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Successful Intermale Aggression and Conditioned Place Preference in Mice

MANUELA MARTÍNEZ,¹ FEDERICO GUILLÉN-SALAZAR, ALICIA SALVADOR,
 AND VICENTE M. SIMÓN

Area de Psicobiología, Facultad de Psicología, Universitat de València, Apartado 22109, 46071 Valencia, Spain

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MARTÍNEZ, M., F. GUILLÉN-SALAZAR, A. SALVADOR AND V. M. SIMÓN. *Successful intermale aggression and conditioned place preference in mice*. *PHYSIOL BEHAV* 58(2) 323–328, 1995.—This study assessed the reinforcing properties of successful intermale agonistic encounters between OF1 male mice using the conditioned place preference paradigm. A three compartment apparatus was used and the procedure consisted of three phases: preconditioning (3 days), conditioning (8 days) and postconditioning (3 tests). Individually housed male mice were allocated to two groups. The aggression group confronted docile opponents in the preconditioning “less-preferred” compartment and were left alone in the “preferred” one. The control group was left alone in both compartments. Whereas no significant differences were found between both groups in the time spent in the less-preferred compartment, a separate analysis of animals in function of the color of the less-preferred compartment revealed a clear-cut difference. Mice developed a conditioned place preference for the aggression-paired compartment only if that experience took place in the black one. These findings suggest that the process of establishing a conditioned place preference with successful intermale aggression as reinforcer is extremely fragile and can be easily disrupted by changing the environmental cues involved.

Intermale aggression Mice Conditioned place preference Environmental cues Experience of victory

INTRODUCTION

THE CONDITIONED Place Preference (CPP) paradigm has been utilised mainly to investigate the rewarding or aversive properties of drugs (4,8,16,20) and endogenously produced substances (6,9). Moreover, this paradigm has been used to study the rewarding or aversive properties of primary behaviors such as feeding (19), maternal (7), sexual (10,15), and social (3) behaviors in rodents. However, no studies have been conducted to investigate if this paradigm could be useful in the research on the rewarding or aversive properties of aggressive behavior.

With respect to the reinforcing properties of aggressive behavior, there have been different opinions. While some authors consider aggression as an appetitive behavior and thus as a positive reinforcer in itself, others consider aggression as an aversive behavior and thus the consequences of the aggressive behavior are what provides the negative reinforcement (see review: 1,18,21). However, very few studies have been concerned with the possible role of intermale aggression as a positive reinforcer, especially the experience of victory during agonistic encounters. One way to demonstrate the appetitive nature and thus the rewarding property of successful intermale aggression is by showing that animals learn and perform operant behaviors to provide themselves with intraspecific attack opportunities when they could easily avoid such interactions by not performing the operant response (18). In this respect, some studies have obtained operant conditioning using the possibility of attacking a docile conspecific male as a reinforcer. The evidence that experience of

victory is rewarding in rodents stems from the following observations: (a) mice learn to press a lever when lever pressing produces a mouse which can be attacked (5); (b) mice learn to discriminate a goalbox in T-mazes in which they have the opportunity to attack a docile male (22,23); and (c) mice run in a run-way for the opportunity to defeat submissive mice (12). However, different explanations have been given for the operant responses found when using intermale aggression as a reinforcer. Some studies have demonstrated that aggression was reinforcing if the subject was given a previous aggressive warm-up stimulation (22). Moreover, such operant responses have been produced even when the animals are allowed to engage in aggressive behaviors which are preparatory to biting attacks but are not permitted to bite (13), which suggests that the stimuli eliciting aggression are the reinforcer. Finally, other studies, using the T-maze procedure, suggest that the social contact is the reward and not the opportunity to attack (11,21).

Although some questions remain, the weight of evidence supports the conclusion that, under appropriate conditions, intraspecific aggression can be positively reinforcing for successful aggressors. One interesting aspect is whether the successful aggressive experience in a particular place would produce a preference for that place. In this sense some information has been obtained in some species of fish that showed a preference for the places in which recent fighting occurred (2).

The purpose of the present study was to assess if successful aggressive experience in mice, resulting from an intermale en-

¹ To whom requests for reprints should be addressed.

counter with a nonaggressive opponent, acts as a reinforcer in the CPP paradigm. Then, if the unconditioned effect of the experience of victory is perceived by the animal as rewarding and an association between this experience and the stimuli of the environment in which it took place is established, the animal will increase the time spent in that environment in the absence of the nonaggressive opponent. The establishment of a CPP could be used to study the biological substrates of the rewarding properties of the experience of victory in male mice.

MATERIALS AND METHODS

Subjects

Commercially acquired OF1 male mice ($n = 62$) arrived in the laboratory at 42 days of age. Twenty subjects (experimental) were individually housed in plastic cages measuring $15 \times 15 \times 10$ cm (Leticia S.A.) during a period of 6 wk to induce aggression. A large group of subjects ($n = 42$) were housed in groups of six animals in opaque plastic cages measuring $24.4 \times 24.5 \times 15$ cm (Panlab S.L.) and utilised as nonaggressive opponents. All the animals lived under a reverse lighting schedule with a 12:12 h light/dark cycle (dark onset at 07:00 h local time) and were maintained at 18–21°C. Food and water were supplied ad lib.

Apparatus for CPP

The apparatus used in the CPP procedure (Fig. 1) consisted of a plastic (Plexiglas) unit ($70 \times 30 \times 30$ cm) divided into three compartments: one neutral brown center section ($10 \times 12 \times 30$ cm) separated by guillotine doors of two cue-distinct end chambers ($30 \times 30 \times 30$ cm). Each of the two large compartments was distinctive in floor, top and wall color, in floor texture and in brightness. These physical differences allowed for distinction by two senses, viz., tactile (floor texture) and visual (color and brightness). One of the large compartments (black one) consisted of a black floor, walls and top, a grey plastic fine mesh floor (20 mm) and was illuminated by white light located 31 cm above the floor. The other large compartment (white one) consisted of a white floor, walls and top, a white rough mesh floor (60 mm) and was illuminated by white light located 56 cm above the floor. The central compartment was brown, nondistinctive and without direct light. The apparatus was covered by an opaque top with a hole for each compartment which allowed light (25 watt. bulb) to enter and also permit the entry or removal of the animals. One wall (in front of the camera) was translucent allowing the observation and recording of the position of the animals.

All behavioral testing was carried out during the dark phase (between 2nd to 9th h of dark period) of the light/dark cycle. Subjects were adapted to the light for some minutes before introducing them into the test apparatus during all phases of the conditioning procedure. Two identical apparatus were employed but not simultaneously. Each animal was randomly assigned to either apparatus in which all phases of the procedure for the mice occurred.

Behavioral Procedure

The conditioning schedule consisted of a series of preconditioning, conditioning and postconditioning sessions. *The Preconditioning phase* was 3 consecutive days in duration (Days 1–3). On these days, each isolated male was placed once a day into the middle compartment (brown choice area) of the apparatus. After 30 s, the guillotine doors were raised and each animal was allowed to explore all three interconnected compartments of the apparatus for 10 min. On the third preconditioning trial, the time spent inside each of the different compartments

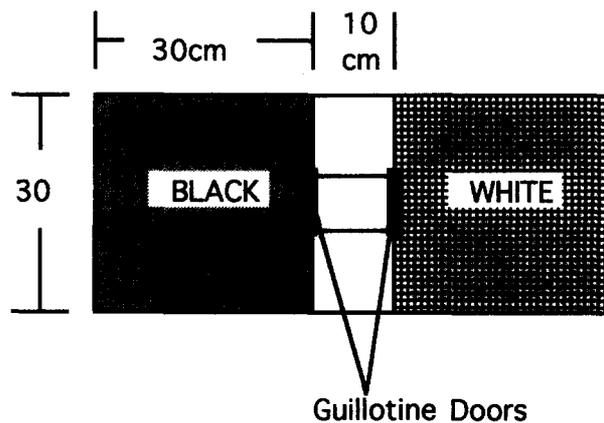


FIG. 1. Top view of the floor plan of the apparatus used for the Place Preference Conditioning in mice.

(black, white and brown) was recorded using a microprocessor (COMMODORE). It was established that an animal was inside when the four paws were in a compartment. The number of seconds spent in each compartment provided a measure of preconditioning preference for the black or white compartment. Mice spent very little time in the brown compartment during this test. The less-preferred compartment was that one in which an individual mouse had spent less time. Twelve mice preferred the black compartment, whereas the other eight preferred the white one. Subsequently, subjects were allocated to two groups (Control and Aggression groups). Each one consisted of four animals with a less-preference for the black compartment and six animals with a less-preference for the white one. Subjects were distributed in such a manner that there were no significant differences in the time both groups spent in the less-preferred and preferred compartments.

The Conditioning phase occurred over the next 8 consecutive days (Days 4–11). On the first and all subsequent odd-numbered days (1,2,3,5, and 7) each experimental male was placed into the initially less-preferred compartment with the door down. Subjects of the Aggression group were left alone for 2 min to ensure that they had ample exposure to the cues of the aggression-paired compartment of the apparatus. Subsequently, a fur-marked opponent was introduced and the subject was allowed to attack it during a period of 10 min. All animals showed aggression towards opponents during all encounters. Each animal was then returned to its home cage. On the second day of conditioning and all subsequent even-numbered days (2,4,6, and 8) each subject was placed in the initially preferred compartment and left alone for 12 min. Animals of the Control group remained alone for 12 min both in the less-preferred and the preferred compartments.

The Postconditioning phase consisted of three tests conducted 1, 6 and 12 days following conditioning (Days 12,18,24). Each male was placed into the brown compartment with the doors lowered. After 30 s, the guillotine doors were raised and the time spent inside the three compartments during the next 10 min was measured. No opponent was present during these tests. Although usually only one preference test is used, in the present experiment the number of days of preference testing was extended to determine the duration of the CPP over 12 days after the conditioning phase.

To eliminate olfactory cues any urine and feces were sponged from the floor and walls of the apparatus between tests and, moreover, was cleaned with 90% ethanol. The mesh was changed after

TABLE 1
TIME (MEAN ± D.S.) SPENT BY CONTROL AND AGGRESSION GROUPS IN THE INITIALLY LESS-PREFERRED COMPARTMENT OF THE PLACE PREFERENCE APPARATUS BEFORE AND AFTER CONDITIONING

Groups	Test for Place Preference			
	Pre Conditioning	Post Conditioning		
		1	2	3
Control	230,3 ± 19,4	239,8 ± 47,3	230,3 ± 31,3	238,1 ± 53,8
Aggression	230,1 ± 15,1	267,5* ± 46,3	249,6 ± 23,0	238,5 ± 42,2

No differences were found between groups.
* Differs from Preconditioning test of the Aggression group ($p < 0.05$), Newman-Keuls post-hoc comparisons.

every test. This procedure was maintained during all the experiment.

STATISTICAL ANALYSIS

Data of the time (s) spent in the initially less-preferred compartment before and after conditioning were subjected to a mixed analysis of variance (ANOVA). The design consisted of one between-subjects factor (GROUP with two levels: Control and Aggression groups) and one within-subjects factor (TEST for preference with 4 levels: one preconditioning and three postconditioning tests). Additionally, data were also submitted to a factorial [2 × 2 × (4)] analysis of variance (ANOVA). The design consisted of two between-subjects factors (one was the GROUP with 2 levels: Control and Aggression groups, and the other was the COLOR SUBGROUPS with 2 levels: Black Subgroup with initially less-preference for the black compartment and White Subgroup with initially less-preference for the white one) and one within-subjects factor (TEST for preference with 4 levels: one preconditioning and three postconditioning tests). Additional ANOVAs for separate GROUPS and COLOR SUBGROUPS of animals were used. Post hoc comparisons were made using Newman-Keuls tests. Student's test was used when necessary.

RESULTS

Time (mean ± SD) spent by Control and Aggression groups in the initially less-preferred compartment (LPC), before and after conditioning, are presented in Table 1. No differences were found between groups in the time spent in the LPC over tests. However, the time animals of the Aggression group spent in this

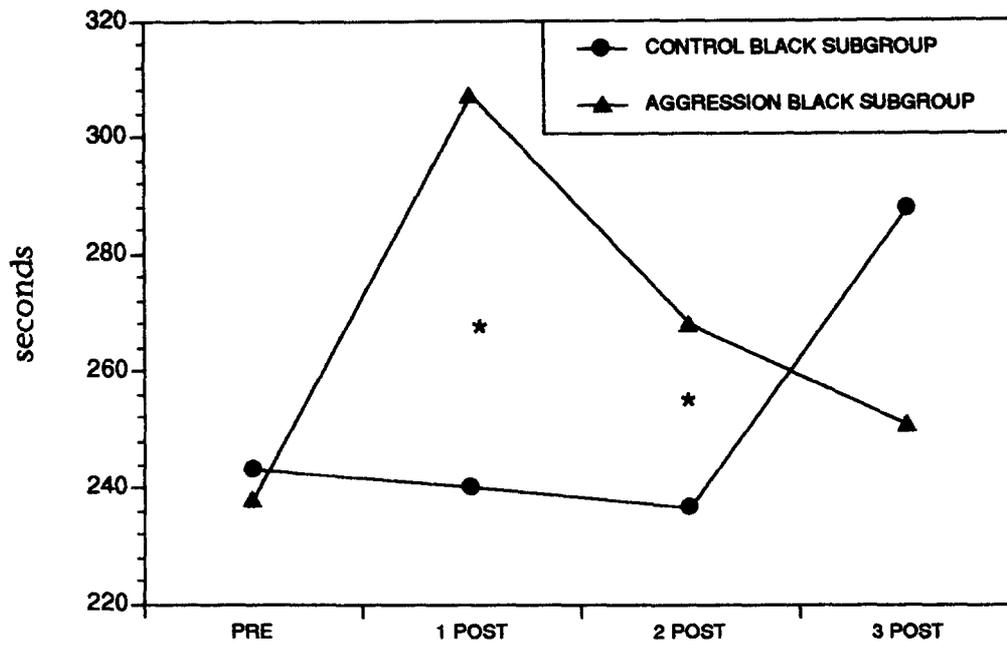
compartment paired with aggression varied over tests [$F(3, 27) = 2.860, p = 0.055$]. Post hoc comparisons revealed that they spent more time in this compartment during the first postconditioning test than during the preconditioning test ($p < 0.05$). On the contrary, no differences were found in the time animals of the Control group spent in the LPC over tests.

A closer observation of individual animals of the Aggression group seemed to indicate that the establishment of a conditioned place preference to the compartment paired with aggression was influenced by the color (black or white) of this compartment. To corroborate statistically this hypothesis, animals of each group were distributed in two Color Subgroups (Black Subgroups with an initially less-preference for the black compartment, and White Subgroups with an initially less-preference for the white one). Time (mean ± SD) spent by Control and Aggression Subgroups in the initially less-preferred compartment, before and after conditioning, is presented in Table 2.

A new ANOVA carried out on these data revealed a significant GROUP × COLOR SUBGROUP × TEST interaction effect $F(3, 48) = 4.013, p < 0.02$. Thus the statistical analysis corroborated that the color of the initially less-preferred compartment paired with aggression influenced the results. A separate ANOVA carried out only on BLACK SUBGROUPS showed a significant SUBGROUP × TEST effect $F(3, 18) = 5.46, p < 0.01$. As can be seen in Fig. 2, there was a significant difference between Control and Aggression Black subgroups in time spent in the LPC during the first ($p < 0.05$) and second postconditioning tests ($p < 0.05$). The differences between subgroups disappeared on the third postconditioning test. Moreover, simple effects revealed that the time the Aggression Black subgroup spent in the LPC paired with aggression varied over encounters $F(3,$

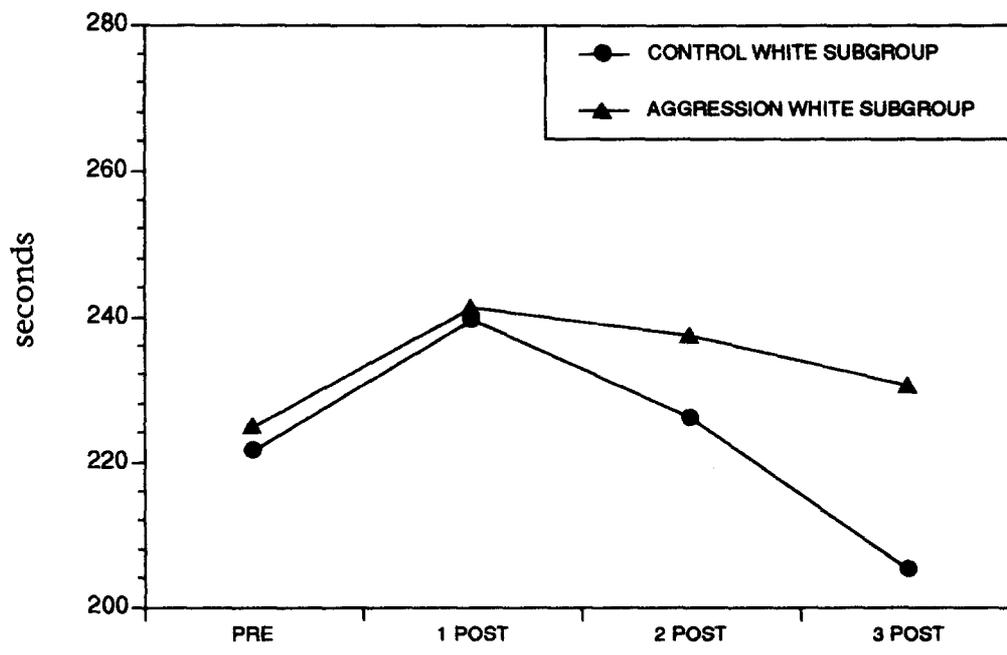
TABLE 2
TIME (MEAN ± D.S.) SPENT BY CONTROL AND AGGRESSION SUBGROUPS IN THE INITIALLY LESS-PREFERRED COMPARTMENT OF THE PLACE PREFERENCE APPARATUS BEFORE AND AFTER CONDITIONING

Groups	Subgroups	Test for Place Preference			
		Pre Conditioning	Post Conditioning		
			1	2	3
Control	Black	243,1 ± 13,52	240,12 ± 31,1	236,62 ± 23,16	287,41 ± 24,63
	White	221,68 ± 18,61	239,63 ± 58,64	226,02 ± 37,21	205,67 ± 40,07
Aggression	Black	238,03 ± 17,45	306,88 ± 40,68	267,87 ± 10,83	250,75 ± 50,53
	White	224,87 ± 12,01	241,27 ± 28,19	237,38 ± 20,82	230,33 ± 38,73



TEST FOR PREFERENCE

FIG. 2. Time (mean) spent by Control and Aggression Black subgroups in the initially less-preferred compartment of the place preference apparatus before and after conditioning. *Differences between subgroups ($p < 0.05$).



TEST FOR PREFERENCE

FIG. 3. Time (mean) spent by Control and Aggression White subgroups in the initially less-preferred compartment of the place preference apparatus before and after conditioning. No differences were found between subgroups.

TABLE 3

TIME (MEAN \pm S.D.) SPENT BY CONTROL AND AGGRESSION SUBGROUPS IN THE PREFERRED AND LESS-PREFERRED COMPARTMENTS DURING PRE-CONDITIONING TEST

Groups	Subgroups			
	White Subgroups (with less-preference for the white compartment)		Black Subgroups (with less-preference for the black compartment)	
	Less-preferred (White)	Preferred (Black)	Less-Preferred (Black)	Preferred (White)
Control	221,7* \pm 18,6	304,7 \pm 31,1	243,1 \pm 13,5	281,6 \pm 20,9
Aggression	224,9† \pm 12,0	284,2 \pm 23,7	238,0 \pm 17,5	262,8 \pm 5,4

* Differs from the preferred black compartment of the Control White Subgroup ($p < 0.006$, paired samples two tailed Student's test). † Differs from the preferred black compartment of the Aggression White Subgroup ($p < 0.005$, paired samples two tailed Student's test).

18) = 4,516, $p < 0.02$, while no significant differences were found for Control Black subgroup. Thus, animals of the Aggression group acquired a CPP for the compartment paired with aggression when it was the black one.

Finally, a separate ANOVA carried out on White subgroups showed no significant SUBGROUP \times TEST effect. As can be seen in Fig. 3, both Control and Aggression White subgroups spent similar time in the LPC over tests. Thus, animals of the Aggression group did not acquire a CPP for the compartment paired with aggression when it was the white one.

DISCUSSION

The present results suggest that environmental cues are an important variable in the establishment of a CPP using successful intermale aggression as a reinforcer. In this experiment mice acquired a CPP for the initially less-preferred compartment paired with aggression only if this experience took place in the black compartment but no CPP was evident in the white one. Thus, environmental cues (color of the wall, top and floor, kind of mesh floor and brightness) chosen in this study to differentiate both large compartments of the apparatus influenced the acquisition of a CPP. The realization of three preference tests over a period of 12 days after conditioning has made it possible to study the extinction of the CPP to the black compartment, that, after being established in the first postconditioning test, was extinguished in the third.

Several explanations of the fact that a CPP was only established in the black compartment paired with aggression are now discussed.

Aversion to The White Compartment

A possible explanation could be that some environmental cues associated with the white compartment (presumably aversive) during conditioning phase neutralized the reinforcing character of the experience of victory. However, animals did not show any aversion for the white compartment during preconditioning phase. A selection of the stimuli that do not result in any strong bias for one compartment over the other was obtained after a pilot study during which all those stimuli that were aversive for the animals were eliminated (17). In general, although the difference was significant ($p < 0.05$), the time subjects spent in the white compartment (242.35 \pm 29.1) was not much shorter than the time spent in the black one (272.9 \pm 35.8).

Additionally, the level of emotionality was measured by the defecation level, counting the amount of fecal excretions inside the white and black compartments. Mean number of fecal excre-

tions in the black side was 2.2 \pm 2.6; 2.5 \pm 2.0; 2.7 \pm 1.9 and 2.2 \pm 2.4 during preconditioning, first, second and third postconditioning tests, respectively. Mean number of fecal excretions in the white side was 3.1 \pm 2.8; 2.0 \pm 1.9; 2.1 \pm 1.7 and 2.5 \pm 1.8 during preconditioning, first, second and third postconditioning tests, respectively. A statistical analysis (student's test) showed no differences in the defecation level during preconditioning and postconditioning tests between white and black compartments. Moreover, no differences were found in the defecation level in the same compartment over tests. Thus, animals showed the same level of emotionality in both compartments, suggesting that the white one was not specially aversive. Thus, it is difficult to attribute the impossibility of establishing a CPP for the white side when paired with aggression to an aversion to this compartment during conditioning phase.

A Strong Bias Unconditioned Preference

Another possible explanation is that the initial preference for the black compartment was much stronger than the initial preference for the white one. In this case, those animals conditioned to the less-preferred white compartment (White subgroups) would have to overcome an initial strong preference for the black compartment. Thus, whereas a change in the place preference would be possible when the initially preferred compartment was the white one (Black subgroups), that would not be possible when it was the black one (White subgroups). This explanation has been appropriate for other studies in which a change of preference was not established in animals with a high preference for one compartment, even when the stimulus was a well known aversive one (14). In the present study, in spite of the fact that 12 animals showed an unconditioned preference for the black and 8 for the white sides, no differences were found on the strength of the initial unconditioned preference. As can be seen in Table 3 the time animals spent inside the preferred side during preconditioning test was similar, independently of the color of the compartment. This suggests that there was not a stronger preference for the black compartment than for the white one that could influence the establishment of a change in the preference. Moreover, the same similarity seen for the preferred compartment was also noted for the less-preferred. This suggests that the white compartment was not less-preferred than the black one. However, as can be seen in Table 3, the differences between time spent in the preferred and less-preferred compartments were greater when the preferred was the black (White subgroups) than when it was the white one (Black subgroups). Control White subgroup spent more time in the black preferred compartment than in the white less-preferred one ($p < 0.006$). And the same

happened with the Aggression White subgroup ($p < 0.005$). But, on the contrary, no differences were found in both groups between the preferred and less-preferred compartments when the preferred was the white one (Black subgroups). Thus, in spite of these differences, the present findings cannot be explained by considering that those animals conditioned to the less-preferred white compartment had to overcome an initial strong preference for the black compartment or an initial strong less-preference for the white one.

Associative Bias

The CPP paradigm is based on the assumption that the animal will establish an association between an internal state or an experience lived in one place and the environmental cues of that place, and later will be able to remember this association. This raises the issue of "associative bias" which refers to the phenomenon that some associations are learned more readily than others (4). This suggests that it would be possible that the association between successful aggressive experience and the black compartment was more ready than between this experience and the white one. To demonstrate that this is what happened in the present study, it is necessary to carry out another experiment in which a well known reinforcing stimulus such as food in a deprived animal is used. To this respect, an experiment is in progress in our laboratory to study the CPP using food as a reward. If the phenomenon seen in the present study (the selective CPP to the aggression-paired black compartment) is found again it will indicate that it is a general phenomenon and it will be necessary to find out if it is due to the version of the place preference method

used in the present experiment (characteristics of the CPP apparatus, procedure), to the species and strain employed or other variables (animal housing, etc.). On the contrary, if this phenomenon is not found with food as a reward, it will be considered specific for the establishment of a CPP using successful aggressive experience as reinforcer.

It is important to have in mind that in the present study the distinctive stimuli between the big compartments have been of a tactile and visual nature. Therefore, it would be interesting to carry out a study in which olfactory stimuli were also used, knowing that in this species the olfactory sense is an important source of information.

In conclusion, the present study demonstrates that successful intermale aggression can act as a reinforcer in the Conditioned Place Preference paradigm. However, it seems that the process of establishing this kind of conditioning using experience of victory as a reinforcer is extremely fragile and can be easily disrupted by changing the properties of the environmental cues involved. The reason for this is not clear. Due to the fact that only a partial Conditioned Place Preference has been obtained, more research is needed to demonstrate that this paradigm is a sensitive measure to study the reinforcing properties of aggressive behavior.

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