Psicológica (2004), 25, 207-216.

Latent inhibition as a function of US intensity in a two-stage CER procedure

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An experiment is reported in which the effect of unconditioned stimulus (US) intensity on latent inhibition (LI) was examined, using a two-stage conditioned emotional response (CER) procedure in rats. A tone was used as the pre-exposed and conditioned stimulus (CS), and a foot-shock of either a low (0.3 mA) or high (0.7 mA) intensity was used as the US. A 2 x 2 factorial design was employed. The first factor was the pre-exposure condition (72 pre-exposures or non-pre-exposure) and the second was foot-shock intensity (low or high). A more durable LI effect was observed in the low-intensity condition than in the high-intensity condition during conditioning trials. The authors discuss the possibility that US intensity modulates either a process of restoring attention to the CS during conditioning or a contextual change, responsible for attenuating the LI effect in the high-intensity condition with respect to the low-intensity condition.

It is well known that non-reinforced pre-exposure to the to-be-conditioned stimulus (CS) retards the acquisition process of the conditioned response (CR) during subsequent conditioning. This phenomenon is termed *latent inhibition* (LI; Lubow and Moore, 1959). By definition, a demonstration of the LI effect requires, at least, a two-stage procedure: a pre-exposure stage in which the CS is presented in isolation and a conditioning stage in which the CS is paired with an unconditioned stimulus (US). Usually, the magnitude of the LI effect is measured by comparing the CR showed by a pre-exposed group to that showed by a control group without prior experience with the CS. This comparison can be made during the conditioning stage itself (two-stage procedure) or in a additional test stage following the conditioning period (three-stage procedure).

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* Acknowledgements: This research project was supported by grants from the Basque Government (PI97/35) and from the Spanish Ministry of Science and Technology (PB98-0230). A Predoctoral Fellowship from the University of the Basque Country (UPV/EHU) also supported the research of the first author. Thanks are due to A. S. Lombas for his helpful comments and support. Requests for reprints should be addressed to G. Alonso, Facultad de Psicología, Universidad del País Vasco, Avenida de Tolosa 70, 20018 San Sebastián, Spain. E-mail: pbpalmag@ss.ehu.es

The experiment reported here examined the effect of US intensity on the magnitude of LI. Given that LI originates during the pre-exposure stage, it could be thought that the intensity of the US presented during conditioning should not affect the magnitude of this phenomenon. Nevertheless, LI reflects a balance between what the subject learns about the CS during both pre-exposure and conditioning. The impact of that learnt during conditioning might modulate the magnitude of the LI effect, affecting not so much its genesis, but rather its detection. The least interesting, but nevertheless plausible, possibility is that the use of a very intense US may mask the LI effect. In Pavlovian conditioning, it is widely accepted that the more intense the US used, the higher both the rate at which conditioning occurs and the asymptotic level of the CR (e.g. Rescorla and Wagner, 1972). In a LI experiment, if the maximum level of performance is reached after just a few conditioning trials, differences in acquisition of conditioning performance between pre-exposure and non-pre-exposure conditions could be obscured by a ceiling effect. In this case, the implication of the effect of US intensity on LI would be reduced to a simple operational question: the use of a relatively intense US might minimize sensitivity for detecting the effects of pre-exposure. Nevertheless, the demonstration of an effect produced by US intensity, thereby dismissing the possibility of masking, might reveal the intervention of another type of process in the detection of LI.

Few studies have examined the effect of US intensity on LI (De la Casa and Lubow, 2000; Ruob, Weiner and Feldon, 1998; Weiner, Bernasconi, Broersen and Feldon, 1997a; Weiner, Tarrasch, Bernasconi, Broersen, Rüttiman and Feldon, 1997b). All these studies used three-stage procedures. De la Casa and Lubow (2000; Experiments 1 and 2) used the conditioned taste aversion procedure with rats, examining the effect of the time interval between the conditioning and test stages on LI at two US intensity levels. The three remaining studies used the conditioned emotional response (CER) procedure in licking with rats, examining the effects of administering haloperidol (Ruob et al., 1998) or amphetamine (Weiner et al., 1997a, 1997b) on LI at two US intensity levels. Only one of the experiments reported by De la Casa and Lubow (2000; Experiment 2) provided data which points to the existence of a relationship between US intensity and LI. In this experiment, a greater LI was observed in the high-intensity condition than in the low-intensity condition when testing was conducted 21 days after conditioning; US intensity was observed to have no effect on LI when testing was conducted just 2 days after conditioning.

Given that all the evidence regarding the effect of US intensity on LI comes from studies which have used three-stage procedures, the experiment reported here was designed, as a preliminary study, to explore that effect in a two-stage procedure, in order to confirm and extend the generalization of the aforementioned results. A 2 x 2 factorial design was used in a CER paradigm in lever press response with rats, one factor being the pre-exposure condition (pre-exposure or no-pre-exposure) and the other the intensity of the foot-shock employed as the US during conditioning (high or low). The effects of pre-exposure to a tone were measured during 6 trials conducted in a single

conditioning session, in which the tone was paired with either a strong or weak foot-shock.

METHOD

Subjects. The subjects were 32 male Wistar rats with a mean ad lib. weight of 395 g (range: 321-515 g). They had previously been used in a conditioned flavor-aversion experiment, but they were naive to the procedure and stimuli used in the present experiment. The animals were maintained at 80% of their free-feeding weights by daily restricted feeding. They were housed in pairs in standard stainless steel and wire mesh cages located in vivarium maintained on a 12: 12-h light: dark cycle with light on at 08:00 AM. All the experimental procedures were conducted in a room away from the vivarium during the light portion of the cycle.

Apparatus. Eight identical Skinner boxes supplied by Coulbourn Instruments were used. Each chamber was housed in a light- and sound-attenuating box and contained a single lever located to the left-hand side of a food tray connected to an external 45-mg pellet dispenser. The floor of the chamber consisted of 16 stainless steel rods, 6 mm in diameter and spaced 1.5 cm apart. A speaker was located over the lever, through which a 4.5 kHz tone of 85 dB could be delivered. A ventilation fan provided a 40-dB background masking noise. The chamber was not illuminated during the course of the experiment. Equipment programming and data recording were computer-controlled.

Procedure.

Pre-training. Rats initially received magazine training sessions. Each session had a maximum duration of 60 min. In each, food pellets were delivered on a variable-time (VT) 60-sec schedule while lever press responses were continuously reinforced. Each rat finished magazine training when it made 100 lever press responses.

Training of the lever press response (baseline). Rats received twelve further sessions training the lever press response. Responding was reinforced by a single food pellet delivered according to a variable interval (VI) 30-sec schedule during the first session. In the remaining sessions, reinforcement was delivered according to a VI 60-sec schedule.

Pre-exposure. The rats were then randomly assigned to four groups (n = 8). For the next twelve sessions all groups responded in accordance with the VI 60-sec schedule. During each of these sessions two groups received six 90-sec presentations of the tone CS without presentations of the foot-shock US that was used later in the conditioning stage (PE condition). The inter-trial interval (ITI) was variable, with a mean of 360 sec. The other two groups (NPE condition) received equal lever press experience but did not receive the tone.

Conditioning. During the following session conditioning was conducted for all groups with the tone CS. Six on-baseline trials were given. The ITI was variable, with a mean of 360 sec. In each trial, the tone was followed immediately by the US (foot-shock). One group from each pre-exposure condition received either a 0.3-sec, 0.3 mA foot-shock (Groups PE-Low and NPE-Low) or a 0.3-sec, 0.7 mA foot-shock (Groups PE-High and NPE-High).

Measurement and analysis of suppression. Suppression to the CS was measured by a ratio A/(A + B), where A represents the number of lever presses made during the 90-sec CS and B the number of lever presses made during the 90-sec period immediately prior to the onset of the stimulus (the pre-CS scores). A ratio of 0 indicates complete response suppression during the CS, whereas a ratio of 0.5 indicates no suppression during the CS. The reliability of the suppression ratios and the pre-CS scores was assessed against a Type I error rate of $p = .05$.

RESULTS

Figure 1 presents mean suppression ratios to the tone for the pre-exposed (PE) and non-pre-exposed (NPE) groups during conditioning trials with low-intensity (panel a) or high-intensity (panel b) shocks. As can be seen in the figure, in general, higher shock intensity produced stronger suppression (i.e. a lower ratio) than lower intensity. In addition, the figure suggests a more durable LI effect (i.e. lower suppression of the PE in comparison with the NPE Group) in the low-intensity condition than in the high-intensity condition. These impressions were confirmed by a three-way analysis of variance (ANOVA), examining the pre-exposure condition (PE vs. NPE), shock intensity (low vs. high) and trial. The ANOVA revealed significant main effects of the pre-exposure condition, $F(1, 28) = 6.78$, shock intensity, $F(1, 28) = 34.36$, and trial, $F(5, 140) = 21.18$. The pre-exposure condition x shock intensity and shock intensity x trial interactions were significant, $F(1, 28) = 5.12$, and $F(5, 140) = 6.86$, respectively. The pre-exposure condition x trial interaction was non-significant, $F(5, 140) = 1.32$. The three-way pre-exposure condition x shock intensity x trial interaction was significant, $F(5, 140) = 2.97$. Subsequent tests then demonstrated that the effect of the pre-exposure condition was significant in Trial 2, $Fs(1, 28) = 15.41$, but not in Trials 1, 3, 4, 5 and 6, $Fs < 3.1$, $ps > 0.09$. Furthermore, there was a significant pre-exposure condition x shock intensity in Trials 3, 4 and 5, $Fs(1, 28) > 6.19$, but not in Trials 1, 2 and 6, $Fs(1, 28) < 1$. Interaction in those trials was due to an effect of pre-exposure on conditioning with the low-intensity shock, $Fs(1, 14) > 7.12$, but not with the high-intensity shock, $Fs < 1.18$, $ps > .29$. That is, a more durable LI was observed in the low-intensity condition than in the high-intensity condition.

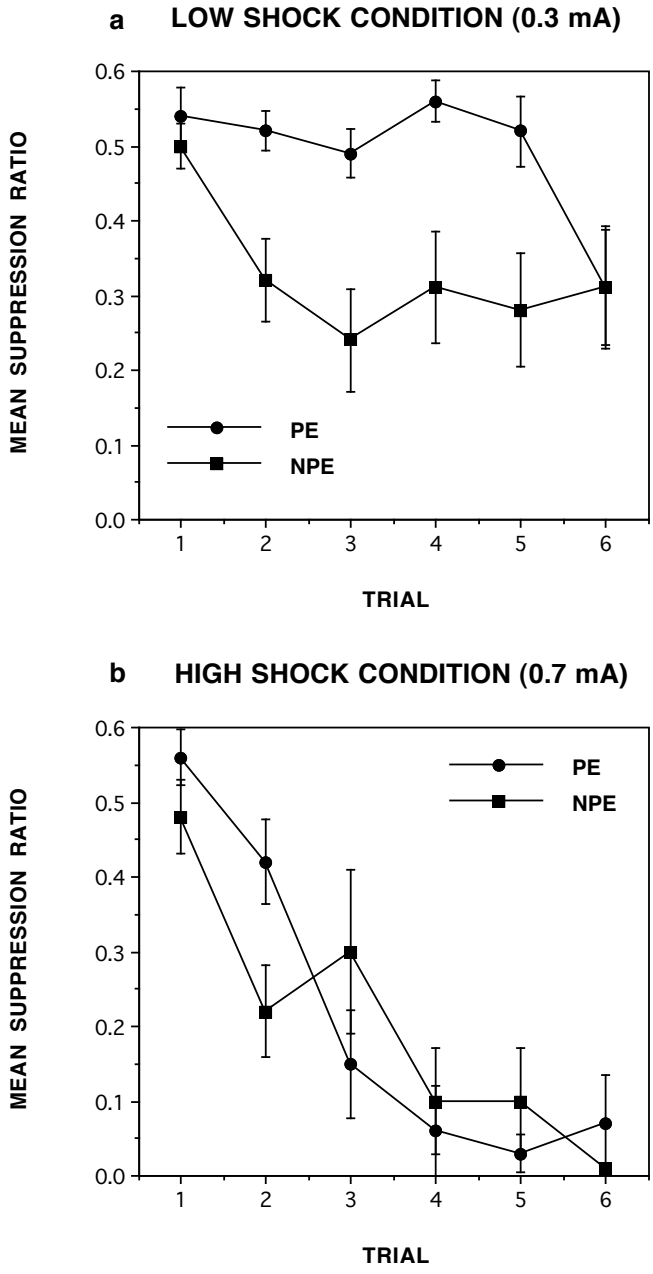

Figure 1. Means and standard errors of suppression ratios during conditioning trials for pre-exposed (PE) and non-pre-exposed (NPE) animals conditioned with low (panel a) or high (panel b) shock intensity.

The mean pre-CS rates of lever pressing during the conditioning session (computed by pooling the scores for the six pre-CS periods) were 22.4, 21.3, 11.7 and 18.7 responses per min, for Groups PE-Low, NPE-Low, PE-High and NPE-High, respectively. There is some indication that pre-CS rates were lower in the high-intensity condition than in the low-intensity condition, and this difference could be reflecting a greater contextual conditioning in the former than in the latter. However, a pre-exposure condition x shock intensity ANOVA using these rates revealed no significant main effects or interaction, $\underline{F}s < 2.03$, $\underline{p}s > .16$, suggesting that our results were not critically influenced by any differential responding during the pre-CS period.

Planned comparisons of the performance of the NPE-Low and NPE-High control groups indicated that it is unlikely that the shorter duration of the LI in the high-intensity condition was the result of a masking or ceiling effect. The intensity of the shock determined the maximum level of response in these groups: the final suppression level showed by the NPE-High Group in Trial 6 was higher than that demonstrated by the NPE-Low Group in any of the six conditioning trials, $\underline{F}s(1, 14) > 10.94$. This difference in the maximum level of response of control groups might have hindered the detection of LI to a greater extent in the lower-intensity condition. Since the animals in the NPE-Low Group showed a lower margin of performance (from a ratio of around 0.5 in the absence of suppression to a suppression ratio of around 0.3, as the apparent asymptotic level of conditioning) than those in the NPE-High Group, the sensitivity to detecting a retard in the acquisition of CR might have been lower in the low-intensity condition than in the high-intensity condition.

Furthermore, unexpectedly, the NPE-Low Group reached its corresponding maximum level of response (a suppression ratio of around 0.3) in less trials than the NPE-High Group (who showed a suppression ratio of around 0 as a maximum level of suppression). The effect of trial was significant in both groups, $\underline{F}s (5, 35) > 3.31$, demonstrating that both shock intensities used as the US resulted in effective conditioning. Post-hoc comparisons between pairs of trials using the t-test revealed that the suppression level showed by the NPE-Low Group in Trial 1 was significantly lower than the level showed in the rest of the trials. No differences were observed between the suppression levels demonstrated by this group in Trials 2, 3, 4, 5 and 6. That is, the NPE-Low Group apparently reached its maximum suppression level in only one trial. Nevertheless, the suppression level showed by the NPE-High Group in Trial 1 was significantly lower than that demonstrated in the rest of the trials, and the suppression level shown in Trials 2 and 3 was also significantly lower than that shown in Trial 6. In other words, the NPE-High Group reached its maximum suppression level in more trials than the NPE-Low Group. This result suggests that the less durable LI observed in the high-intensity condition cannot be attributed to a ceiling effect.

DISCUSSION

The results obtained in this experiment suggest the existence of an inversely proportional relationship between the intensity of the US employed during conditioning and the magnitude of the LI effect in a two-stage procedure. The lower the intensity of the US, the greater the LI effect observed. The relationship found is difficult to explain in terms of a masking of the LI effect in the high-intensity condition. The performance of the non-pre-exposed groups seems to indicate that the conditions for detecting LI were less favorable in the low-intensity condition (since the control group in that condition showed a narrower margin of performance and reached their corresponding maximum suppression level in less trials than the control group in the high-intensity condition). In this sense, the results could perhaps be more easily explained if we assume that US intensity controlled some process capable of affecting the magnitude of LI. There are at least two potential mechanisms by which these results could be explained.

The first possibility is that US intensity may have modulated the amount of attention paid to the CS during conditioning. LI has been most often attributed to a decrement in attention to (or in the associability of) the CS, which prevents the formation of a strong CS-US association (e.g. Lubow, Weiner and Schnur, 1981; Mackintosh, 1975; Pearce and Hall, 1980; Wagner, 1981). According to this account of latent inhibition, the unexpected occurrence of the US during conditioning restores attention to the CS that was lost during pre-exposure. This mechanism would allow the formation (albeit somewhat retarded) of the CS-US association and is proportional to the intensity of the US. In other words, the more intense the US employed during conditioning, the more readily and effectively the effects of pre-exposure are counteracted. According to this hypothesis, pre-exposed subjects from the high-intensity condition will have redirected their attention to the CS during conditioning more quickly than subjects from the low-intensity condition. This in turn will have facilitated the formation of the CS-US association, thereby attenuating the LI effect more in the former than in the latter.

A second possibility is that the US intensity may have exerted a contextual control over LI. It is well established that LI is context-specific and as such, pre-exposure to a CS in one context has little or no effect on subsequent conditioning in a different context (e.g. Hall and Minor, 1984; Hall and Channell, 1986; Lovibond, Preston and Mackintosh, 1984). This evidence is congruent with those theories which assume that the cause of LI is the formation of an association between the pre-exposure context and the CS (McLaren and Mackintosh, 2000; Miller and Matzel, 1988; Wagner, 1981), or a retrieval failure in which the context plays a key role as a retrieval cue of conflictive associations (Bouton, 1993). In order to assume that US intensity may exert a contextual control over LI, it is necessary to first accept that the introduction of a US during conditioning itself constitutes a contextual change. From the first conditioning trial onwards, the after-effects of the US may generate a novel context, which would be different from the pre-exposure context (Hall, 1991, p. 204). Evidence consistent with this notion comes from

the study reported by Killcross and Dickinson (1996). This study found that although 12 stimulus pre-exposures did not produce a measurable LI effect in normal circumstances, a significant retardation was found of both excitatory and inhibitory conditioning when pre-exposures to the CS occurred during a session in which subjects were also exposed to the US explicitly unpaired with the CS. Killcross and Dickinson (1996) interpreted these results in terms of contextual specificity of LI. The presence of the US during the pre-exposure stage would have equated the pre-exposure and conditioning contexts, therefore facilitating the transfer of the learning that mediates LI. If we accept that the occurrence of the US during conditioning may generate a contextual change, it seems logical to assume that the more intense the US, the more easily distinguishable the pre-exposure and conditioning contexts would be. According to this hypothesis, in the high-intensity condition of our experiment a more pronounced contextual change would have occurred than in the low-intensity condition, and therefore the transfer of the learning about the CS during pre-exposure might have been lower in the former than in the latter. This hypothesis could also explain the results found by De la Casa and Lubow (2000; Experiment 2), even though these are opposite from the results found in our experiment. It should be remembered that in the experiment reported by De la Casa and Lubow a three-stage procedure was used. If the US intensity exerted a contextual control over LI in this experiment, then the conditioning context would have been more distinguishable from the pre-exposure and test contexts in the high-intensity condition than in the low-intensity condition. If this was so, then a poorer transfer to the test of that learned during conditioning (CS-US association), coupled with a better transfer of that learned during pre-exposure, might have occurred in the high-intensity condition than in the low-intensity condition. This would explain the direct relationship observed between US intensity and LI. The fact that this relationship was detected when the test was conducted 21 days after conditioning and not when it was conducted 2 days after conditioning might also reflect the effect of the time interval on discriminability between the different experiment stages. A longer time interval between the conditioning and test stages might result in an enhancement of discrimination between the two (Bouton, 1993, p.91), thereby enabling the effect of US intensity on LI to be observed.

In summary, the results of our experiment suggest that the intensity of the US used during conditioning is a crucial variable which may determine the magnitude of the LI effect. The possibility that US intensity may control either a process of restoring attention to the CS during conditioning or a contextual change requires more in-depth, empirical analysis in the future, due to its important implications for LI theories and its possible relationship with the processes these theories identify as the cause of the phenomenon.

RESUMEN

Inhibición latente en función de la intensidad del EI en un procedimiento REC de dos fases. En el experimento que presentamos se examinó el efecto de la intensidad del estímulo incondicionado (EI) sobre la inhibición latente (IL), empleando un procedimiento de respuesta emocional condicionada (REC) de dos fases, con ratas como sujetos experimentales. Se utilizó un tono como estímulo preexpuesto y estímulo condicionado (EC), y una descarga, de intensidad baja (0.3 mA) o alta (0.7 mA), como EI. Se empleó un diseño factorial 2 x 2. El primer factor fue la condición de preexposición (72 preexposiciones o sin preexposición) y el segundo factor fue la intensidad de la descarga (baja o alta). Se observó un efecto de IL más duradero en la condición de intensidad baja que en la condición de intensidad alta. Se discute la posibilidad de que la intensidad del EI module o bien un proceso de restauración de la atención al EC durante el condicionamiento o bien un cambio contextual, responsable de la atenuación del efecto de IL en la condición de intensidad alta, respecto a la condición de intensidad baja.

REFERENCES

Bouton, M. E., 1993. Context, time and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin 114*, 80-99.

De la Casa, L. G., Lubow, R. E., 2000. Super-latent inhibition with delayed conditioned aversion testing. *Animal Learning & Behavior 28*, 389-399.

Hall, G., 1991. *Perceptual and Associative Learning*. Clarendon Press, Oxford.

Hall, G., Minor., H., 1984. A search for context-stimulus associations in latent inhibition. *Quarterly Journal of Experimental Psychology 36B*, 145-169.

Hall, G., Channel, S., 1986. Context specificity on latent inhibition in taste aversion learning. *Quarterly Journal of Experimental Psychology, 38B*, 121-139.

Killcross, S., Dickinson, A., 1996. Contextual control of latent inhibition by the reinforcer. *Quarterly Journal of Experimental Psychology 49B*, 45-59.

Lovibond, P., Preston, G. C., Mackintosh, N. J., 1984. Contextual control of conditioning and latent inhibition. Journal of Experimental Psychology: *Animal Behavior Processes 10*, 360-375.

Lubow, R.E., and Moore, A. U., 1959. Latent inhibition: the effect of nonreinforced pre-exposure to the conditional stimulus. *Journal of Comparative and Physiological Psychology, 52*, 451-419.

Lubow, R. E., Weiner, I., Schnur, P., 1981. Conditioned attention theory. In: Bower, G. H. (Ed), *The Psychology of Learning and Motivation, vol 15.* Academic Press, New York, pp. 1-49.

Mackintosh, N. J., 1975. A theory of attention: Variations in the associability with reinforcement. *Psychological Review 82*, 276-298.

McLaren. I. P. L., Mackintosh, N. J., 2000. An elemental model of associative learning: I. Latent inhibition and perceptual learning. *Animal Learning & Behavior 28*, 211-246.

Miller, R. R., Matzel, L. D., 1988. The comparator hypothesis: A response rule for the expression of associations. In: Bower, G. H. (Ed), *The Psychology of Learning and Motivation, vol 22.* Academic Press, San Diego, CA, pp. 51-92.

Pearce, J. M., Hall, G., 1980. A model for Pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review 87*, 532-552.

Rescorla, R. A., Wagner, A. R., 1972. A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In: Black, A. H., Prokasy, W. F. (Eds), *Classical conditioning II: Current research and theory*. Appleton-Century-Crofts, New York, pp. 64-99.

Ruob, C., Weiner, I., Feldon, J., 1998. Haloperidol-induced potentiation of latent inhibition: interaction with parameters of conditioning. *Behavioural Pharmacology 9*, 245-253.

Wagner, A. R., 1981. SOP: A model of automatic memory processing in animal behavior. In: Spear, E., Miller, R. R. (Eds), *Information processing in animals: Memory mechanisms*. Erlbaum, Hillsdale, NJ, pp. 5-47.

Weiner, I., Bernasconi, E., Broersen, L. M, Feldon, J., 1997a. Amphetamine-induced disruption of latent inhibition depends on the nature of the stimulus. *Behavioural Pharmacology 8*, 442-457.

Weiner, I., Tarrasch, R., Bernasconi, E., Broersen, L.M., Rüttiman, T.C., Feldon, J., 1997b. *Pharmacology Biochemistry and Behavior 56,* 817-826.

(Manuscript received: 27 January 2004; accepted: 1 April 2004)