ACTA DE INVESTIGACIÓN PSICOLÓGICA, 2011, 1 (1), 14-22

Effects of Scopolamine on Conditioning of Lever Pressing

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Abstract

The aim of this work was to determine the effects of scopolamine, a cholinergic antagonist, on the conditioning of an instrumental response and the contextual conditioning of this response. Five groups of rats were trained to lever-press on a Variable Interval 30 s schedule in context A. Scopolamine was administered 15 min before each conditioning session to AB 0.01 mg/kg, AB 0.10 mg/kg and AB 1.00 mg/kg groups. The AA Saline and AB Saline groups received saline injections. Contextual conditioning of the lever-pressing response was assessed in one extinction session. The AA group received this extinction session in the conditioning context (A), while the AB groups received this session in a different context (B). Results showed that scopolamine impaired the conditioning of the lever-pressing response but no effects on contextual conditioning were found.

Key Words: Scopolamine, Instrumental conditioning, Rats, Context.

Efectos de la Escopolamina en el Condicionamiento de Presiones a la Palanca

Resumen

El propósito de este trabajo fue determinar los efectos de la escopolamina, un antagonista colinérgico, sobre el condicionamiento de una respuesta instrumental así como sobre el condicionamiento contextual de esta respuesta. Se entrenó a cinco grupos de ratas a presionar una palanca bajo un programa de Intervalo Variable 30 s en el contexto A. 15 min antes de cada sesión de condicionamiento se administró escopolamina en dosis de 0.01, 0.10 y 1.00 mg/kg a grupos diferentes en el contexto A, mientras dos grupos de ratas recibieron solución salina en el contexto A. El condicionamiento contextual de la respuesta de palanqueo se evaluó en una sesión de extinción en el contexto A o en un contexto B diferente. Los resultados mostraron que la escopolamina obstaculizó el condicionamiento de la respuesta de presionar la palanca pero no se encontraron efectos sobre el condicionamiento contextual.

Palabras Clave: Escopolamina, Condicionamiento instrumental, Ratas, Contexto.

[1] The Dirección General del Personal Académico, UNAM, grant PAPIIT IN307509, conceded to Javier Nieto financed this research. Correspondence regarding this paper may be addressed to Livia Sánchez-Carrasco to lyvia.sanchez@gmail.com.

Forgetting has been defined as the inability to remember or retrieve information previously learned (Baddeley, 1999; Ebbinghaus, 1885) or the total and permanent loss of information (Loftus & Loftus, 1980); however, current theories suggest that the information is preserved when it is properly learned and codified (Capaldi & Neth, 1995; Rescorla, 2001). Thus, forgetting is featured as a temporal failure in the retrieval of information, and according to McGeoch (1932) it is determined by "alterations in the stimulus or environmental conditions". Likewise, extinction is a classic example in which removing reinforcement produces a decrement of responding. Some authors (e.g., Rescorla & Wagner, 1972) have argued that such a reduction in responding results from the weakening or loss of associations established in the brain as the result of prior conditioning. Nevertheless, Capaldi (1994) and Bouton (1994) have proposed that such information is preserved and that the environmental cues (i.e., the context) determine its retrieval. In short, forgetting and extinction might be considered as a result of changes in the contextual cues rather than the simple loss of information (Bouton, 1994; Capaldi & Neth, 1995).

In agreement with these ideas, a large body of evidence (see Bouton, 1994; Delamater, 2004; Rescorla, 2001; Sánchez-Carrasco & Nieto, 2009) supports the claim that learning not evident in a moment might be available in a different circumstance or time. Therefore, the study of phenomena such as desinhibition, spontaneous recovery, renewal, and reinstatement where the recovery of an extinguished response is observed, might provide significant insights about the mechanisms (e.g., cognitive, pharmacological, neurobiological and neuroanatomical) of remembering and forgetting (Bouton, 1993; Quirk & Muller, 2008; Rescorla, 2001).

As we mentioned, contextual cues appear to have ubiquitous effects on the retrieval of memories (Bouton, 1994; McGeoch, 1932). For example, there is some evidence that shows recovery of a conditioned response, either in Pavlovian or instrumental procedures, by exposing subjects to a different context to that employed during extinction (contextual renewal, Bouton & Bolles, 1979; Bouton & Ricker, 1994). Additionally, some experiments have shown a reduction in the magnitude of the conditioned response by the mere change of context (Bouton & Peck, 1990; Hall & Honey, 1989).

In relation to the neural and pharmacological mechanisms, some experiments support hippocampal involvement in acquiring and consolidating the context representation. Since acetylcholine contributes to the production of hippocampal theta rhythm, some researchers have proposed that learning alterations produced by cholinergic antagonism result from the blockade of atropine-sensitive theta rhythm impairing hippocampal function (Vanderwolf, Kramis, & Robinson, 1978). For example, disruption of theta rhythm by cholinergic antagonists attenuates hippocampal plasticity in vitro, while the facilitation of theta rhythm enhances significantly contextual fear conditioning and hippocampal long-term potentiation (LTP) (Maren & Fanselow, 1995). Thus, these findings provide a possible mechanism for the observed effects of cholinergic antagonist on memory (Huerta & Lisman, 1995). Accordingly, tasks that are sensitive to hippocampal damage are also impaired by cholinergic antagonism. For example, Anagnostaras,

Maren, and Fanselow (1995) and Rudy (1996) have designed some experiments to analyze the physiological and pharmacological processes involved in contextual conditioning. Particularly, they have analyzed the role of cholinergic systems using scopolamine, an antagonist of cholinergic systems, on contextual fear conditioning.

Anagnostaras et al. (1995) designed an experiment to evaluate the effects of cholinergic antagonism in a contextual fear-conditioning paradigm; rats were given scopolamine (1.00 mg/kg) for 3 days either before or three days after the conditioning session in which a tone was paired with an aversive footshock. Fear to both context and tone was assessed in independent test sessions. Their results showed that scopolamine injections impaired contextual fear conditioning if administered prior to, but not after conditioning; while, conditioning of the tone was not affected in either condition. Also, Rudy (1996) found that the same dose of scopolamine disrupted contextual fear conditioning more than fear conditioning to the tone when administered prior to conditioning. However, Rudy found that post-conditioning administration of scopolamine also impaired contextual fear conditioning.

The present study was designed to provide further evidence on the participation of cholinergic systems on the acquisition of lever pressing, as well as on conditioning of contextual cues. Specifically, it was designed to assess the effects of scopolamine injections on contextual conditioning using an instrumental appetitive procedure with rats. In this experiment subjects were assigned to five groups, which received one of three different doses of scopolamine or of saline 15 min prior to the onset of each of the two acquisition sessions. During the acquisition phase all groups were trained to lever press in Context A. Afterwards, all groups received one extinction session in Context A for one group and in Context B for the other groups.

Method

Subjects

45 three-month old male Wistar rats obtained from the School of Psychology own breeding colony were used; they were food-deprived at 80% of their ad libibitum body weights, and were housed in individual cages with free access to water and restricted access to food.

Apparatus

Six experimental chambers (model ENV-001, MED® Associates) for rats were used. The chambers measured 20.8 (height), 21.0 (wide) and 28.2 (length) cm. The walls and ceiling were of acrylic and the frontal and rear panels were stainless steel. Each chamber had a floor made of 0.5 cm diameter stainless steel rods that were separated 1.7 cm. A lever (4.5 cm length by 0.1 cm thick) in the frontal panel was placed 7.1 cm from the floor and 14 cm from the left sidewall, it required a force of 10 g to be operated. The food-magazine was placed at the

center of the front panel where 45 mg Noyes pellets were delivered. General illumination was provided by a house light (28v DC), that was located at the upper left side of the frontal panel. Two loudspeakers provided a 90 dB white noise. All chambers were connected to a MED® associates interface, model SG-502, and a computer that controlled and registered all environmental and behavioral events.

Contextual cues

The experimental chambers were prepared to provide two different contexts, which featured olfactory, tactile and localization cues. Three of the chambers represented the vinegar–rods context that contained a recipient with 10 ml of vinegar (commercially available) placed outside of the chamber but under the food-magazine and the floor was made up of the stainless steel rods. The three remaining chambers represented the menthol-sandpaper context, that contained a recipient with 1 g of menthol (Richardson- Vick, Inc., Shelton, CT) placed outside of the chamber but under the food-magazine, the stainless steel rods were covered with coarse sandpaper. Context exposition was counterbalanced between subjects as Context A and B.

Procedure

All subjects were trained to press a lever on a continuous reinforcement schedule in the absence of the target contextual cues. Then, subjects were assigned to five groups and the experimental phase started.
The experiment consisted of two phases: acquisition and extinction. During the acquisition phase, animals were trained in Context A to lever press under a VI 30 s schedule for two sessions. Half of the subjects in each group were trained in the vinegar scented and stainless steel rods context, while the other half were trained in the menthol scented and sandpaper floor context. After that, the extinction session started and lever pressing was extinguished, that is, lever presses no longer produced pellets. The AA saline group received this phase in the acquisition context A, while the AB groups received it in a different context (Context B). Table 1 shows the procedure used in the present experiment.

Drug administration

Scopolamine hydrobromide (S0929, Sigma-Aldrich Chemicals) was dissolved in saline solution to provide doses of 0.01, 0.1, and 1.0 mg/kg a dose was intraperitoneally injected 15 min prior to the start of each acquisition session.

Table 1
Procedure used in the Experiment. A and B stand for the context in which each phase was conducted. R indicates response and O the outcome

Group	Phase Acquisition VI 30s	 Test Extinction
AA Saline	A:R-O	A: R-
AB Saline	A:R-O	B: R-
AB 0.01 mg/kg	A:R-O	B: R-
AB 0.1 mg/kg	A:R-O	B: R-
AB 1.0 mg/kg	A:R-O	B: R-

Statistical analyses

The number of lever presses during the acquisition and testing sessions were used as an index of learning. Response rates of the groups in the last training session were used to determine learning differences between groups, these rates were analyzed with ANOVAs. In order to analyze the effect of changing the context on response rates, an extinction ratio was calculated for each group. The extinction ratio was obtained by dividing the response rates observed in the extinction session over the response rate recorded in the last acquisition session plus the response rate observed in the extinction session. An extinction ratio close to zero shows a decrease in the response rate in the extinction session relative to the last conditioning session, while values near to 0.50 show no changes in the response rate.

Results

Figure 1 shows the mean response rates observed in the last acquisition and test sessions. Saline groups and the AB 0.01mg/kg group had the highest response rate in the last acquisition session, while the AB 0.10 mg/kg and the AB 1.00 mg/kg groups showed response rates close to 10 responses per min. Figure 1 also shows an abrupt reduction in the response rates during the test session for all groups, down to five responses per min. These findings were confirmed by a ANOVA with Phase and Group as factors, the results showed significant principal effects of group $F(4,40) = 17.04$, $p < 0.05$, and phase $F(1,40) = 262.00$, $p < 0.05$. The interaction between Phase and Group was also significant $F(4,40) = 8.23$, $p < 0.05$. A post hoc analysis (Tukey HSD) of the principal effect of group showed significant differences of the response rates during the last acquisition session among both saline groups and between AB 0.01mg/kg group, and the AB 1.00mg/kg and AB 0.10 mg/kg groups.

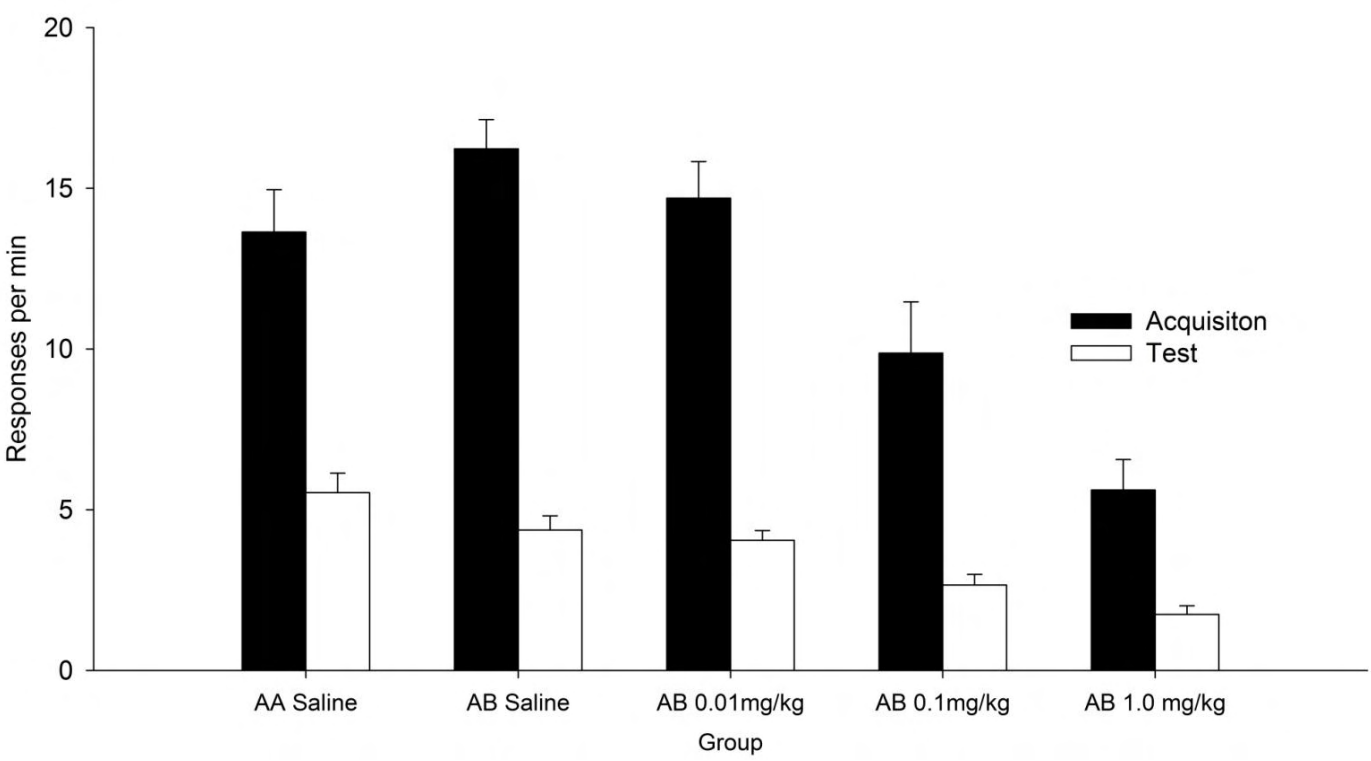

Figure 1. Responses per min for each group in the last acquisition session and the test session.

As groups differed in response rates, to assess the effects of context changes, an extinction ratio was computed for each subject in each group. The extinction ratio was obtained by dividing the rate during extinction over that rate plus the rate during the last acquisition session. The mean extinction ratios for each group are shown in Figure 2. The figure shows similar extinction ratios for all groups. A one-way ANOVA confirmed this finding $F(4, 40) = 0.74$, $p > 0.05$.

Discussion

The present results showed that moderate doses of scopolamine (e.g., AB 0.10 mg/kg and AB 1.00 mg/kg) prior to acquisition attenuated the rate of lever pressing. These findings are consistent with previous reports showing that moderate pre-training doses of scopolamine (1mg/kg, 10 mg/kg and 100 mg/kg) impair learning in fear conditioning procedures (Anagnostaras, Maren, Sage, Goodrich & Fanselow, 1999). Additionally, we should mention that the neural structures that mediate instrumental learning remain poorly understood; however, it has been suggested that the hippocampus may be critical for the detection of causal relationships in the enviroment, and that removal of the hippocampus renders rats insensitive to changes in the instrumental contingency, resulting in behavior that is determined primarily by event contiguity (Devenport, 1979, 1980;

Devenport and Holloway, 1980). In conclusion, and in agreement with Rudy´s (1996) findings, instrumental conditioning, tone-fear conditioning, and context fear conditioning seems to depend, at least with the parameters used in these experiments, on central colinergic systems.

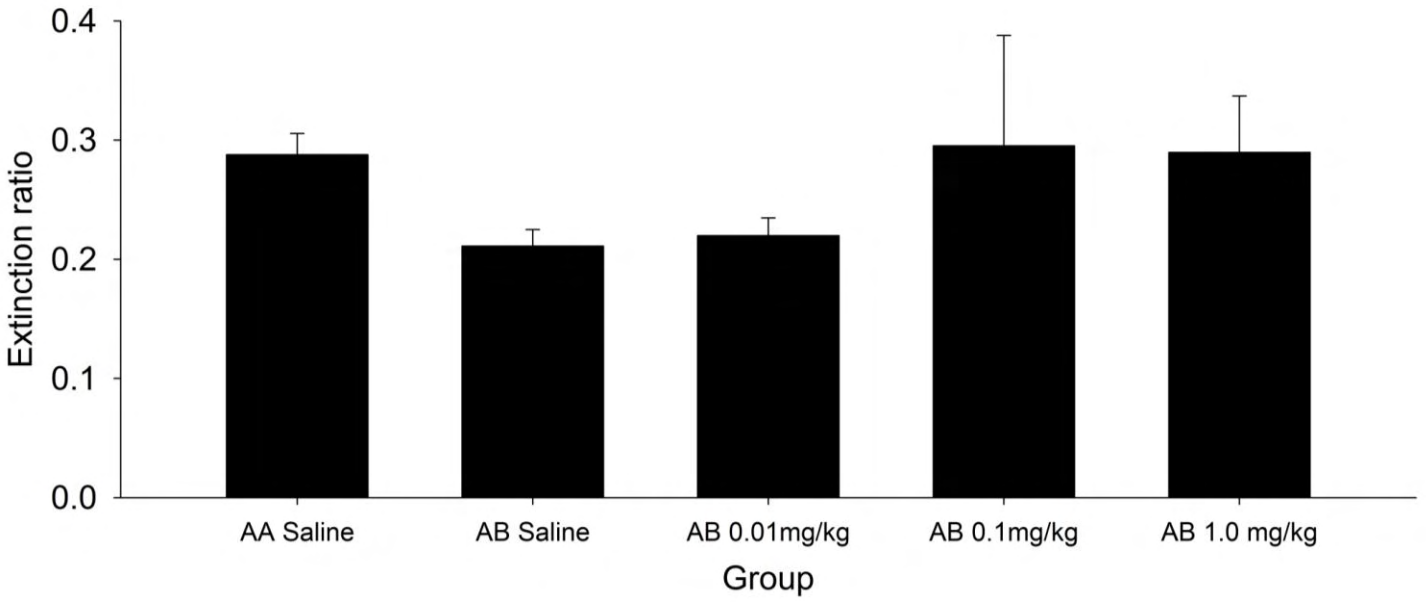

Figure 2. Extinction ratio for each group in the test session.

The second purpose of the present experiment was to extend Rudy´s (1996) findings that pre-training doses of scopolamine impair contextual learning; that is, when tone-footshocks are presented in context A, and then the subjects are exposed to the context alone and the tone in a new context, both produce freezing. However, some experiments have found that fear elicited by the context is attenuated when scopolamine is administered (Anagnostaras et al., 1999; Rudy, 1996), wheras other experiments have found that freezing elicited by the tone is also attenuated (Rudy, 1996). Our results did not showed effects of context changes, even in the saline groups. Thus, we were unable to assess the effects of scopolamine on context conditioning. However, it is important to stress that the lack of evidence of effects on context conditioning cannot be attributted to the abscense of scopolamine effects, as shown by the reduction in the rates of response, but to other factors. For example, when a drug is administered prior to a learning experience one can never be sure that the observed behavioral impariment was due to the drugs' action on learning and memory processes per se. A drug given prior to the learning experience could impair the sensory systems that mediate the detection of the relevat features of the experience. Thus, the effects of scopolamine administration after the conditioning session should be

analyzed. Furthermore, it is also important to consider that the moment when scopolamine is administered, either prior or after conditioning, may impair different phases in the memory process. For instance, the pre-conditioning administration has an effect on the acquisition process, whereas post-conditioning administration disturb the consolidation process, while pre-test administration affects the recovery process. Additionally, it is also important to acertain that the effects of scopolamine on behavior are produced by scopolamine´s action on the central colinergic systems, and not by its peripheral systems. Thus, in this type of experiments it is important to include an additonal control group that receives methyl-scopolamine, which is thought to not penetrate into the central nervous system (Feldman & Quinzer, 1984).

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