CONTEXT LEARNING IN THE MARSUPIAL (*Lutreolina crassicaudata* Red Opossum)

Mauricio R. Papini, Alba E. Mustaca, Gustavo Tiscornia, and Marieta DiTella

ABSTRACT: Context learning was studied in the Red Opossum, the marsupial, *Lutreolina crassicaudata*. In Experiment 1 the animals received four trials per day in two different boxes (contexts): X and Y. Half of the animals received periodic deliveries of a sugar solution (+) in one box (X+), but not in the other (Y-); the rest received the opposite training (X-), (Y+). Several behavioral categories were recorded during the final trial in each context. Animals approached the feeder significantly more in the positive context. Experiment 2 was designed to determine the extent to which the number of trials per day affected acquisition. Two groups of animals received differential training with either four or one trial per day. No differences between groups were observed, although in both of them, approach to the feeder was significantly higher in the positive context. The results are discussed in relation to both the role of practice distribution on learning in marsupials, and their potential value of this species for the study of learning processes.

RESUMEN: Se estudió el aprendizaje contextual en la zarigueya colorada *Lutreolina crassicaudata*. En el Experimento 1 los animales recibieron cuatro ensayos por día en dos diferentes cajas de condicionamiento (contextos): X e Y. La mitad de los animales recibían periódicamente un monto de solución azucarada en una caja, X+, pero no en la otra, Y-; el resto de los animales recibían la contingencia opuesta, X-, Y+. Durante el último ensayo en cada caja se registraron varias categorías de comportamiento. Los animales se acercaron al bebedero significativamente más en el contexto positivo. El Experimento 2 fue diseñado para determinar hasta que punto el número de ensayos por día afectó la adquisición. Dos grupos de animales recibieron entrenamiento diferencial ya sea con uno o cuatro ensayos por día. No se observaron diferencias entre grupos, aunque en ambos casos el acercamiento al bebedero fue significativamente más frecuente en el contexto positivo. Los resultados se discuten tanto en relación con el papel de la distribución de la pràctica sobre el aprendizaje en marsupiales, como con el valor potencial de estas especies para el estudio de los procesos de aprendizaje.

Associative and cognitive capabilities of marsupials are poorly known despite the potential importance of this group from the comparative point of view (Bitterman, 1986; Papini, 1986). For instance, most of the experiments on Pavlovian conditioning in marsupials lack appropriate

Correspondence should be sent to M. R. Papini, Laboratorio de Fisiologia del Comportamiento, Instituto de Biologia y Medicina Experimental, Obligado 2490, 1428-Buenos Aires, Argentina.

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control conditions to eliminate nonassociative factors (Papini, 1986). Recent evidence, however, suggests that at least two species, *Lutreolina crassicaudata* (Red Opossum) and *Didelphis albiventris* (White-eared Opossum) can be trained under several standard situations, such as differential conditioning, summation, discrimination reversal, and single alternation (Papini, in press a, b). It is worth considering here that these results encourage not only the comparative study of learning, but also open the way for a systematic research on two aspects to which marsupials are well suited: developmental and neuropsychological processes of learning.

In the present article, we report a successful attempt at studying another standard conditioning phenomenon: context learning. Learning about the static, continuously present cues of the environment began to be investigated to a large extent because of a successful model of Pavlovian conditioning that assumed contextual and discrete stimuli to be equivalent (Rescorla & Wagner, 1972). Because the events of any learning situation always occur on a background, the potential relevance of context learning is general (Balsam, 1985). Moreover, if contextual and discrete stimuli are equivalent, the principles of Pavlovian conditioning may be considerably more general than it was previously thought.

EXPERIMENT 1

Animals. Eight red opossums (*Lutreolina crassicaudata*), four males and four females, all adult, wild-caught, and experimentally naive, were studied. Initial weights were as follows: 900-2150 g for males; 500-600 g for females. Sexual dimorphism in body size is usual in this species. They lived in individual cages with food continuously available. One week before the first session, the opossums began to receive a limited amount of water daily (100 ml); during the experiment, subjects were allowed to drink during a maximum of 30 m per day. Access to water occurred at least 15 m after the session was over.

Apparatus. Two conditioning boxes (i.e., X and Y) were used, which differed in terms of visual, auditory, and tactile cues. These were located at different heights from the floor and were differently oriented in the room. Table 1 summarizes the features of each box.

Delivery of the unconditioned stimulus (US: 3% commercial sugar mixed by weight with tap water) was automatically controlled by standard relay equipment located in an adjacent room. Control and experimental rooms were separated by one-way windows.

Procedure. The animals received four trials per day with an intertrial interval (ITI) of approximately 20 m during which they were placed back in their cages. Each trial lasted 10 m. In the initial two trials of day one, the opossums were exposed to each context (order of exposure counterbalanced) without receiving

Table 1

Characteristics of the Boxes (Contexts) Used in Experiments 1 and 2

	Context X	*Context Y*
Long x Wide x High	54 x 33.5 x 31 cm	42 x 35.5 x 55 cm
Floor	Translucent acrylic	Aluminum bars
Lateral Walls	Red vertical lines on white background	Aluminum
House Light	25 W	3.6 W
Light Module	Off	Two yellow lights, 3.6 W each
Feeder	2 cm diameter, to the right side of the box's door	6 cm diameter, to the left side of the box's door
White Noise	70 dB, S.P.L.	Off
Distance from the Room Floor	110 cm	150 cm

Legend. White noise was on in context Y during Experiment 2. Context validity was counterbalanced within groups in both experiments.

USs. In trials three and four, the animals were placed in the positive context, half the animals in X+, and half in Y+. They received a total of 30 ml of sugar solution in five deliveries.

From day two to six, animals received four trials per day; two trials were reinforced (R) and two nonreinforced (N). One of the following sequences of trials was used in each day: RRNN, RNRN, NRRN, NRNR, and RNRN. In an R trial animals received 20 USs, 1.4 ml each, according to a variable time (30 s) schedule. In an N trial subjects remained in the box during 10 m and received no USs.

Behavioral recordings were carried out during the last two trials of the last day of training. During the initial 2 m, and before any US was delivered in the case of positive contexts, two experimenters recorded the following categories:

1. Orient—head oriented toward the frontal wall with at least the forelimbs within the half of the floor closest to the feeder.
2. Feeder—head on the feeder.
3. Quiet—complete absence of movements.
4. Ambulation—movement of at least two limbs.
5. Grooming—scratch or wash any part of the body with hindfoot.

6. "Sleep"—quiet with eyelids closed.
7. Head-Jerks—rhythmic head movements in the saggital plane.
8. Rear—standing on hindlegs; forelegs may or may not be touching a wall.

Recordings were based on an instantaneous sampling of each box every 10 s, which gives 12 recording periods for each animal during the first two minutes. Only one category was recorded in a given sampling period. Overall interexperimenter reliability was 96.4%.

Table 2

Mean Proportion of Recording Periods in which each Behavioral Category was Recorded in Relation to Experiment 1

Category	*In the Positive Context*	*In the Negative Context*
Orient*	.50	.05
Feeder	.26	.05
Quiet	.52	.54
Ambulation	.01	0
Grooming	.08	.25
"Sleep"	0	.05
Head-Jerks	.01	.08
Rear	.02	0

*P < 0.01

RESULTS AND DISCUSSION

The results obtained in each dependent measure are presented in Table 2. Two categories yielded consistent results across subjects: Orient and Feeder. In both cases, the proportion of recording periods in which each category was recorded in relation to the total number of recording periods was higher in the reinforced than in the nonreinforced context. One-factor, repeated measures analyses of variance for each category indicated that the proportion of Orient behavior was significantly higher in the positive context [$F(1, 7) = 13.96$; $p < 0.01$]; Feeder was only marginally different across contexts [$F(1, 7) = 4.17$; $p < 0.10$]. None of the other categories yielded significant differences.

This experiment shows that associatively based context conditioning can be observed in red opossums and that it is measured by a tendency to orient and approach the feeder in the positive context. Apparently, contextual stimuli do not control head-jerk movements, a behavior observed in conditioning experiments with discrete visual and auditory signals (Papini, in press a). These results also contrast with those obtained in pigeons (Durlach, 1983) and rats (Mustaca, Gabelli, Charabuki, & Papini, 1987) which in analogous situations display conditioned activity in positive contexts.

EXPERIMENT 2

In two prior investigations in our laboratory, we attempted to contextually condition red opossums using one trial per day in visually different contexts or in olfactorily different contexts. We were unable to do so. The negative results may have been obtained because (1) the contexts were not sufficiently discriminable, or (2) the training was too spaced. The choice of one trial per day was based on a similar experiment on pigeons reported by Rescorla, Durlach, and Grau (1985), where they observed rapid context discrimination using activity as the dependent measure. However, there are some suggestions in the literature that opossums (*D. virginiana*) learn faster under massed rather than spaced trial conditions (Friedman & Marshall, 1965; Tilley, Doolittle, & Mason, 1966). For instance, Friedman and Marshall found little within session improvement in a successive reversal task with few trials per day, but relatively good performance when subjects received as many trials as needed to achieve a behavioral criterion. Although these experiments are difficult to interpret because they did not control for the effect of accumulated training per se, it is still possible that spaced practice adversely affects performance in opossums (see also Cone & Cone, 1970).

The results of Experiment 1 suggested that our prior unsuccessful attempts to obtain contextual learning in red opossums might have been caused either by the use of relatively similar contexts or by the use of a relatively distributed practice. The latter factor seemed worthwhile pursuing further in light of the seemingly beneficial effect of massed practice on discrimination learning in Virginia opossums (Friedman & Marshall, 1965; Tilley et al., 1966). Also, the literature suggests that at least in some situations (i.e., autoshaping of the pigeon's key-peck response) acquisition speed is inversely related to the number of trials per session under both continuous and partial reinforcement (Papini & Overmier, 1984, 1985). Experiment 2 was designed to replicate differential conditioning and to compare acquisition under either one trial (spaced) or four trials (massed) per day.

Animals and Apparatus. Eight adult, experimentally naive, wild-caught red opossums (4 males, 4 females) were studied. Initial weights were: 400-700 g for males and 280-390 g for females. Maintenance and deprivation conditions were similar to those in Experiment 1 except that after five days of training, because signs of satiety were observed, access to water in the cage was restricted to 15 m for the Spaced Group (one trial per day) and 5 m for the Massed Group (four trials per day). Conditioning chambers were the same as those used in Experiment 1 except that background white noise was delivered in both of them (Table 1).

Procedure

Training was divided into two phases. In phase one, the procedure was that used in Experiment 1 except that behavior was recorded during the initial 90 s of each trial, and that only the following categories were noted: a combination of Orient plus Feeder; Quiet; and Grooming. In addition, animals received a 10% sugar solution rather than a 3% sugar solution as in Experiment 1. Half the animals received four trials a day as in Experiment 1, and half received one trial a day (Massed Group and Spaced Group respectively). During Phase 1, animals received 20 USs (1.4 ml each) in R trials, on a VT 30 s schedule.

Some procedural modifications were introduced in Phase 2 because we observed signs of satiety in the Massed Group and because the opossums approached the feeder mainly after the noise produced by the solenoid valve that delivered the US. Accordingly, the number of USs was reduced from 20 to 10 per trial (trial length was reduced to 5 m) and the solenoid valve was activated during both positive and negative trials, but only in the former was the US delivered. Behavioral recordings were similar to those of Phase 1. Phase 2 lasted 20 trials (10 R and 10 N trials). Interobserver reliability was calculated on 15% of the trials, and it was 95%.

RESULTS AND DISCUSSION

During Phase 1, some animals from the Massed Group were reluctant to drink the sugar solution, particularly during the third and fourth trial of each day. Because sugar solutions of even lower concentrations are known to be potent USs (Papini, in press a) we interpreted this observation as a sign of satiety. The approach to the feeder only after the noise produced by the solenoid valve suggested that there was in fact a salient discrete signal in the situation that could possibly block context conditioning (e.g., Durlach, 1983). Despite these problems, Orient and Feeder behavior appeared consistently and significantly more in positive than in negative contexts in both groups [$F(1, 6) = 8.70$; $p < 0.025$], although groups were not different from each other in this behavior. These results are presented in Table 3. As expected from Experiment 1, neither Quiet nor Grooming exhibited differential scores between contexts or between groups (p's > 0.05).

Table 3 also shows the results of Phase 2. No evidence of satiety and control by the noise of the solenoid were observed in Phase 2, with ten (instead of 20) USs per trial and the solenoid activated in R and N trials (instead of only in R trials). Again, there was a significantly higher proportion of Orient behavior in positive contexts [$F(1, 6) = 26.87$; $p < 0.01$] but the groups were not statistically different from each other. As Table 3 also shows, however, the difference in Orient

Table 3

Group Means for each Behavioral Category Recorded in Experiment 2

Category	*Spaced Group*		*Massed Group*	
	+*	–	+	–
Phase 1				
Orient**	.39	.08	.21	.11
Quiet	.18	.14	.27	.13
Grooming	.24	.48	.32	.30
Phase 2				
Orient***	.61	.04	.41	.16
Quiet	.20	.39	.18	.12
Grooming	.06	.33	.19	.23

*+: Positive context; –: Negative context.
**P< 0.025
***P< 0.01

behavior between contexts was larger for the Spaced Group than for the Massed Group, a result evident from a marginally significant Groups by Contexts interaction [$F(1, 6) = 4.36$; $p < 0.10$]. No differences were found in Quiet and Grooming.

GENERAL DISCUSSION

The present experiments were intended to provide evidence of contextual learning in red opossums. The observation of differential conditioning together with the appropriate counterbalance of context validity across subjects supports the conclusion that behavioral changes were associatively based. Therefore, the range of conditioning phenomena is expanded as demonstrated in this didelphid marsupial, (see also Papini, in press) using training procedures which are flexible enough to allow the use of these species for comparative, developmental, and neuropsychological studies of learning.

Durlach (1983) observed more activity in a positive context in pigeons and rats. The increased activity cannot be attributed to US delivery since it was measured at the start of each session and before the animal received the first US. Moreover, it cannot be explained in nonassociative terms because they used differential training procedures. The present results agree with their general conclusions thus showing that direct control of performance by contextual cues seems to be a fairly general phenomenon.

In addition, these results show that general activity may not be the only index of contextual learning in appetitive situations. The red opossums showed no differences in ambulation but they oriented and approached the feeder mainly in the positive context. We still do not know whether these performance differences are a function of the kind of stimuli in each context, described in rats for discrete signals of food (Holland, 1977), the type of US, or if they simply depend on the freezing tendency of wild red opossums. For example, in discrete-trial differential training with visual and auditory signals, red opossums reared under laboratory conditions showed goal approach in positive trials and goal withdrawal in negative trials; however, goal withdrawal during negative trials was substantially reduced in wild-caught red opossums (Papini, in press a). The topography of anticipatory responses to the sugar solution also differs between contextual and discrete signals, whereas in the former red opossums simply orient and approach the feeder, in the latter they display rhythmic movements of the head in the saggital plane directed toward the feeder (Papini, in press a).

The absence of a trials-per-session effect is not in agreement with discrimination experiments with Virginia opossums (Friedman & Marshall, 1965; Tilley et al., 1966) that found better performance under massed practice i.e., many trials per session. Thus, the claim that Virginia opossums in particular, and marsupials in general, differ from other mammals in their memory mechanisms (cf. Tilley et al., 1966) is not supported by the present results. If anything, it appears that contexts were more discriminable under spaced then under massed practice; the lack of a significant interaction (see Table 3, Phase 2), might be related to the small sample size or to possible differences in motivational level across groups.

These results also contrast with the effect of the number of trials per session found in discrete-trial experiments with pigeons (Papini & Overmier, 1984, 1985). There are important procedural differences that prevent a full understanding of this discrepancy, such as the use of different species (pigeons versus red opossums) and different kinds of signals (discrete versus contextual). This pattern of results suggests the possibility that learning about contextual and discrete signals might be differentiated, at least with regard to the distribution of trials per session.

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