

# Adrenalectomy eliminates the extinction spike in autoshaping with rats

Brian L. Thomas<sup>a</sup>, Mauricio R. Papini<sup>b,\*</sup>

<sup>a</sup>*Department of Psychology, University of Massachusetts, Amherst, MA 01003, USA*

<sup>b</sup>*Department of Psychology, Texas Christian University, Box 298920, Fort Worth, TX 76129, USA*

Received 27 June 2000; received in revised form 4 October 2000; accepted 13 November 2000

## Abstract

Experiment 1, using rats, investigated the effect of adrenalectomy (ADX) on the invigoration of lever-contact performance that occurs in the autoshaping situation after a shift from acquisition to extinction (called the extinction spike). Groups of rats with ADX or sham operations were trained under spaced and massed conditions [average intertrial intervals (ITI) of either 15 or 90 s] for 10 sessions and then shifted to extinction. ADX did not affect acquisition training but it eliminated the extinction spike. Plasma corticosterone levels during acquisition were shown in Experiment 2 to be similar in rats trained under spaced or massed conditions. Adrenal participation in the emotional arousal induced by conditions of surprising nonreward (e.g., extinction) is discussed. © 2001 Elsevier Science Inc. All rights reserved.

*Keywords:* Arousal; Rats; Adrenalectomy; Corticosterone; Extinction spike; Surprising nonreward; Trial-spacing effect; Schedule-induced polydipsia

## 1. Introduction

Removal of the adrenal glands (adrenalectomy, ADX) leads to behavioral effects that suggest a reduction in the organism's general arousal level (i.e., degree of generalized behavioral activation; Ref. [11]). For example, ADX prevents the development of schedule-induced polydipsia, i.e., excessive drinking induced by periodic food delivery [15]; ADX decreases the frequency of freezing responses in contextual fear conditioning situations [19]; and ADX also reduces exploratory behavior [13]. Moreover, rats that spontaneously develop polydipsia tend to have significantly larger adrenal glands than rats that do not develop polydipsia [6].

The extinction of appetitively reinforced behavior is often characterized by an invigoration of behavior during the early trials that can be understood as a temporary increase in arousal caused by the omission of an expected reward [1,11,17]. For example, we have routinely observed that rats shifted from autoshaping acquisition to extinction exhibit a transient increase in response rate during the initial session of extinction [12], a phenomenon we have labeled the extinction spike. In autoshaping with rats, a lever (conditioned stimulus, CS) is presented for a fixed time

period, and its retraction is paired with the response-independent delivery of a food pellet (unconditioned stimulus, US). Although rats are not required to press the lever in order to obtain food, they nonetheless approach and contact the lever in anticipation of food delivery [18]. Furthermore, the occasional omission of an otherwise expected reward facilitates autoshaping performance [7,8]. Arousal effects on autoshaping are also evident in terms of circadian variations [20]. For example, autoshaping performance tends to be higher when animals are trained in the evening hours (19:00 h) rather than in the morning (09:00 h).

Additional evidence from other appetitive situations suggests that the early extinction trials are arousing. For example, extinction is accompanied by an elevation in plasma levels of pituitary–adrenal hormones, well-established markers of emotional stress [2,5,14]. Extinction and unpredictable nonreinforcement also increase levels of agonistic behavior in rats, pigs, and humans [4,10,21]. The experiments reported in this paper explored adrenal participation in the acquisition and extinction of appetitively motivated behavior.

## 2. Experiment 1

Experiment 1 studied the effect of ADX on the extinction spike in autoshaping with rats. We hypothesized that the

\* Corresponding author. Tel.: +1-817-257-6084; fax: +1-817-257-7681.

E-mail address: m.papini@tcu.edu (M.R. Papini).

extinction spike should be reduced or eliminated by ADX. In addition, training was administered in either massed or spaced conditions, that is, with relatively short or long intertrial intervals (ITI), respectively. It is well known in a variety of learning situations, including autoshaping in rats [16], that spaced training leads to faster acquisition rate, a phenomenon called the trial-spacing effect (TSE). Given the dependency of both the TSE and polydipsia on the spaced administration of practice (i.e., both effects required relatively long ITIs to occur) and the adrenal involvement in polydipsia [15], it was relevant to determine whether ADX affects autoshaping acquisition as well as extinction. Faster acquisition in the spaced condition could reflect arousal caused by infrequent reward administration.

## 2.1. Method

### 2.1.1. Subjects

The subjects were 28 experimentally naive, male Wistar rats. Ad libitum weights averaged 375 g. These rats were purchased from Harlan Laboratories, where they had been subjected to ADX ( $n=12$ ) or sham surgery ( $n=16$ ) 25 days before the start of the experiment (the experiment ended 53 days after surgery). The ADX rats received exogenous saline (0.09%) supplements in their water supply to compensate for any deficit in salt retention due to the surgery. All rats were maintained at 85% of their ad libitum weights. Animals were individually housed in a colony room with a 12-h light/dark cycle (light on at 07:00 h). Water was continuously available in the cage during the course of this experiment. Daily sessions were administered between 12:00 and 14:00 h.

### 2.1.2. Apparatus

Four standard conditioning chambers were used, each enclosed in a sound-attenuating cubicle. The internal dimensions of each chamber were 20.1 cm wide, 28 cm long, and 20.5 cm high. The floor of each chamber was made of stainless steel bars 0.4 cm in diameter and spaced 1.6 cm apart, center to center. Located in the center of the front wall was a recessed magazine, 2 cm from the floor, into which the pellets (45-mg Noyes rat formula A/I) were delivered automatically. An aluminum, retractable lever (4.8 cm wide, 1.9 cm deep, and 7 cm above the floor) was located 2 cm to the left of the magazine. Insertion (or retraction) of the lever took 0.2 s. A light bulb (GE 1820) attached to the ceiling of the chamber provided diffuse illumination and was positioned opposite the magazine. A speaker and fan provided background noise (75 dB, SPL, scale B, measured in front of the magazine) and ventilation, respectively.

The third phase of the experiment (see Procedure section) involved measurement of drinking behavior in the conditioning boxes. Each conditioning box was equipped with a stainless steel drinking spout inserted 2 cm to the right of the food cup and 7 cm above the floor. Animals could obtain

free 0.09% sodium solution (ADX rats) or water (sham rats) by licking at this spout.

### 2.1.3. Procedure

ADX and sham rats were randomly assigned to one of two groups depending upon the ITI enforced during training. For Groups M/ADX ( $n=6$ ) and M/Sham ( $n=8$ ; M stands for massed training), the mean ITI was 15 s (range: 10–20 s), whereas for Groups S/ADX ( $n=6$ ) and S/Sham ( $n=8$ ; S stands for spaced training), the mean ITI was 90 s (range: 60–120 s). All rats received two sessions of habituation to the conditioning chamber during which the house light was turned on but no other stimulus was presented. The duration of these habituation sessions was equal to that of the training session that follows: 5 min long for the massed groups and 18 min long for the spaced groups.

Thereafter, rats were trained in three successive phases: acquisition, extinction, and fixed-time food delivery. In the first phase (acquisition), each of the 10 sessions started with the onset of the house light and ended when the house light was turned off. There were 10 training trials per session separated by a variable ITI as described above. Before the first trial and after the last trial in each session, there was an interval of duration and range equal to that of the ITI. Each trial started with the insertion of the retractable lever for 10 s (the CS). A computer recorded lever-contact responses while the lever was inserted in the chamber. At the end of the 10 s, the lever was retracted, and five pellets were delivered on the magazine cup at a rate of one pellet per 0.2 s (the US). Each rat consumed 50 45-mg pellets per session. In the second phase (extinction), the training conditions were the same for five additional sessions, except that all food delivery was withheld. The number of responses recorded in each trial was transformed into a rate measure, responses per minute, and subjected to mixed-model analysis of variance with session as the repeated-measures factor.

The third phase (fixed-time training) was introduced as a means of validating the effects of the ADX treatment by replicating its known deleterious effect upon schedule-induced polydipsia [15]. A procedure similar to that used by Levine and Levine [15] was instrumented for this phase of the experiment. Six ADX and six sham animals were randomly selected from those trained in the prior phases. In each of the two groups thus formed, three rats had received massed training and three had spaced training. During the subsequent five daily sessions (starting 4 days after the last autoshaping extinction session), the levers remained retracted, and animals had continuous access to a 0.09% saline solution (ADX rats) or water (sham rats) in the conditioning box. Five 45-mg Noyes pellets were programmed according to a fixed-time 60-s schedule. Each session ended after the delivery of 60 such reinforcements; thus, animals consumed a total of 300 pellets per session, and sessions were approximately 1 h long. The amount of liquid consumed during the 23 h before the session in the home cage (HC) and the amount consumed during the

session were measured volumetrically. Fluid intake data were subjected to mixed-model analysis of variance, with sessions as the repeated-measures factor.

## 2.2. Results

As shown in Fig. 1, a strong TSE emerged rapidly in both the ADX and sham groups. There seems to be a slightly faster rate of acquisition in the sham groups compared to the ADX groups, but the size of the TSE is roughly equivalent. A Group (ADX, Sham)  $\times$  ITI (S, M)  $\times$  Session analysis indicated a highly significant ITI  $\times$  Ses-Session interaction [ $F(9,216)=5.56$ ;  $P<.01$ ]. This interaction resulted from the relatively faster and greater increase in response rate in the spaced training groups than in the massed training groups. There were also significant simple effects for ITI [ $F(1,24)=29.84$ ;  $P<.001$ ] and for Session [ $F(9,216)=6.21$ ;  $P<.01$ ]. All other effects were nonsignificant ( $P_s>.05$ ).

Fig. 1 also shows the performance of these groups during the extinction phase of this experiment. The transient facilitation of lever-contact behavior (i.e., the extinction spike) was observed during the initial extinction session in both sham control groups relative to both their own performance in the last acquisition session and the performance of the ADX groups, none of which exhibited such behavioral activation. A comparison of the mean response rate during the last acquisition session and first extinction session indicated that 13 of 16 (81%) sham rats increased their response rate, whereas only 3 of 12 (25%) ADX rats did so. A Group (ADX, Sham)  $\times$  ITI (S, M)  $\times$  Session analysis captured the effect of ADX on the extinction spike as a significant Group  $\times$  Session interaction [ $F(4,96)=3.34$ ;  $P<.03$ ]. The significant ITI  $\times$  Session interaction [ $F(4,96)=6.14$ ;  $P<.01$ ] reflects the large differences across spaced and massed conditions present at the start of extinction. Significant ITI [ $F(1,24)=25.79$ ;  $P<.001$ ] and

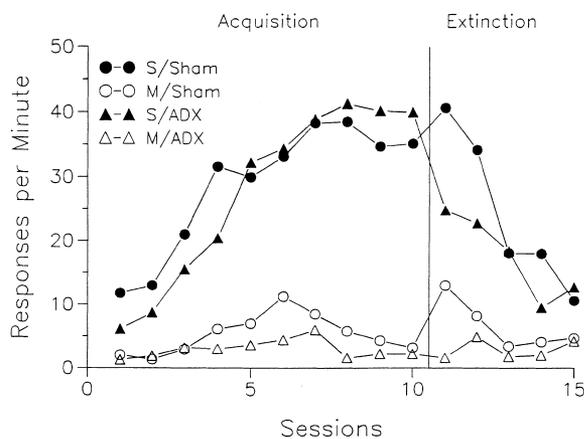


Fig. 1. Responses (lever contacts) per minute in groups receiving spaced (S) or massed (M) autoshaping training and given ADX or sham treatment. Extinction sessions were equal to those of acquisition, except that the reinforcers were never administered.

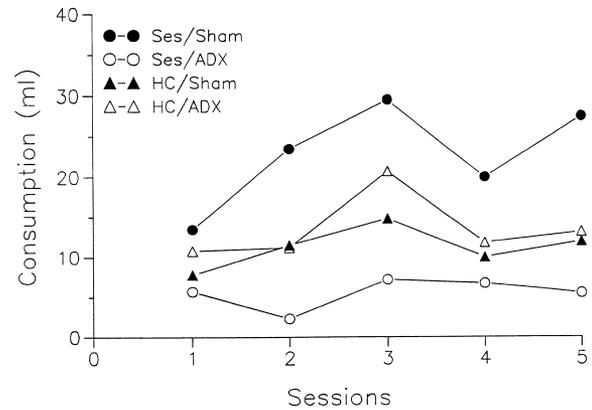


Fig. 2. Fluid intake (water in shams, saline solution in ADX animals) in the HC or session chamber (Ses) across five sessions of response-independent presentations of food pellets.

Session effects [ $F(4,96)=10.13$ ;  $P<.001$ ] were also obtained. All other effects were nonsignificant ( $P_s>.05$ ).

SIP was rapidly induced during the final phase of this experiment in sham animals, but it failed to occur in rats that had undergone ADX treatment. Fig. 2 shows the amount of fluid consumed by ADX and sham rats during the training sessions (Ses) and in the intervening 23 h while in their home cage (HC). Consumption during the session was higher than that observed in the cage for sham animals, thus indicating the development of schedule-induced polydipsia, but the opposite held for ADX animals. A Group (ADX, Sham)  $\times$  Drinking (Ses, HC)  $\times$  Session analysis supported that conclusion in terms of a significant triple interaction [ $F(4,40)=4.67$ ;  $P<.025$ ]. There were also significant effects for the Group  $\times$  Drink-Drinking interaction [ $F(1,10)=56.64$ ;  $P<.001$ ] and Group  $\times$  Session interaction [ $F(4,40)=2.97$ ;  $P<.05$ ], and for the simple main effects of Group [ $F(1,10)=6.03$ ;  $P<.05$ ] and Session [ $F(4,40)=10.18$ ;  $P<.001$ ]. All other effects were nonsignificant ( $P_s>.05$ ).

## 3. Experiment 2

Experiment 1 provides the first demonstration that the transient invigoration of behavior characteristic of the early stages of appetitive extinction in the autoshaping situation, or extinction spike, is eliminated by ADX. The effect of ADX appears to be specific to extinction since acquisition performance was not affected. Experiment 2 was designed to provide information on the possible adrenal participation in acquisition using a different approach, that is, by determining plasma levels of corticosterone immediately after training sessions. Corticosterone levels are known to be elevated during extinction of appetitive behavior [2,14], but there is little information on their levels during acquisition training. A reason to be cautious about discarding an adrenal involvement in autoshaping lies in the similarity between

the spaced training conditions used in the present experiments and the training conditions that typically generate schedule induced polydipsia. In both cases, relatively long interreinforcement intervals are involved, and as already noted, there is evidence of adrenal participation in polydipsia [15].

### 3.1. Method

#### 3.1.1. Subjects and apparatus

The subjects were 16 experimentally naive, male Wistar rats, with an average ad libitum weight of 400 g. Rats were purchased from Harlan Laboratories, where they had been cannulated via the jugular vein. Each day, the heparinized glycerol solution (333 U/ml) in the cannula from the previous day was withdrawn and replaced after a sterile saline flush with fresh solution. This procedure was performed immediately after each training session as part of the blood sampling procedure (which required less than 30 s per animal to complete). Rats were trained daily between 16:00 and 18:00 h. Maintenance conditions and the conditioning boxes used during training were as described in Experiment 1.

#### 3.1.2. Procedure

The experiment started with two sessions of habituation to the context similar to those described in Experiment 1. Rats were randomly assigned to Groups S and M ( $n = 8$ ) and exposed to 10 sessions of training, each one involving 10 trials. The training parameters were exactly those used in Experiment 1 for groups trained under spaced and massed conditions. A small blood sample (0.3 ml) was collected, via cannula, at several times during the experiment. Samples were obtained in the HC prior to any exposure to the conditioning chamber immediately after each of the two context habituation sessions and immediately after each of the 10 daily training sessions. Blood samples were allowed to clot and were then centrifuged at 2000 rpm for 45–60 s. The serum supernatant was isolated and kept at  $-20^{\circ}\text{C}$  for future processing. Final serology involved conventional radioimmunoassay techniques (ICN Biomedicals; Costa Mesa, CA). Corticosterone concentrations ( $\mu\text{g}/\text{dl}$ ) were assessed for samples obtained for each animal at the end of each training session.

The average response rate and corticosterone concentrations over the last three sessions of training (Sessions 8–10) were the primary dependent variables in this study. Data were analyzed in terms of independent-sample two-tailed  $t$  tests applied to each of these variables.

### 3.2. Results

The TSE developed again as expected in terms of lever-contact responses. Spaced training led to a fast acquisition of lever-contact behavior and a high performance level during the last three sessions of training, both contrasting with the usual impairment observed under massed condi-

tions. However, corticosterone levels tended to be higher during the initial samplings, perhaps because the sampling procedure itself was somewhat aversive. Consequently, the analyses presented below were restricted to the final three sessions of training, which is when the differences among conditions were expected to be maximal.

The mean number of lever-contact responses per minute provided evidence for the TSE. The average ( $\pm$ S.E.M.) response rates were 42.5 ( $\pm$ 5.0) and 4.9 ( $\pm$ 0.8) responses per minute for Groups S and M, respectively, a significant difference [ $t(14) = 7.37$ ;  $P < .001$ ]. By contrast, mean plasma concentration levels of corticosterone during the same sessions of acquisition were 161.1 ( $\pm$ 30.3) and 160.3 ( $\pm$ 35.3)  $\mu\text{g}/\text{dl}$  for Groups S and M, respectively. This difference was not significant ( $t < 1$ ).

## 4. Discussion

The extinction spike, or transient invigoration of appetitive performance early in extinction, was eliminated by ADX treatment under both spaced and massed conditions of training. This result suggests that the extinction spike is based on a transient state of arousal induced by a shift from expected reward to surprising nonreward. This finding fits both theoretical developments regarding the drive-inducing properties of frustrative nonreward [1], as well as a variety of empirical results including the response invigoration that follows surprising nonreward [7,8] and an increase in plasma corticosterone levels during early appetitive extinction [2,5,14].

The deleterious effect of ADX on the extinction spike occurred in the absence of any obvious adrenal participation on acquisition. In Experiment 1, neither the triple interaction nor the ADX  $\times$  ITI interaction was significant in the analysis of acquisition data. In Experiment 2, levels of plasma corticosterone during acquisition were found to be equivalent under both spaced and massed conditions of training. Therefore, adrenal participation in autoshaping under the present training conditions appears to be relatively specific to extinction.

The fact that ADX disrupted schedule-induced polydipsia confirms a previous report [15] and indicates that the conditions of training used in Experiment 1 compare to those used in other laboratories. This effect of ADX on polydipsia also validates the glandular lesion procedure and suggests that the results of ADX on the extinction spike and polydipsia may share the same underlying mechanism. Further experiments will be necessary to determine whether the effects of ADX on the extinction spike depend on the resulting decrease in circulating corticosteroids. Corticosteroid hormones (e.g., corticosterone and aldosterone) have been shown to play a role in behavioral deficits induced by ADX. For example, ADX-induced deficits in polydipsia are eliminated by corticosterone replacement [15], whereas ADX-induced deficits in

spatial learning are reversed by the administration of aldosterone [3]. It would also be of interest to determine if other situations involving surprising omissions or reductions in reward magnitude are also modulated by ADX. For example, a shift from a more preferred to a less preferred reward deteriorates performance below the level exhibited by an unshifted control group always exposed to the less preferred reward. This phenomenon, called successive negative contrast, is also accompanied by elevated levels of plasma corticosterone in rats [9]. It is possible that this contrast effect is also reduced or even eliminated by ADX, as it is the case with the extinction spike.

### Acknowledgments

The research reported in this article was partially supported by TCU/RCAF grant 5-23625.

### References

- [1] Amsel A. Frustration theory. Cambridge: Cambridge Univ. Press, 1992.
- [2] Coe CL, Stanton ME, Levine S. Adrenal responses to reinforcement and extinction: role of expectancy versus instrumental responding. *Behav Neurosci* 1983;4:654–8.
- [3] Conrad C, Lupien SJ, Thanasoulis LC, McEwen BS. The effects of Type I and Type II corticosteroid receptor agonists on exploratory behavior and spatial memory in the Y maze. *Brain Res* 1997;759:3–5.
- [4] Dantzer R, Arnone M, Mormede P. Effects of frustration on behaviour and plasma corticosteroid levels in pigs. *Physiol Behav* 1980;24:1–4.
- [5] Dantzer R, Gonyou HW, Curtis SE, Kelley KW. Changes in serum cortisol reveal functional differences in frustration-induced chain chewing in pigs. *Physiol Behav* 1987;39:775–7.
- [6] Devenport LD. Schedule-induced polydipsia in rats: adrenocortical and hippocampal modulation. *J Comp Physiol Psychol* 1978;92:651–60.
- [7] Dudley RT, Papini MR. Pavlovian performance of rats following unexpected reward omissions. *Learn Motiv* 1995;26:63–82.
- [8] Dudley RT, Papini MR. Amsel's frustration effect: a Pavlovian replication with control for frequency and distribution of rewards. *Physiol Behav* 1997;61:627–9.
- [9] Flaherty CF, Becker HC, Pohorecky L. Correlation of corticosterone elevation and negative contrast varies as a function of postshift day. *Anim Learn Behav* 1985;13:309–14.
- [10] Frederiksen LW, Peterson GL. Schedule-induced aggression in humans and animals: a comparative parametric review. *Aggressive Behav* 1977;3:57–75.
- [11] Gray JA, McNaughton N. The neuropsychology of anxiety. 2nd ed. Oxford: Oxford Univ. Press, 2000.
- [12] Huneycutt DC. Paradoxical effects and Pavlovian contingencies: effects of shifts in reward magnitude on autoshaped responding in rats. Unpublished Thesis, Texas Christian University, 1996.
- [13] Islam A, Henriksson B, Mohammed A, Winblad B, Adem A. Behavioural deficits in adult rats following long-term adrenalectomy. *Neurosci Lett* 1995;194:49–52.
- [14] Kawasaki K, Iwasaki T. Corticosterone levels during extinction of runway response in rats. *Life Sci* 1997;61:1721–8.
- [15] Levine R, Levine S. Role of the pituitary–adrenal hormones in the acquisition of schedule-induced polydipsia. *Behav Neurosci* 1989;103:621–37.
- [16] Papini MR, Brewer M. Response competition and the trial-spacing effect in autoshaping with rats. *Learn Motiv* 1994;25:201–15.
- [17] Papini MR, Dudley RT. Consequences of surprising reward omissions. *Rev Gen Psychol* 1997;1:175–97.
- [18] Peterson GB, Ackil JE, Frommer GP, Hearst E. Conditioned approach and contact behavior toward signals for food or brain-stimulation reinforcement. *Science* 1972;177:1009–11.
- [19] Pugh CR, Tremblay D, Fleshner M, Rudy JW. A selective role for corticosterone in contextual-fear conditioning. *Behav Neurosci* 1997;111:503–11.
- [20] Thomas BL, Huneycutt D, Papini MR. Reward magnitude, but not time of day, influences the trial-spacing effect in autoshaping with rats. *Physiol Behav* 1998;65:423–7.
- [21] Thompson T, Bloom W. Aggressive behavior and extinction-induced response-rate increase. *Psychon Sci* 1966;5:335–6.