor activity.

Training step-through latencies differences among all the groups used were not significant (TSTL = 11 (6–4) s; $H_{(15)} = 6.35$; $p > 0.05$), and all the groups showed similar spontaneous motor activity, irrespective of the treatment employed (Table 1, $p > 0.05$).

Animals were carefully observed during the 30 min elapsed between the injection and the retention test. Animals injected with PIC did not evidence signs of convulsions (it is of worth pointing out that the dose of PIC used in these experiments is approximately 18 times lower than the DE_{50} for its convulsive effect in mice). Mice injected with DZP did not show unspecific alterations either.

Table 1
Spontaneous motor activity, measured immediately after the retention test (counts, arbitrary units) of mice tested using the platform or the box

	SS	DZP (0.5 mg/kg)	PIC (0.3 mg/kg)	DZP + PIC
Platform				
Shocked mice	320.3 ± 20.2	353.1 ± 19.5	332.9 ± 18.6	331.1 ± 19.4
Unhocked mice	333.5 ± 26.2	345.8 ± 24.4	324.9 ± 22.4	329.6 ± 23.5
Box				
Shocked mice	331.7 ± 17.9	339.3 ± 18.8	329.6 ± 20.0	335.3 ± 16.9
Unhocked mice	329.6 ± 22.4	349.8 ± 26.3	320.2 ± 22.5	331.5 ± 18.5

Data are means ± S.E. ($N = 20$ for shocked groups and $N = 10$ for unshocked groups). $p > 0.05$, in all cases compared with SS treated mice.

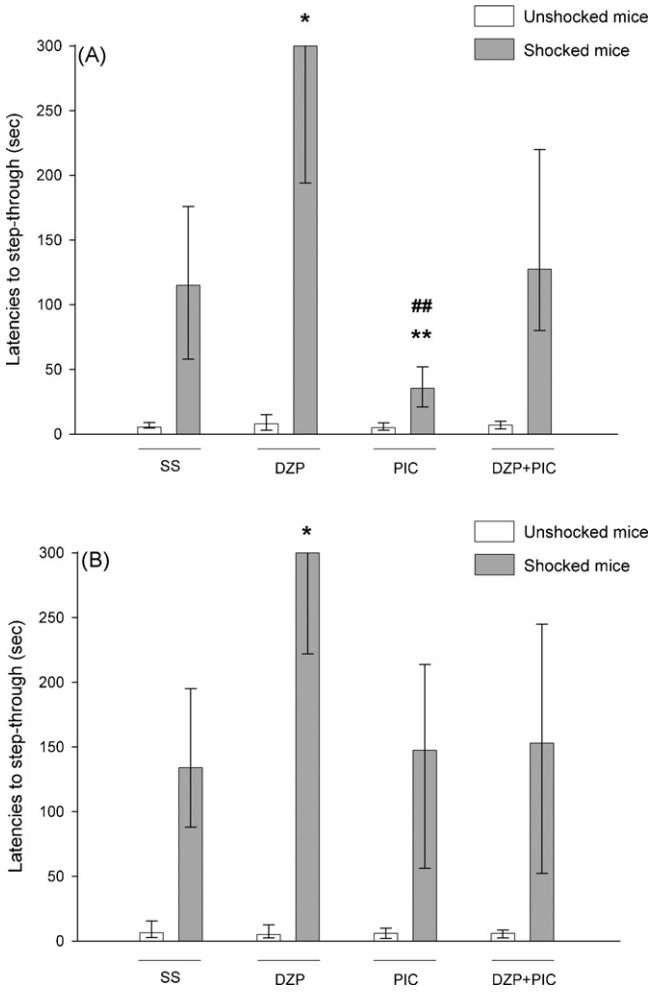

Fig. 2. Effects of saline (SS), diazepam (DZP) (0.5 mg/kg), picrotoxin (PIC) (0.3 mg/kg) or DZP + PIC given 30 min before the retention test in mice trained with or without footshock. Mice were trained and tested using the box (A) or the platform (B). Data are expressed as medians and interquartile ranges. $^{**}p < 0.01$, $^{*}p < 0.05$, compared with SS-treated mice; $^{\#\#}p < 0.01$, comparing shocked vs unshocked groups. $N = 10$ for unshocked groups; $N = 20$ for shocked groups.

The results are shown in Fig. 2. In the groups trained and tested using the platform, the pre-test injection of PIC significantly impaired performance ($p < 0.01$), whereas DZP significantly increased latencies to step-through ($p < 0.05$, in both cases compared with the saline-treated mice). When given together, DZP and PIC cancelled each other its effects on retention performance (Fig. 2A).

On the contrary, in the groups trained and tested using the box, only the pre-test injection of DZP significantly increased latencies ($p < 0.05$, compared with the saline-treated group), but no significant difference was observed in the group of mice injected with PIC. This effect of DPZ was prevented by PIC (Fig. 2B).

No significant differences were found in the eight groups of mice trained without footshock ($p > 0.05$).

At first glance, the present results show critical differences on retention performance between the two variants of an inhibitory avoidance task employed. The pre-test injection of DZP (0.5 mg/kg ip), increased latencies in both forms of the task, but a subconvulsive dose of PIC (0.3 mg/kg ip) impaired retention performance only in those mice trained and tested using the platform, but not with the box. In this sense, Castellano and McGaugh [5] demonstrated that even a dose of 1 mg/kg of PIC given from 3 to 30 min prior to the retention test, did not affect retention performance of an IA task in mice. In that paper, the authors used an IA apparatus with an illuminated compartment similar to our box-type, and here we report similar findings, confirming their results. However, when the platform was used, the pre-test injection of 0.3 mg/kg of PIC produced a severe impairment on retention performance.

Since mice injected with PIC and tested using the box performed as well as controls, it is quite clear that PIC is not able to erase memory on its own. Hence, the poor performance of mice tested on the platform under the effects of PIC seems to be due to an impairment to access to the memory trace.

Moreover, it is noteworthy that unshocked animals injected with DZP did not show significant increments of retention latencies, suggesting that DZP did not affect retention performance on its own. In addition, mice shocked and tested 48 h later under the effects of DZP showed enhanced retention performance on both types of the task. These results indicate that avoidance memory is required for this effect. So, the injection of DZP could be actually modulating the expression of memory, but is not a sufficient condition to control behavior.

Altogether, these data suggest that the effects of DZP and PIC on retention performance could be attributable to a modulation of memory retrieval.

There are three major ways to modulate retrieval processes [4]. The first involves aminergic and cholinergic systems involved in the perception of, and the response to, stress, anxiety, fear and aversiveness. The multiple neurotransmitter systems involved in this modulation might explain why memory retrieval is so dependent on emotional states [4].

The second major mechanism is hormonal. The stress hormones enhance memory retrieval at low to moderate doses [1,21], but impair it at high doses [8].

The third mechanism involves other contextual information presented near the time of memory retrieval. Exposition to novel information seems either to be able to enhance retrieval [16] or to generate false memories [19].

It is quite probable that more than one of the mechanisms above mentioned were involved in the effects of DZP and PIC described here. Although we cannot discard any of these mechanisms, as it is well known that gabaergic pathways are involved in modulation of anxiety levels, it is possible that some participation of "anxiety" of mice during memory retrieval contribute to the effects observed. In this sense, Ennaceur et al. [9] found that exposition of animals to open spaces produce fear-induced anxiety, whereas the exposition to enclosed ones develop fear-induced avoidance behavior [9]. Since the platform might resemble an open space, mice exposed to the platform might be developing increased anxiety; however, as the box is an enclosed space, mice could develop avoidance, in accordance with Ennaceur's observation.

So, since gabaergic antagonists produce an increment of anxiety-like behavior [7], it is probable that increased "anxiety" might lead mice to perform poorly. In this sense, as PIC injected mice performed poorly only in the platform, but not in the box, it may be argued that the platform is more "anxiogenic" than the box, and that the combined "anxiogenic" effects of the injection of PIC and the platform led to an interference that makes memory less available during the retrieval session.

During retrieval processes, memory traces influence performance, but other processes are, indeed, critical in controlling the subsequent behavior [4].

It is quite clear that the two variants of step-through inhibitory avoidance task used here have much in common, but the differences elicited by injection of PIC emphasize that small differences in the set up can dramatically change the outcome in this test, and because of this, one should be very careful at the time of mak-

ing generalizations with results obtained using one task to other tasks.

Acknowledgments

This work was supported by grant B028 from the University of Buenos Aires to CMB. MMB and CMB are members of CONICET.

References

[1] D.M. Barros, L.A. Izquierdo, J.H. Medina, I. Izquierdo, Bupropion and sertraline enhance retrieval of recent and remote long-term memory in rats, Behav. Pharmacol. 13 (2002) 215–220.
[2] M.M. Boccia, G.B. Acosta, M.G. Blake, C.M. Baratti, Atropine, an anticholinergic drug, impairs memory retrieval of a high consolidated avoidance response in mice, Neurosci. Lett. 345 (2003) 97–100.
[3] J.D. Brioni, J.L. McGaugh, Post-training administration of GABAergic antagonists enhances retention of aversively motivated tasks, Psychopharmacology 96 (1988) 505–510.
[4] M. Cammarotta, L. Bevilacqua, D. Barros, M. Vianna, L. Izquierdo, J.H. Medina, I. Izquierdo, Retrieval and the extinction of memory, Cell. Molec. Neurobiol. 25 (2005) 465–474.
[5] C. Castellano, J.L. McGaugh, Retention enhancement with post-training picrotoxin: lack of state-dependency, Behav. Neural Biol. 51 (1989) 165–170.
[6] D.S. Charney, S.J. Mihic, R.A. Harris, Hypnotics and sedatives, in: L.L. Brunton, J.S. Lazo, K.L. Parker (Eds.), Goodman and Gilman's The Pharmacological Basis of therapeutics, 11th ed., McGraw-Hill, New York, 2006, pp. 401–427.
[7] Y. Clement, G. Chapouthier, Biological bases of anxiety, Neurosci. Biobehav. Rev. 22 (1998) 623–633.
[8] M.A. De Almeida, I. Izquierdo, Effect of the intraperitoneal and intracerebroventricular administration of ACTH, epinephrine, or beta-endorphin on retrieval of an inhibitory avoidance task in rats, Behav. Neural. Biol. 40 (1984) 119–122.
[9] A. Ennaceur, S. Michalikova, P.L. Chazot, Models of anxiety: responses of rats to novelty in an open space and an enclosed space, Behav. Brain Res. 171 (2006) 26–49.
[10] W.B. Essman, H. Alpern, Single trial conditioning methodology and results with mice, Psychological reports 14 (1964) 731–740.
[11] M.S. Fanselow, G.D. Gale, The amygdala, fear, and memory, Ann. N.Y. Acad. Sci. 985 (2003) 125–134.
[12] M. Fendt, M.S. Fanselow, The neuroanatomical and neurochemical basis of conditioned fear, Neurosci. Biobehav. Rev. 23 (1999) 743–760.
[13] D.N. Franz, Central nervous system stimulants, in: A. Goodman Gilman, L.S. Goodman, A. Gilman (Eds.), The Pharmacological Basis of Therapeutics, third ed., Macmillan Publishing Co., New York, 1980, pp. 585–591.
[14] P.E. Gold, The use of avoidance training in studies of modulation of memory storage, Behav. Neural Biol. 46 (1986) 87–98.
[15] I.B. Introini-Collison, J.L. McGaugh, Epinephrine modulates long-term retention of an aversively motivated discrimination, Behav. Neural Biol. 45 (1986) 358–365.
[16] L.A. Izquierdo, D.M. Barros, J.H. Medina, I. Izquierdo, Exposure to novelty enhances retrieval of very remote memory in rats, Neurobiol. Learn. Mem. 79 (2003) 51–56.
[17] M. Jarvik, R. Kopp, An improved one-trial passive avoidance learning situation, Psychological Reports 21 (1967) 221–224.
[18] S.R. Kopf, C.M. Baratti, Memory modulation by post-training glucose or insulin remains evident at long retention intervals, Neurobiol. Learn. Mem. 65 (1996) 189–191.
[19] E.F. Loftus, D. Davis, Recovered memories, Annu. Rev. Clin. Psychol. 2 (2006) 469–498.
[20] M.B. Parent, M. West, J.L. McGaugh, Memory of rats with amygdala lesions induced 30 days after footshock-motivated escape training reflects degree of original training, Behav. Neurosci. 108 (1994) 1080–1087.
[21] B. Roozendaal, Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval, Neurobiol. Learn. Mem. 78 (2002) 578–595.
[22] S. Siegel, Non-Parametric Statistics for the Behavioral Sciences, McGraw-Hill, New York, 1956, 344 pp.
[23] M.R. Tinsley, J.J. Quinn, M.S. Fanselow, The role of muscarinic and nicotinic cholinergic neurotransmission in aversive conditioning: comparing pavlovian fear conditioning and inhibitory avoidance, Learn. Mem. 11 (2004) 35–42.