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INHIBITORY AVOIDANCE WITH A TWO-WAY SHUTTLE-BOX

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The possibility of carrying out inhibitory avoidance experiments, also called passive avoidance, with a two-way shuttle-box was tested in thirty-five OF1 male mice. Two intensity levels of shock (0.3 and 0.6 mA) and a non-shocked group of animals were employed in this experiment. Inhibitory avoidance conditioning was observed in the 0.6 mA group (statistically significant differences between the response latencies of Day 2 versus Day 1) but not in the case of the 0.3 mA group. These results allow us to conclude that it is possible to obtain inhibitory avoidance in a shuttle-box.

Evitación inhibitoria con una caja de escape-evitación de dos sentidos. Se estudió, en treinta y cinco ratones macho OF1, la posibilidad de ejecutar experimentos de evitación inhibitoria, también llamada evitación pasiva, con una caja de escape-evitación de dos sentidos. Se empleó shock de dos niveles de intensidad (0.3 mA y 0.6 mA) y un tercer grupo de animales que no recibió shock. Se observó condicionamiento de evitación inhibitoria en el grupo de 0.6 mA (diferencias estadísticamente significativas entre las latencias de respuesta del Día 2 con respecto a las del Día 1) pero no en el grupo de 0.3 mA. Nuestros resultados permiten concluir que es posible obtener condicionamiento de evitación inhibitoria con una caja de escape-evitación de dos sentidos.

In inhibitory avoidance experiments (also called passive avoidance), the animal precludes the aversive stimulus by not showing a behavior regularly shown in a particular environment. Inhibitory avoidance is acquired in only one trial, where the subject is punished for doing something such as entering a new compartment (test). Then, in a second session (retest), behavioral inhibition, for example not entering the compartment, is measured (see Bureš, Burešová and Huston, 1983, for the usual procedures). This procedure is frequently used in memory experiments (e.g., Berger-Sweeney, Arnold, Gabeau and Mills, 1995; for a review, Izquierdo and Medina, 1997), perhaps because it is not a time consuming procedure. Also and more important, it is easy to decide the appropriate moment for pharmacological treatment taking into account the learning processes of acquisition, consolidation and retention (Heise, 1987).

Separate equipment exists for experiments of inhibitory (passive) and active avoidance. Nevertheless, mixed procedures alternating trials of both are performed with two-way shuttle-boxes and are well known in scientific literature. These procedures in-

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volve discrimination between *go* (active) and *no-go* (passive) trials as described by Alleva, Laviola, Tirelli and Bignami (1985). The present experiment was designed in part to check for the first time a complete inhibitory avoidance protocol for mice with a two-way shuttle-box, an apparatus commonly used for active avoidance conditioning. In addition to these technical questions, a deeper theoretical matter was considered. In inhibitory avoidance experiments inhibition is defined as the increase of response latency in the retest session with respect to that of the first test. That is to say, the control for the inhibition is the performance of the same subjects in a previous situation. This is a within subject design. We believe that the appropriate control for the behavior of the subjects in the retest session are subjects that in the test session did not receive shock when entering the other compartment. This is a between subject design.

Other details of the experiment that follows are inspired by Bureš' et al. (1983) report on the *step-through* type of passive avoidance task.

Method

Animals

Thirty-five OF1 male mice were obtained from CRIFFA (Lyon, France). They were housed in groups of five in standard plastic cages stored in a temperature controlled room (21 ± 2 ºC). Food and water were freely available and a reversed light-dark cycle was in effect (0730-1930 lights off). The tests were always carried out during the dark phase of the light cycle.

Apparatus

A two-way shuttle-box with acrylic walls and steel floor bars was used (Shuttle Scan, Model SC-II, Omnitech Electronics, Inc., Columbus, Ohio, USA). The box, 44 x 20 x 19 cm, is bisected by a vertical partition with an opening in the middle that allows the animal to move freely from one side to the other. Eight infrared light beams detect the position of the animal. The equipment was controlled by computer using the RMS Version 2.06 of the Omnitech Electronics software.

Procedure

Mice were randomly assigned to one of three groups: control, $shock_1$, and $shock_2$. Control mice (n = 12) were trained without footshock. $Shock_1$ mice (n = 12) received a footshock of 0.3 mA for 10 seconds if the mouse stepped into the dark compartment and remained there. The animal could return to the initial (light) compartment at will. $Shock_2$ mice (n = 11) received a footshock of 0.6 mA in the same conditions as the $shock_1$ group.

All the animals were individually subjected to 2 minutes of adaptation to the apparatus, in which the mouse could explore the light compartment and move about freely. This adaptation was repeated 30 minutes later. After an additional period of 30 minutes the test session began. The mouse was placed in the box and if it stepped into the dark compartment (maximum 300 sec), no-shock, a footshock of 0.3 mA ($Shock_1$) or 0.6 mA ($Shock_2$), depending on the group, was delivered (maximum 10 sec). If the mouse returned to the light compartment the shock disappeared. If the animal belonged to the control group the shock was always turned off. Response latencies were measured in all cases. Eight of the forty-two animals did not enter the compartment and were removed from the experiment. These mice were not numbered as subjects of the study.

The animals were returned to their home cages immediately after the acquisition test.

Twenty-four hours later, in the second phase (retest) each mouse was again placed in the shuttle-box and the latency to step through was recorded (maximum 300 sec). Again, the mouse received no-shock, 0.3 or 0.6 mA of shock. We measured crossing response latencies.

Analysis

Data were subjected to analysis of variance (ANOVA), with Treatment as a *between* factor with three levels: no-shock, $shock_1$ and $shock_2$ and Day as a *within* factor with two levels: Day 1 and Day 2. We used the Newman-Keuls-test as post-hoc analysis. Statistical analyses for individual groups of shock were done with Student's *t*-test for related samples to compare the duration of the shock received on Day 1 versus Day 2. Analyses were performed with the Statistica package, version 4.3 for Windows (StatSoft, Inc., 1993).

Results

The Treatment and Day effects were statistically significant $F(2,32)= 4,27$, $p < .023$; $F(1,32)= 5.58$, $p < .024$, respectively, as was Treatment X Day interaction $F(2,32)= 5.75$, $p < .007$. The response latencies in the $Shock_2$ group were higher than in any other group (in any of the days): Control-Day 1($p < .001$), control-Day 2 ($p < .001$), $Shock_1$-Day 1 (p (.001), $shock_1$-Day 2 (p (.001), and $Shock_2$-Day 1 ($p < .002$). The remaining comparisons were not statistically significant.

The escape from shock in the Day 2 tended to be quicker than in the Day 1 but did not reach significance level: $Shock_1$: $t(11)=1.58$, $p < .14$ and $Shock_2$: $t(10)= 2.03$, $p < .069$ (see Figure 1). Mean latencies and mean shock received (in seconds) are summarized in Table 1.

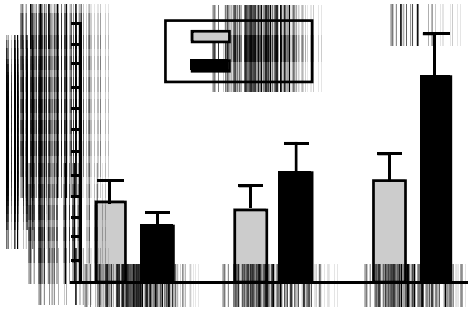

Figure 1. Mean latencies (+ *SEM*) of a each group of mice during Day 1 (test) and Day 2 (retest): Control: mice which were trained without footshock; 0.3 mA: mice which received this intensity of footshock for 10 seconds if they stepped into the dark compartment, and 0.6 mA: mice which received this intensity of footshock. **p* < .002 compared to the rest of the groups

Table 1

	Day 1: Test	**Day 2: Retest**
Control $n = 12$	76.12 (19.63	51.23 (12 .21
$Shock_1$ $n = 12$	68.35 (19.49 Mean shock: 4.15	99.34 (28.71 Mean shock: 1.71
$Shock_2$ $n = 11$	93.89 (23.43 Mean shock: 3.43	191.85 (32.86 Mean shock: 1.35

Discussion

It can be said that a shock intensity of 0.6 mA was high enough to produce inhibitory avoidance conditioning, as measured by crossing latencies: on Day 2 they were longer than on Day 1. In the present experiment the 0.3 mA group ($Shock_1$) did not show statistically significant inhibitory avoidance. It is well known that the higher the shock the quicker the inhibitory avoidance acquisition.

Standard procedure for inhibitory avoidance experiments involves performance comparison of the same subjects in two situations usually called test and retest. We think that the behavior of subjects on Day 2 (retest) is influenced, of course, by the

shock experienced on Day 1, and by adaptation to the apparatus, which is greater on Day 2. The way to control this supposed intervening variable consists of making a *between* comparison instead of a *within* one. The performance of subjects on Day 2 (those that had received shock on Day 1) have to be compared with that of a control group also on Day 2 (animals that were on Day 1 in the same situation as the experimental group unless they did not received shock when crossed to the other side of the box). Some authors have used this kind of control (e.g., Rush, 1988; Castellano, Cestari, Cabib and Puglisi-Allegra, 1993; Pavone, Fagioli and Castellano, 1993). In general, control group subjects show statistically indistinguishable latencies on Day 1 and Day 2 but the Day 2 latencies tend to be shorter than that of Day 1. This fact can facilitate obtaining statistically significant differences.

Nineteen per cent of the animals did not cross to the dark compartment during 300 seconds on Day 1. This number of discarded animals may be considered too high. In a different study carried out in our laboratory with a similar procedure the percentage was 6%, perhaps because the mice were individually housed (Everss, 1997).

In summary, we have found that the two-way shuttle box is a useful instrument to carry out inhibitory avoidance experiments. This broadens its utility already demonstrated in active avoidance experiments (e.g., Arenas, Parra and Simón, 1995; Monleón and Parra, 1997; Vinader-Caerols, Aguilar, Pérez-Iranzo, Miñarro, Parra and Simón, 1996).

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