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Equivalent aversiveness of back- and tail-handling in the rat

CARLO AMBROGI LORENZINI, CORRADO BUCHERELLI, SUSANNA FALCHINI, ALDO GIACHETTI, and GIOVANNA TASSONI
Università degli Studi di Firenze, Firenze, Italy

(Vincent M. LoLordo, *Sponsor*)

Using a two-box apparatus, it was shown that back-handling, when temporally close to a step-through latency test, causes a conditioned avoidance response that inhibits the exploratory behavior of the rat. The amount of inhibition due to back-handling was compared with that due to tail-handling (previously published). No significant differences were found between the inhibitory effects of these two methods of handling rats. This result is discussed in relation to sensory input and predation simulation.

We have observed that in many published papers on the behavior of rats, no information is given on how the rats had been handled; in fact, most authors simply state that the animals "were placed" inside an apparatus. It has recently been shown that handling rats to place them in an experimental apparatus causes a conditioned inhibition of their exploratory behavior (Ambrogi Lorenzini, Bucherelli, Giachetti, & Tassoni, 1990). Thus, the effects of handling can interfere with the results of an experiment, and must be taken into account (Candland, Horowitz, & Culbertson, 1962).

In our previous experiments, rats were handled by the tail (Ambrogi Lorenzini et al., 1990). Clearly, this is only one of the possible ways of handling a rat. Back-handling (i.e., grasping the animal by the back) seems to be the most likely alternative. With reference to the effects of back-handling on the hooded rat, at least one report has stated that this method of handling is less aversive than tail-handling (McAllister, McAllister, Hampton, & Scoles, 1980).

The aim of the present work was to ascertain, using the experimental apparatus described by Ambrogi Lorenzini et al. (1990), whether or not back-handling is sufficiently aversive to cause a conditioned inhibition of exploration in Wistar rats. Should this be the case, the next question is: Are there significant differences in aversiveness to tail- and back-handling? We shall try to answer this by comparing the already published results of tail-handling with those of back-handling, in order to provide both qualitative methodological information (i.e., is there a preferable way of handling rats?) and more quantitative information on the effects of handling (Barnett, 1975).

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EXPERIMENT 1

The aim of this experiment was to ascertain whether back-handling could cause a conditioned inhibition of exploratory behavior, measured as step-through latency in a two-box apparatus.

Method

Subjects. Seventy-two 60-day-old naive male adult Wistar rats (purchased from Morini, Italy) served as subjects. The rats were housed singly and received food and water ad lib. The animals were not handled by us before the experiment, and we ascertained that the rats had not received any previous gentling at the breeding facilities.

Apparatus. The two-box apparatus (Ambrogi Lorenzini et al., 1990) consisted of two Plexiglas chambers of equal dimensions (30 × 21 × 15 cm) with stainless steel bar floors. The chambers were connected by a guillotine door (8 × 6 cm). The walls of one of the chambers were solid white; those of the other were vertically striped black and white (black stripe: 1.4 cm; white stripe: 3 cm). Both chambers were covered by transparent lids. The apparatus was placed in an acoustically isolated room, maintained at a constant temperature (20° ± 1° C). Lighting inside the apparatus was 60 lux.

Procedure. In all trials, the subjects were manually taken out of the home cage by the back (the hand of the experimenter closing gently around the thorax, just behind the forelegs). They were then placed inside a plastic container, and were slid gently into the startbox of the apparatus. Manual step-through latency measurements started immediately after the lid of the start chamber was closed, and were taken for up to 180 sec. Step-through latency was taken when the animal had placed all four paws in the goal chamber. During this interval, the number of attempts to enter the goal chamber as well as instances of grooming were counted, and freezing duration was measured. Attempts were defined as incomplete passages into the goal chamber (up to three paws inside it). Freezing was defined as motor inhibition resulting in immobility, ending when voluntary movements were performed. All measurements were made by human observers. For all animals, the start chamber was the solid white one, while the striped box was the goal chamber.

The experiment consisted of six consecutive daily trials. The animals underwent one trial each day, starting at 0900 h. The animals were randomly divided into six groups of 12 subjects each. The subjects in the first group (D_0) were not detained within the goal chamber. Immediately after these subjects had entered the goal chamber or at the end of a 180-sec period, they were returned to their respective home cages. After their spontaneous entry into the goal chamber or at the end of a 180-sec period, the subjects of the other five groups were detained

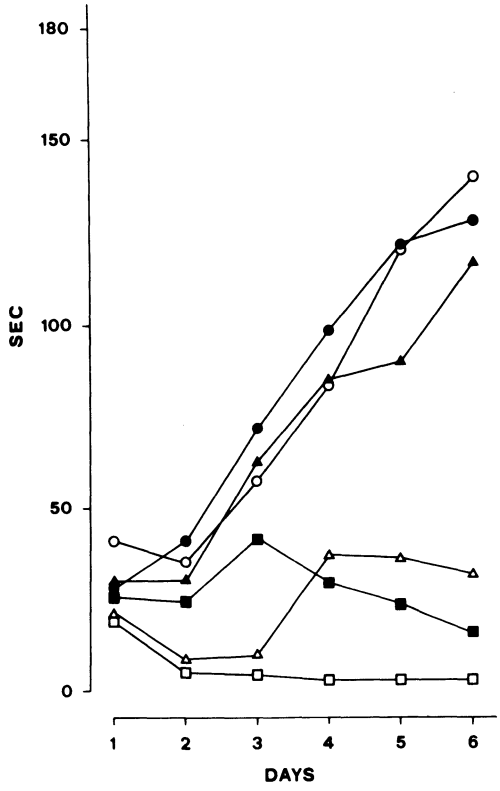

Figure 1. Mean daily step-through latency values (in seconds) of undetained (D_0, ●) and detained (D_{10}, ○; D_{30}, ▲; D_{60}, △; D_{300}, ■; and D_{600}, □) groups of subjects in Experiment 1.

inside the goal chamber for 10, 30, 60, 300, or 600 sec before being returned to their home cages (D_{10}, D_{30}, D_{60}, D_{300}, and D_{600} subjects). The results of the experiment were analyzed by means of a two-way analysis of variance (ANOVA) with repeated measures on trials. Paired comparisons between groups were performed by means of the post hoc Tukey test (Tukey, 1949). Trial 1 step-through latencies of all subjects were subjected to independent statistical analysis, since these latencies were measured before group differentiation in relation to the duration of goal-chamber detention.

Results

Step-through latency. As shown in Figure 1, in Trials 2-6, the subjects that did not undergo detention (D_0) and those that underwent short-lasting detention (D_{10} and D_{30}) exhibited a progressively higher step-through latency than did the subjects of the other three groups. The results were analyzed by means of a groups (6) × trials (5) ANOVA. There were significant differences between groups [$F(5,66) = 10.15$, $p < .001$] and between trials [$F(4,264) = 28.02$, $p < .001$]. Significant differences were also found between Group D_0 and each of Groups D_{60}, D_{300}, and D_{600} (Tukey test, $p < .001$). Among the detained groups, significant differences were found between Groups D_0 and D_{30} and each of Groups D_{60}, D_{300}, and D_{600} ($p < .01$). There were no significant differences in Trial 1 step-through latency between any of the groups [$F(5,66) = 0.96$, n.s.].

Attempts. As shown in Table 1, in the groups that exhibited a progressive increase in step-through latency from Trial 2 to Trial 6 (Groups D_0, D_{10}, and D_{30}), there was also a progressive increase in the number of attempts. D_0 subjects exhibited the highest number of attempts. A groups (6) × trials (5) ANOVA showed that there were significant differences between groups [$F(5,66) = 7.24$, $p < .001$] and between trials [$F(4,264) = 22.49$, $p < .001$]. Significant differences were also found between Group D_0 and each of Groups D_{30}, D_{60}, D_{300}, and D_{600} (Tukey test, $p < .01$), and between Group D_{10} and each of Groups D_{60}, D_{300}, and D_{600} ($p < .01$).

Grooming. As shown in Table 1, although there was a great amount of variability in all groups of subjects, the highest mean grooming counts occurred in the groups with the lowest detention durations (D_0 and D_{10}). A groups (6) × trials (5) ANOVA showed that there were significant differences between groups [$F(5,66) = 4.16$, $p < .001$] and between trials [$F(4,264) = 5.22$, $p < .001$]. Significant differences were found between Group D_{10} and each of Groups D_{60}, D_{300}, and D_{600} (Tukey test, $p < .01$).

Table 1
Mean (±*SE*) Number of Attempts and Groomings of Undetained (D_0) and Detained (D_{10}, D_{30}, D_{60}, D_{300}, D_{600}) Groups of Subjects in Experiment 1

	Trial											
	1		2		3		4		5		6	
Group	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
						Attempts						
D_0	0.00	0.00	0.17	0.11	0.42	0.19	0.92	0.29	1.25	0.33	1.00	0.37
D_{10}	0.00	0.00	0.08	0.08	0.25	0.18	0.75	0.25	1.33	0.50	1.58	0.56
D_{30}	0.08	0.08	0.08	0.08	0.33	0.19	0.75	0.28	0.50	0.19	0.08	0.37
D_{60}	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.11	0.08	0.08	0.17	0.11
D_{300}	0.00	0.00	0.00	0.00	0.17	0.11	0.00	0.00	0.08	0.08	0.08	0.08
D_{600}	0.00	0.00	0.00	0.00	0.08	0.08	0.17	0.11	0.00	0.00	0.25	0.13
						Groomings						
D_0	0.00	0.00	0.25	0.18	0.50	0.29	0.50	0.26	0.33	0.19	1.25	0.41
D_{10}	0.00	0.00	0.00	0.00	0.17	0.17	0.42	0.23	1.08	0.42	1.00	0.37
D_{30}	0.08	0.08	0.00	0.00	0.08	0.08	0.25	0.13	0.50	0.29	0.42	0.29
D_{60}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.13	0.08	0.08
D_{300}	0.00	0.00	0.00	0.00	1.17	1.17	1.17	1.17	0.08	0.08	0.00	0.00
D_{600}	0.00	0.00	0.00	0.00	1.17	1.11	0.00	0.00	0.25	0.13	0.08	0.08

Freezing. No freezing was observed in any of the subjects.

Discussion

The results show that there was a progressively increasing inhibition of step-through latency in Groups D_0, D_{10}, and D_{30}, in which there was close or fairly close temporal contiguity between stepping from the starting into the goal chamber and handling. Thus, there was an inhibition of exploratory behavior due to the aversive character of back-handling, as was shown to be the case for tail-handling.

The uniform behavior of all animals on Trial 1 is to be taken as proof of the behavioral uniformity of the subjects employed. Thus, the exhibited behavioral differences must be taken as being due to the effects of the experimental paradigm. It must be emphasized that in all subjects, only exploratory behavior was inhibited, whereas locomotion and/or other activities remained unimpaired. This statement is borne out by attempts and instances of grooming, and by the absence of freezing.

EXPERIMENT 2

The aim of Experiment 2 was to ascertain whether the aversive effects of back-handling, once established, can undergo extinction subsequent to goal-chamber detention. Five initial trials were undertaken. During these trials, the animals were handled by the back immediately after they entered the goal chamber (as was the case for the D_0 subjects in Experiment 1). Six additional trials were carried out, in which one group of rats continued to be handled as before (i.e., as soon as they entered the goal chamber) and the other five groups were detained in the goal chamber for different lengths of time (10, 30, 60, 300, and 600 sec).

Method

Subjects. Sixty 60-day-old naive male adult Wistar rats (purchased from Morini, Italy) served as subjects. They were housed singly and received food and water ad lib. The rats were not handled by us before the experiment, and we ascertained that they had not received any previous gentling at the breeding facilities.

Apparatus. The apparatus used in Experiment 1 was also used in this experiment.

Procedure. The animals were randomly divided into six groups of 10 rats each. The experiment consisted of 11 consecutive daily trials. The animals underwent one trial each day, starting at 0900 h. On Trials 1-5, all subjects underwent the same treatment as the D_0 subjects in Experiment 1 (i.e., they were handled by the back and returned to their home cages either as soon as they had gone into the goal chamber or at the end of a 180-sec period). In Trials 6-11, the subjects in the first group (D_0-0) were not detained inside the goal chamber, but underwent the same procedure as in Trials 1-5. The subjects in the other five groups were detained in the goal chamber for 10, 30, 60, 300, or 600 sec before being returned to their home cages (D_0-10, D_0-30, D_0-60, D_0-300, and D_0-600 subjects).

The results were subjected to the same statistical analysis as in Experiment 1. In the same way, step-through latencies of all subjects in Trial 1 were subjected to independent statistical analysis. Trial 6 step-through latencies of all subjects were statistically treated together with those of Trials 2-5, since they were obtained before group differentiation according to the duration of goal-chamber detention. In addition, latencies of Trials 7-11 were combined for statistical analysis.

Results

Step-through latency. As shown in Figure 2, in Trials 2-6 (before any of the subjects were detained in the goal chamber), all groups exhibited a similar progressive increase in step-through latency. The results were analyzed by means of a groups (6) × trials (5) ANOVA. There were no significant differences between groups [$F(5,54) = 0.34$, n.s.], but there were significant differences between trials [$F(4,216) = 47.60$, $p < .001$]. Following the detention of five of the groups of subjects in the goal chamber, as shown in Figure 2, there were differences in step-through latency, which were already present by Trial 7 and were maintained through Trial 11. Step-through latency values for Groups D_0-0 and D_0-10 remained the highest, whereas latency values for Groups D_0-300 and D_0-600 (the longest detentions) were almost always the lowest. These results were analyzed by means of a groups (6) × trials (5) ANOVA. There were significant differences between groups [$F(5,54) = 3.35$, $p < .01$], but there were no significant differences between trials [$F(4,216) = 1.51$, n.s.]. The Tukey test showed that there were significant differences between Group D_0-0 and Groups D_0-300 ($p < .01$) and D_0-600 ($p < .05$). With regard to the detained groups, there were significant differences between Group D_0-600 and Groups D_0-10 and D_0-60 ($p < .05$). There were also significant differences between Group D_0-300 and Groups D_0-10 and D_0-60 ($p < .05$). There were no significant between-groups differences in step-through latencies [$F(5,54) = 0.57$, n.s.].

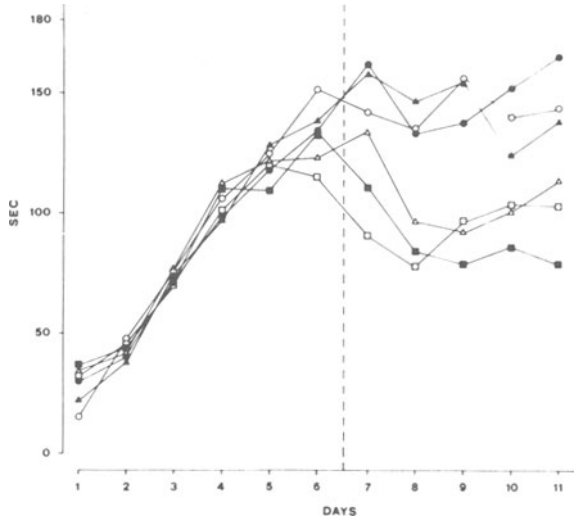

Figure 2. Mean daily step-through latency values (in seconds) of undetained (D_0-0, ●) and detained (D_0-10, ○; D_0-30, ▲; D_0-60, △; D_0-300, ■; and D_0-600, □) groups of subjects in Experiment 2.

Discussion

The results of this experiment confirm that back-handling inhibits exploratory behavior, and that this inhibition undergoes extinction when a sufficient delay is interposed between a step-through test and handling.

Tail- versus back-handling. By means of ANOVA, no significant differences were found between the step-through latencies of all subjects on Trial 1 in Experiment 1 of the present work and on Trial 1 of Experiment 1 of the study by Ambrogi Lorenzini et al. (1990) [$F(11,132) = 1.33$, n.s.]. The results of the present experiments and those of our previous study were analyzed by means of a two-way ANOVA with repeated measures on trials. Paired comparisons between homologous groups (i.e., those with the same detention times) were performed using post hoc the Tukey test (Tukey, 1949).

The results of Experiment 1 of the present work and of Experiment 1 of the previously published paper (Trials 2-6) were analyzed by means of a groups (12) × trials (5) ANOVA. There were significant differences between groups [$F(11,132) = 4.25, p < .001$] and between trials [$F(4,528) = 20.51, p < .001$]. No significant differences were found between homologous groups of tail- and back-handled rats (Tukey test).

The statistical analysis of the results of Experiment 2 of the present work and of Experiment 3 of the previously published paper, for Trials 2-6 [groups (12) × trials (5) ANOVA], revealed no significant differences between groups [$F(11,108) = 0.19$, n.s.]. However, there were significant differences between trials [$F(4,432) = 149.02, p < .001$]. For Trials 7-11, there were significant differences between groups [$F(11,108) = 3.93, p < .001$], but there were no significant differences between trials [$F(4,432) = 0.55$, n.s.]. No significant differences were found between homologous groups of tail- and back-handled rats (Tukey test).

GENERAL DISCUSSION

As in previous reports, all subjects exhibited a low step-through latency on Trial 1 of Experiments 1 and 2. This may be taken as an index of the behavioral uniformity of the animals employed, in addition to confirming the neophilia of Wistar rats.

The results of Experiment 1 show that back-handling, even if performed with the greatest possible care (i.e., without unduly squeezing or constricting the animals), causes a progressive inhibition of exploratory behavior. This inhibition, measured by the increase in step-through latency, appears to be stronger the shorter the interval is between the passage into the goalbox and handling.

The results of Experiment 2 show that, for all subjects, inhibition of exploration caused by back-handling in close temporal proximity with a step-through test can be significantly diminished during subsequent trials if a sufficient delay is interposed between the test and back-handling. In other words, the present experiments show that back-handling causes a conditioned inhibition of exploratory behavior in the rat, and that this conditioned response undergoes extinction.

It must be emphasized that only exploratory behavior was inhibited; locomotion and other motor activities, such as grooming and attempts, were performed by the rats during the step-through latency period. Therefore, we may conclude that back-handling possesses sufficient aversive characteristics to cause a conditioned avoidance response, as has already been shown to be the case for tail-handling, thus confirming other reports (Max et al., 1974; McAllister et al., 1980).

At this point, we may proceed to compare the aversiveness of these two methods of handling rats. Such a comparison can be made for several reasons. First, in the present study, we employed naive rats, since rats already subjected to tail-handling could not be used to investigate back-handling. Second, the present experiments employed the same procedure and apparatus, and the same source, age, housing, alimentation, sex, and strain of rats were used as in the previous study. In fact, the behavioral uniformity of the Wistar rats employed in both sets of experiments is shown by the absence of statistical differences between the step-through latency values of Trial 1 of all the experiments.

Why compare these two methods of handling rats? Back-handling has been recommended as being "better" (Pessotti & Longoni Forges, 1972), and as being less aversive than tail-handling in hooded rats (McAllister et al., 1980). Moreover, it can be surmised that the stimulation of different types and populations of receptors (vestibular, articular, cutaneous) depends on how the animal is handled.

The statistical comparison of the findings of the experiments of Ambrogi Lorenzini et al. (1990) and the present ones did not reveal any significant difference in step-through latency values for homologous groups of rats. Therefore, the conclusion that must be drawn is that, at least for the paradigm employed, and for Wistar rats, there are no differences in aversiveness between tail- and back-handling. Thus, there is no preference for either method of handling. Back-handling may be more suitable when particular manipulations are to be performed (e.g., i.p. administration of drugs). Nevertheless, our results do not confirm the existence of a quantitative difference between the two methods of handling with regard to the acquisition of a conditioned passive-avoidance response.

This equivalent aversiveness may not be due to an equal or balanced sensory input but, rather, to the effect being handled has on the animal. In fact, for a rat (or indeed any small rodent), being handled is very similar to being seized by a predator (Barnett, 1975). It may be the case that the effects of this simulation are so massive that they elicit behavioral responses so elementary as to cause the same conditioned response, irrespective of the possible differences in sensory input due to back- or tail-handling.

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