Physiol. Res. 51: S21-S27, 2002

Taste Aversion Learning and Aging: A Comparison with the Effect of Dorsal Hippocampal Lesions in Rats

I. MORON[1], M. A. BALLESTEROS[1], A. CANDIDO[1], M. GALLO [1,2]

[1]Department of Experimental Psychology and Physiology of Behavior, University of Granada, Granada, [2]Institute of Neurosciences Dr. F. Oloriz, University of Granada, Spain

Summary

The relationship between hippocampal function and aging was explored in Wistar rats using taste aversion learning by comparing the performance of adult dorsal hippocampal lesioned and fifteen-month-old intact rats with that of adult intact rats. In experiment 1 the conditioned blocking phenomenon was absent in the hippocampal and the aging rats. Unlike the adult intact rats, the hippocampal and aging rats were not impaired in acquiring a learned aversion to a cider vinegar solution (3 %) presented as a serial compound with a previously conditioned saccharin solution (0.1 %). In experiment 2 both the hippocampal and the aging rats developed reduced aversions to a saline solution (0.5 %) followed by an i.p. injection of lithium chloride (0.15 M; 2 % b.w.) if the taste solution was previously preexposed without consequences. This latent inhibition effect was similar to that seen in intact adult rats. In both experiments, the aging rats exhibited enhanced conventional learned taste aversions. It is concluded that aging is not a unitary process but induces both hippocampal dependent and hippocampal independent complex changes in the functioning of the neural circuits, implementing taste aversion learning.

Key words

Aging • Conditioned blocking • Hippocampal • Latent inhibition • Taste aversion learning • Rat

Introduction

Aging has been associated with cognitive deficits dependent on the deterioration of hippocampal function that may start well before senescence. A variety of learning and memory tasks impaired by hippocampal lesions in adult rats show a selective decline in aging rats (Gallagher 1997). On the contrary, the ability to acquire new aversions to the taste of foods that have been followed by visceral distress, as well as other Pavlovian conditioned responses (Bureš 1993), is not impaired, but even potentiated by age (Hinderliter Misanin 1995, Misanin Hinderliter1995, Misanin *et al.* 1988). However, the acquisition of conditioned taste aversions (CTA) is profoundly modulated by prior experience and thus represents a valuable behavioral paradigm for the study of complex learning phenomena, such as conditioned blocking (Kamin 1969) and latent inhibition (Lubow 1973, Lubow 1989), that may depend on the hippocampal function (Buhusi *et al.* 1998, Gallo *et al.* 1999). Conditioned blocking (CB) is manifest as a reduced aversion to a taste that was followed by malaise, if during the conditioning trial this taste is presented in compound with another previously conditioned taste. Latent inhibition (LI) is a reduced aversion to a familiar taste that was preexposed without negative consequences before the aversive conditioning trial. Previous data have shown that in the context of taste aversion learning CB, but not LI, is impaired in rats with dorsal hippocampal lesions (Gallo Candido 1995a, Gallo Candido 1995b,

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

Honey Good 1993, Reilly *et al.* 1993). Some evidence has pointed to the fact that CB of taste aversion may be affected by age (Gallo *et al.* 1997). The aim of the present study was to compare CB (Exp. 1) and LI (Exp. 2) of CTA[1] in aging and hippocampal lesioned rats.

Methods

Surgery

Dorsal hippocampal lesions were performed under general anesthesia with sodium pentobarbital (50 mg/kg). Two trephine openings were drilled in each side at -2.3 and -3.3 mm posterior to and 1.5 m lateral to bregma, in order to introduce a 200 µm stainless steel electrode, insulated except the distal cross-section, at four insertion points (two on each side). Bilateral dorsal hippocampal lesions were made at 3.8 mm from the skull surface by passing a 3 mA direct anodal current through the electrode for 30 s. Stereotaxic coordinates were taken from Paxinos and Watson (1986). After the stereotaxic procedure the animals were allowed a ten-day recovery period with water and food avalaible ad libitum, before the behavioral training begun.

Histology

At the end of the behavioral procedures the animals were deeply anesthetized with sodium pentobarbital, and intracardially perfused with physiological saline followed by 10 % formalin. The brains were removed and stored for several days in a 10 % formalin. After being cut with a freezing microtome, coronal sections stained with cresyl violet were studied under a light microscope in order to identify the extent and location of the damage. Microphotographs showing representative hippocampal lesions are shown in Figure 1. No signs of hippocampal damage were seen in aging brains.

Experiment 1 Procedure

In experiment 1 a total of 47 male Wistar rats were assigned to three groups. Intact rats (INT n=16) and hippocampal lesioned rats (HC n=13) were 3-months old (280-350 g.). Fifteen-month-old rats (OLD n=18) weighed 570-630 g. They were provided by the breeding colony of the University of Granada and individually housed in a room with constant temperature and a 12:12 h. light cycle. Food was available ad libitum. The animals were adapted to the water deprivation schedule, with water available for 15 min daily during one week.

The animals in each group were assigned to one of the behavioral procedures, either a blocking procedure (BL) (INT n=8, HC n=7, and OLD n=8) or a control procedure (CTRL) (n= 8, 6, and 10 respectively).

The conditioned blocking paradigm, that has been described in detail elsewhere (Gallo & Cándido, 1995a), used a serial taste compound of sodium saccharin (0.1 %) and cider vinegar (3 %) solutions in tap water (Table 1). The training procedure for the blocking group was scheduled as follows. On Day 1, during the daily drinking period, only sodium saccharin was avalaible for 15 min and the amount that was ingested was recorded. Approximately 10 min after drinking, the rats received an i.p. injection of lithium chloride (0.15 M; 2 % b.w.). On the next two days, the animals were allowed to drink water. On Day 4, the saccharin solution was presented first in a graduated burette and as soon as the rat had drunk 5 ml the tube was removed and another one containing 5 ml. of cider vinegar solution was substituted. If a rat did not drink 5 ml of either liquid, the tube was withdrawn after 10 min. Ten minutes later, the rat was given a lithium chloride i.p. injection. Thus, the training procedure for this group consisted of two experimental sessions: an initial saccharin-lithium pairing and a second serial compound-lithium pairing. The CTRL groups received only the serial-compound conditioning trial session. With respect to the vinegar solution, training was identical in the BL and CTRL groups with a single vinegar-lithium pairing. However, the BL and CTRL groups differed in their previous experience with saccharin since prior to the compound-lithium pairing, saccharin was familiar only to the BL groups.

After two water drinking days, a one-bottle test was performed on Day 7 in both groups. Only one tube containing the vinegar solution was available for 15 min and the intake was recorded. An additional saccharin-vinegar choice-test was given the following day. The saccharin and vinegar tubes were presented simultaneously for 15 min to the rats and a saccharin preference ratio was calculated (100 x saccharin/total fluid intake). A value of 100 would indicate that the animal drank only saccharin, and a ratio of 0 would correspond to an animal drinking only vinegar. A ratio of 50 would indicate equal consumption of saccharin and vinegar.

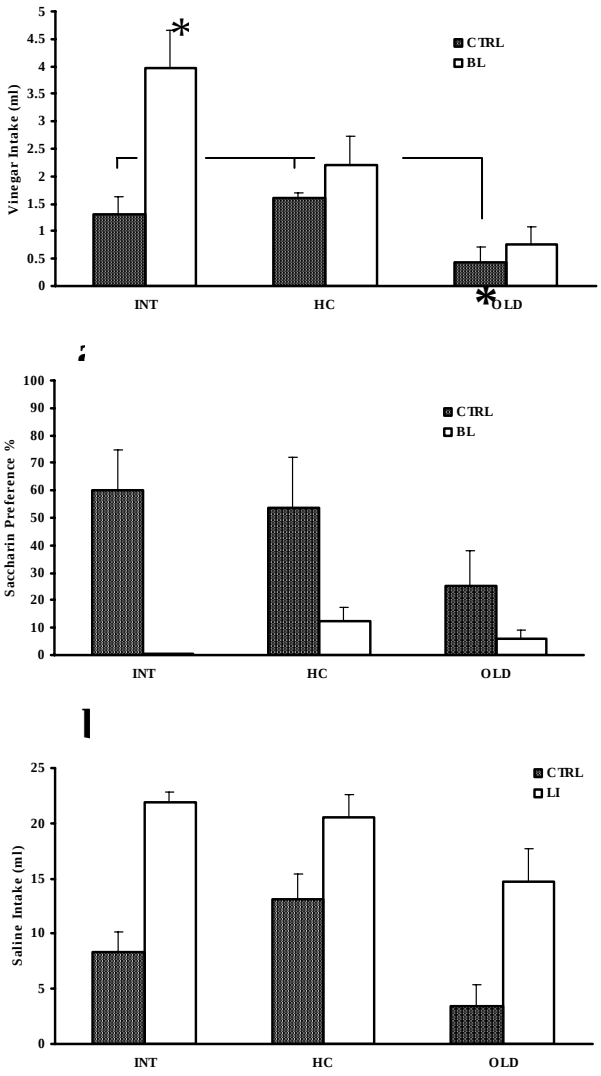

Fig. 1. a) *Mean (S.E.M.) vinegar consumption of the different groups during the one- bottle test in the Exp 1 showing absence of conditioned blocking in hippocampal (HC) and aging (OLD) groups but not in adult (INT)* **b)** *Mean (S.E.M.) saccharin preference rates of the different groups during the saccharin-vinegar choice test in the Exp1* **c)** *Mean (S.E.M.) saline consumption of the different groups during the one-bottle test in the Exp 2 showing the latent inhibition phenomenon in all the groups.*

It was expected that because the BL groups had learned an aversion to saccharin, that they would continue to associate the illness that followed the compound pairing with saccharin and they would show a weaker aversion to the novel vinegar. The CTRL groups on the other hand, would associate the illness with both novel solutions, saccharin and vinegar, showing stronger vinegar aversions.

Results

There were no significant differences between the groups in water intake during the baseline nor the day before testing. No significant differences were found in the saccharin and vinegar intake during the training procedure, except in the saccharin intake during the compound-lithium session. A 3X2 (Group X Behavioral Treatment) ANOVA of the saccharin intake during this session showed a significant effect of group ($F(2,41)=16.275$; $p<0.001$), behavioral treatment ($F(1,41)=171.943$; $p<0.001$), and a significant interaction ($F(2,41)=9.484$; $p<0.001$). BL groups drank less saccharin than CTRL groups, due to the previous saccharin-lithium pairing. There were differences in the INT ($F(1,41)=221.869$; $p<0.001$), HC ($F(1,41)=5.629$; $p<0.04$) and OLD ($F(1,41)=220.200$; $p<0.001$) groups. There were no significant differences among the CTRL groups ($F(1,21)=1.502$; $p<0.2$). However, the saccharin intake differed among the BL groups ($F(1,20)=15.289$; $p<0.001$), due to the fact that the HC group drank more than the INT ($p<0.01$) and the OLD ($p<0.001$) groups. No other difference was significant.

Figure 1a) summarizes the results of the vinegar one-bottle test. As can be seen, CB, with higher vinegar intake, i.e., reduced aversions, in the groups having a previous saccharin-lithium pairing, appeared only in the INT group but not in the HC nor in the OLD groups. A 3X2 (Group X Behavioral Treatment) ANOVA of these results revealed a significant effect of group ($F(2,41)=13.013$; $p<0.00$), behavioral treatment ($F(1,41)=11.74$; $p<0.001$), and a significant interaction ($F(2,41)=4.610$; $p<0.01$). Analysis of the interaction using one-way ANOVA showed a significant effect of the behavioral treatment in the intact groups ($F(1,14)=11.642$; $p<0.00$), but not in the hippocampal groups ($F(1,11)=1.061$; $p<0.32$) nor in the 15-month old groups ($F(1,16)=.568$; $p<0.46$).

A significant effect of group appeared in the CTRL ($F(2,21)=5.061$; $p<0.01$) and the BL groups ($F(2,20)=9.00$; $p<0.001$). Post hoc Newman-Keuls comparisons revealed that the OLD group showed greater aversions than INT in the CTRL ($p<0.05$) and the BL procedures ($p<0.01$). Compared with the HC group, the aversion of the OLD group was greater in the CTRL ($p<0.05$), but did not reach significance in the BL procedure ($p<0.1$). The hippocampal BL groups differed from both intact groups ($p<0.05$) but not from the two CTRL groups.

In summary, it can be concluded that both hippocampus-lesioned and aging intact rats showed an absence of CB that was clearly seen in adult intact rats. However, in the CTRL groups older rats behaved differently than both hippocampal and intact rats, showing a greater vinegar aversion after a single conditioning trial.

The analysis of the total intake during the choice-test revealed a significant effect of group ($F(2,41)=14.428$; $p<0.001$), but no effect of the behavioral treatment ($F(1,41)= 0.081$; $p<0.77$) and no interaction ($F(2,41)=0.563$; $p<0.57$).Old rats drank less than the INT ($P<0.01$) and the HC ($P<0.01$) groups, independently of the behavioral treatment. These results confirm greater aversions to both tastes in aging rats. Then, the use of a choice-test allowed us to explore the relative aversion to both members of the compound avoiding the problem of different total consumption. An 3X2 ANOVA analysis of the saccharin preference ratios (Fig. 1b) showed a significant effect of the behavioral treatment ($F(1,41)=19.009$; $p<0.00$), but no effect of the group ($F(2,41)=1.153$; $p<0.32$) or the interaction ($F(2,41)=1.862$; $p<0.16$). The BL groups showed a stronger aversion to saccharin than the CTRL groups, which could be expected as the BL groups received two saccharin-lithium pairings. However, the similar pattern of results in the aging, the young-adult and the hippocampal groups does not support an explanation for the CB deficit seen in the older animals being based on their greater stimulus generalization. Older rats show stronger aversions but they are able to discriminate between the two taste solutions, as they exhibit aversions of different magnitude to each of them depending on the number of previous training sessions.

Table 1. Behavioral procedures of conditioned blocking (Exp 1) and latent inhibition (Exp 2) Both procedures require a control (CTRL) and a preexposed group: blocking (BL) or latent inhibition (LI) groups (S = Sodium Saccharin; Sal = saline V = Cider Vinegar; W = water; Li = lithium chloride)

		Day							
	Group	1	2	3	4	5	6	7	8
Exp1	CTRL	W	W	W	(S-V) – Li	W	W	V	S vs V
	BL	S - Li	W	W	(S-V) – Li	W	W	V	S vs V
Exp 2	CTRL	W/W	W/W	W/W	Sal – Li	W	W	Sal	------
	LI	Sal/Sal	Sal/Sal	Sal/Sal	Sal – Li	W	W	Sal	------

Experiment 2. Procedure

In experiment 2 forty-five male Wistar rats were divided in three groups as those described above: INT (n=13), HC (n=13) and OLD (n=19). In order to test LI, animals in each group were assigned to one of the behavioral procedures: LI (a = 6, 7, and 10, respectively) or CTRL (a = 7, 6, and 9, respectively). The behavioral procedure is shown in Table 1. The taste solution used was sodium chloride (0.5 %) diluted in tap water. The LI groups received six non-reinforced preexposures to the saline solution. On Days 1, 2 and 3, the saline solution was presented for 15 min twice each day, with a seven hours interval between them. On Day 4, approximately 10 min after drinking the same saline solution, all the animals (LI and CTRL groups) received an i.p. injection of lithium chloride (0.15 M, 2 % b.w.). Thus, CTRL groups received only the acquisition trial without previous preexposures to the saline solution. On Day 5 and 6, all the animals drank water during 15 min. Testing on day 7 was identical for all groups. The saline solution was available for 15 min and the amount ingested was recorded.

Results

No significant differences were seen between the groups in water intake, neither during the baseline nor the day before testing. There were no differences in the saline intake during the behavioral training. Figure 1c shows the results of the one-bottle test. A two way ANOVA showed a significant effect of group ($F(2,2)=6.405$, $p<0.001$) and behavioral treatment ($F(1,2)=33.127$, $p<0.001$), but no interaction ($F(2,39)=0.803$, $p>0.45$). Newman-Keuls analyses showed that the older groups drank less of the taste solution than the INT ($p<0.05$) and the HC ($p<0.01$) groups, again showing greater aversions. However, all groups exhibited the latent inhibition phenomenon, with higher intake of the saline solution in the preexposed groups (LI) compared with their respective non-pre-

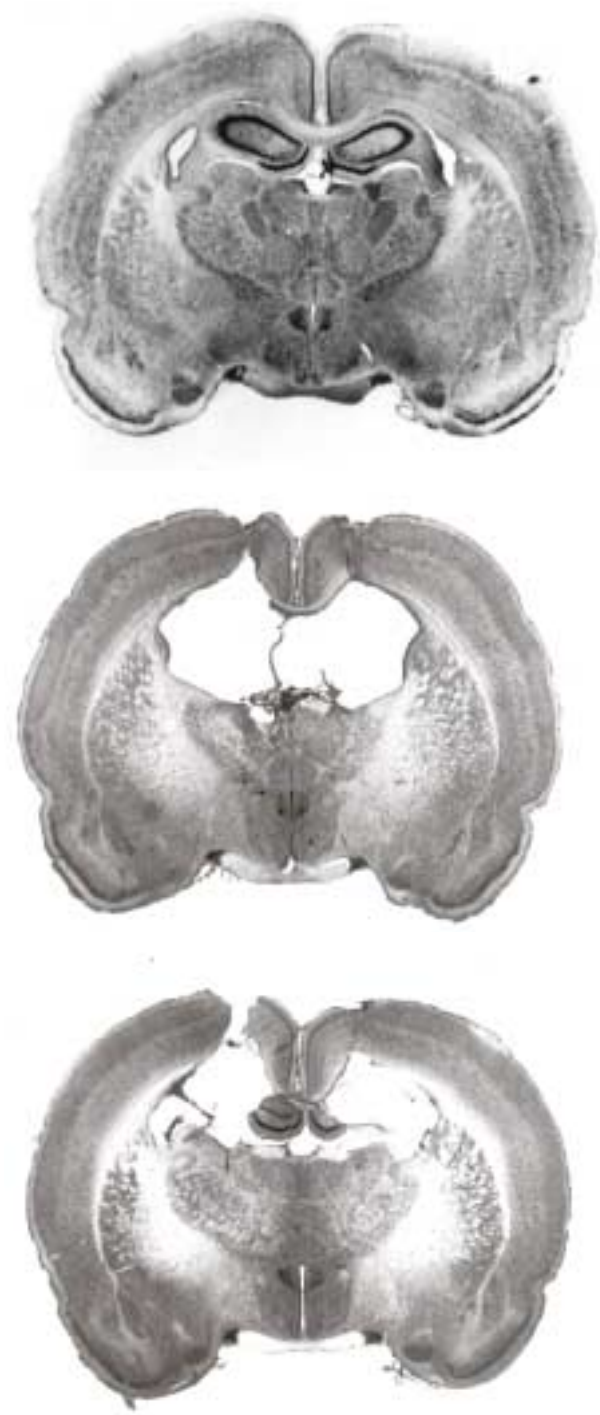

Fig. 2. *Representative coronal sections of intact brains (a) and those showing damage of the dorsal hippocampus in the lesioned groups (b and c) Large lesions were evident affecting in some cases the corpus callosum and cortex dorsal to the hippocampus In some rare cases the lesions extended ventrally slightly affecting thalamic nuclei No correlation was found between the extent of the lesion and the behavioral performance.*

exposed CTRL group (INT: $F(1,11)=41.028$; $p<0.001$; HC: $F(1,11)=6.071$; $p<0.03$; OLD: $F(1,17)=9.586$; $p<0.001$).

Discussion

On one hand, the absence of CB of CTA in aging and hippocampal rats is in agreement with previous results (Gallo Candido 1995a, Gallo Candido 1995b) and supports an explanation of the cognitive impairment induced by aging based on the decline of hippocampal function. Moreover, aging and hippocampal rats exhibit an intact LI phenomenon providing further support for an hippocampal decay explanation of cognitive deficits in senescence. These data demonstrate that normal aging does not induce a general cognitive impairment and add new evidence, consistent with previous studies (Coutoreau *et al.* 1999, Gallo Candido 1995a, Weiner Feldon 1997), to the puzzling results previously reported on the effect of hippocampal lesions in LI (Buhusi *et al.* 1998).

On the other hand, the enhancement of CTA seen in aging animals, is consistent with previous reports (Misanin et al 1988) and cannot be explained by age-related differences in the effect of lithium (Misanin Hinderliter 1994) or context-illness association (Misanin Hinderliter1995). The superior performance of aging rats in CTA seems to be independent of the hippocampal function decay, as it was not seen in hippocampal lesioned animals. It is concluded that aging is not a unitary process and induces complex changes in the functioning of the neural circuits required for taste aversion learning.

Appendix

To better understand our world, science must transcend the contribution of specific individuals. However, each scientist and each field of knowledge recognizes their debt to particular contributions that qualitatively changed the course of events and paved the way for subsequent advances. This has been my experience concerning the influence of Olga Burešova and Jan Bureš. I (M. Gallo) first came into contact with Jan through Olga, who generously, without knowing me personally, helped me with my first paper written in English. Since that time I have had several opportunities to admire Jan´s firmness, unselfishness, humbleness, patience, open mind, sense of humor and enthusiasm, a rare combination in one person. His appealing personality was always offering new

surprises, such as his hidden mastery of Spanish or his tremendous memory for subtle details. Working in his lab in Prague during the early nineties allowed me to meet an interdisciplinary team, showing Jan´s abilities to integrate different techniques and approaches. Moreover, I am proud of having been a witness of a critical discovery in the field of taste aversion, that took place at that time: the location of the associative mechanisms in the parabrachial nucleus. The contribution of Jan's work to this challenging issue can only be appreciated by considering the evolution of the field during the last 30 years. The spreading depression work of Olga and Jan in the early seventies, allowed a cortical location for the associative mechanisms to be excluded and pointed to a lower brain site. In spite of the unproductive predominant belief during the seventies and eighties that excluded the brain stem as a potential location, Jan persevered and took advantage of reversible lesion techniques. In 1990 Ivanova and Bureš were the first to suggest a role for the parabrachial area in the "gustatory trace-poisoning association". Results consistent with the initial findings have been accumulated and the notion is now widely accepted in the field. Thanks to this critical contribution, the study of the neural mechanisms of taste aversion learning is at present an active and rapidly evolving area.

Acknowledgements

This research was supported by the CICYT grants PB95-01182, PB98-1309 and PB98-1362 (Spain). Morón was recipient of a predoctoral grant of the Junta de Andalucía (Spain). The authors are greatly indebted to J. Bureš and A. Fenton for reviewing a draft of this paper.

References

BUHUSI CV, GRAY JA, SCHMAJUK NA: Perplexing effects of hippocampal lesions on latent inhibition: a neural network. *Behav Neurosci* **112**: 316-351, 1998.

BUREŠ J: Critical appraisal of behavioral tests used for evaluation of age-related memory deficits in animals. *Neurosci Res Comm* **13**: S35-S38, 1993.

COUTOREAU E, GALANI R, GOSSELIN O, MAJCHRZAK M, DI SCALA G: Entorhinal but not hippocampal or subicular lesions disrupt latent inhibition in rats. *Neurobiol Learn Mem* **72**: 143-157, 1999.

GALLAGHER M: The use of animal models to study the effects of aging on cognition. *Ann Rev Psychol* **48**: 339-370, 1997.

GALLO M, CÁNDIDO A: Dorsal hippocampal lesions impair blocking but not latent inhibition of taste aversion learning in rats. *Behav Neurosci* **109**: 1-13, 1995a.

GALLO M, CÁNDIDO A: Reversible inactivation of dorsal hippocampus by tetrodotoxin impairs blocking of taste aversion selectively during the acquisition but not the retrieval in rats. *Neurosci Lett* **186**: 1-4, 1995b.

GALLO M, BALLESTEROS MA, MOLERO A, MORÓN I: Taste aversion learning as a tool for the study of hippocampal and non-hippocampal brain memory circuits regulating diet selection *Nutrit Neurosci* **2**: 277-302, 1999.

GALLO M, VALOUSKOVÁ V, CÁNDIDO A: Fetal hippocampal transplants restore conditioned blocking in rats with dorsal hippocampal lesions: Effect of age. *Behav Brain Res* **88**: 67- 74, 1997.

HINDERLITER CHF, MISANIN JR: Age differences and the interstimulus interval context in long-delay taste-aversion conditioning in rats. *Psychol Rep* **76**: 636-638, 1995.

HONEY RC, GOOD M: Selective hippocampal lesions abolish the contextual specificity of latent inhibition and conditioning. *Behav Neurosci* **107**: 23-33, 1993.

KAMIN LJ: Predictability surprise attention and conditioning In: *Punishment and Aversive Behavior*, CHURCH R CAMPBELL B (eds), Appleton-Century-Crofts, New York, 1969, pp 279-296.

LUBOW RE: *Latent Inhibition and Conditioned Attention Theory*. Cambridge University Press, New York, 1989.

LUBOW RE: Latent Inhibition. *Psychol Bull* **79**: 398-407, 1973.

MISANIN JR, HINDERLITER CF: Lack of age differences in context-illness associations in the long-delay taste-aversion conditioning of rats. *Percept Mot Skills* **80**: 595-598, 1995.

MISANIN JR, HINDERLITER CF: Efficacy of lithium chloride in the taste-aversion conditioning of young-adult and old-age rats. *Psychol Rep* **75**: 267-271, 1994.

MISANIN JR, GREIDER DL, HINDERLITER ChF: Age differences in the outcome of long-delay taste-aversion conditioning in rats. *Bull Psychon Soc* **26**: 258-260, 1988.

PAXINOS G, WATSON C: *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego, 1986.

REILLY S, HARLEY C, REVUSKY S: Ibotenate lesions of the hippocampus enhance latent inhibition in conditioned taste aversion and increase resistance to extinction in conditioned taste preference *Behav Neurosci* **107**: 966-1004, 1993.

WEINER I, FELDON J: The switching model of latent inhibition: an update of neural substrates *Behav Brain Res* **88**: 11-25, 1997.

Reprint requests

M. Gallo, Department of Experimental Psychology and Physiology of Behavior, University of Granada, Campus Cartuja, Granada ,E-18071 Spain. Phone:34-58-243771 fax: 34-58-246239 e-mail: mgallo@platonugres