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BRIEF COMMUNICATION

Injection of Vehicle Is Not a Stressor in Porsolt's Swim Test

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HILAKIVI-CLARKE, L. A. *Injection of vehicle is not a stressor in Porsolt's swim test.* PHARMACOL BIOCHEM BEHAV **42**(1) 193–196, 1992.—A single injection or chronic injections with vehicle are reported to induce physical changes in the brain, suggesting this treatment may be stressful. Furthermore, preliminary observations indicate that vehicle injections may interact with the behavioral effects of psychoactive compounds. We investigated the behavioral response to an injection in two tests sensitive to stress: Porsolt's swim test of depressive behavior and the resident-intruder test of aggression. Acute or repeated injections of vehicle did not alter immobility in the swim test in "normal" male NIH Swiss mice, isolated mice, or mice previously exposed to continuous fight stress. Behavior in the resident-intruder test was similar in injected and noninjected mice. The present data suggest that in male NIH Swiss mice a vehicle injection does not induce behavioral changes in tests sensitive to stress, although it is shown to alter various physiological parameters indicative of stress.

Injection Stress Porsolt's swim test Aggression Mice

BOTH acute and repeated exposure to mild stressors induce various physical changes in rodents. It has been reported that handling and weighing increase plasma corticosterone levels in rats (1). Furthermore, a single episode of handling results in a significantly increased corticotropin-releasing factor (CRF) immunoreactivity in the hypothalamus (8) and stimulates hippocampal glucose utilization transiently via NMDA receptors (16). The effects of mild stressors on behavior are largely unexplored. There are some data suggesting that in rats handling and vehicle injections interact with the anxiolytic properties of benzodiazepines in the plus-maze test (4).

The present study investigated whether a vehicle injection alters a behavioral response in two animal models known to be sensitive to stress: Porsolt's swim test (15) and resident-intruder test of aggression (14). In Porsolt's swim test, an animal is put into a cylinder containing water and the time it spends floating motionless is measured. This immobility time in the water is reportedly lengthened by an acute or chronic exposure to various stressors (2,7,10). The time spent in social investigation and aggressive behavior in the social interaction test is shown to be dependent on how stressful the testing equipment is to mice (13). Moreover, aggressive behavior in laboratory animals is often induced by means stressful to animals (14). The present study also examined whether a previous exposure to a stressor alters the effect of a vehicle injection on behavior. Fight-stressed mice are shown to spend a longer time immobile in the water than controls (10). However, according to our preliminary observations the difference in immobility in Porsolt's swim test between fight-stressed and control mice tends to diminish after a vehicle injection (9,12), indicating that injections affect animals and that the effect may be different in stressed animals. In addition, the possibility that increased sensitivity to environmental stimuli may influence behavioral responses to vehicle was examined by isolating animals prior to an injection. Isolation makes animals irritable and more difficult to handle (3, 11,17).

The results revealed that a single or repeated vehicle injection did not significantly affect behavior of mice in the swim test of depressive behavior and ability to cope with stress or in the resident-intruder test of aggression. In addition, previously isolated or fight-stressed animals did not respond to vehicle treatment. These findings suggest that injection does not induce behavioral changes in male NIH Swiss mice, although it is shown to alter various physiological parameters indicative of stress in rodents.

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METHOD

Male NIH Swiss mice, weighing approximately 22–24 g, served as subjects. Animals were housed in groups of 10 per cage and maintained on standard conditions: a 12L : 12D cycle (lights on 6:00 a.m.), room temperature + 22–24°C, and ad lib access to food and water.

Experiment 1

A total of 88 mice were allocated to the following five groups:

1. *No injection*—twenty mice were randomly taken from five cages in which they were housed separately from the other groups.
2. *Acute injection*—twenty-four mice were randomly taken from five cages in which they were housed separately from the other groups. These animals received a single vehicle injection 30 min prior to the swim test.
3. *Control, no injection*—fourteen mice were randomly chosen from cages containing animals that were given chronic injections.
4. *Handling*—fifteen mice were put daily on the lid of their home cage and tail-marked. These mice were housed in the same cage with animals receiving chronic injections.
5. *Chronic injection*—fifteen mice that were housed in five cages were injected daily with vehicle. The chronic injections and handling were carried out over 7 days, last injection or handling occurring 30 min prior to the swim test.

Animals were not weighed to avoid disturbing Group 1; thus, each mouse in the injection groups was given intraperitoneally a standard dose of 0.20 ml distilled water between 10:00–11:00 a.m.

Experiment 2

This experiment studied the effect of vehicle injection on differences in immobility in the swim test of fight-stressed and control mice. Fight-stressed mice were housed with an alpha mouse (10) for 2–3 weeks. We have shown that the alpha mouse (one per cage) attacks all its cage mates but it itself lacks any signs of being attacked (10). All fight-stressed mice, on the other hand, have severe bite marks on their tail and back.

A total of 30 fight-stressed mice, randomly chosen from 8 different cages, and 60 control mice, which arrived into our laboratory at the same time as the fight-stressed animals but lacked any signs of fighting and were randomly chosen from 11 cages, were used. Animals were not weighed, and half received an intraperitoneal injection of 0.20 ml distilled water 30 min prior to the swim test.

Experiment 3

Ten animals were isolated for 7 days. On day 8, five of these animals were given an intraperitoneal injection of 0.20 ml distilled water 30 min prior to the swim test and the other five left undisturbed. Ten group-housed mice were similarly either given a vehicle injection or left undisturbed before the swim test.

Swim Test

Each mouse was placed in a plastic cylinder (height 17 cm, diameter 21 cm) containing 8 cm of water maintained at about 25°C for 10 min. The 10-min period included a 2-min acclimation period at the beginning of the test, immediately followed by an 8-min test. Half the animals were observed during testing and the behavior of the other half was recorded using a camera and a videocassette recorder. A mouse was judged immobile when it was floating, making only those movements necessary to keep its head above the water. The time spent immobile was scored using a keyboard linked to a PDP microcomputer running SKED-11 software.

Resident-Intruder Test

A total of 50 mice were house singly for 10 days. On the eighth day of social isolation, they were weighed. On day 10, 25 mice were given 0.20 ml vehicle 30 min prior to the test and the other 25 mice were left undisturbed. Then, they were confronted in their home cage with a group-housed male intruder. The body weights of the intruders were matched with that of the residents. During the 7.5-min test period, an observer monitored the behavior of the resident with a keyboard interfaced with a PDP microcomputer. Half the animals were observed live and half from the videotape. The behaviors recorded were the number and duration of social investigation (sniffing, following, grooming), aggression (lateral threat, tail-rattle, fighting), and defensive behavior (moving away or withdrawing when sniffed, squealing, startling; occurring in the absence of aggression).

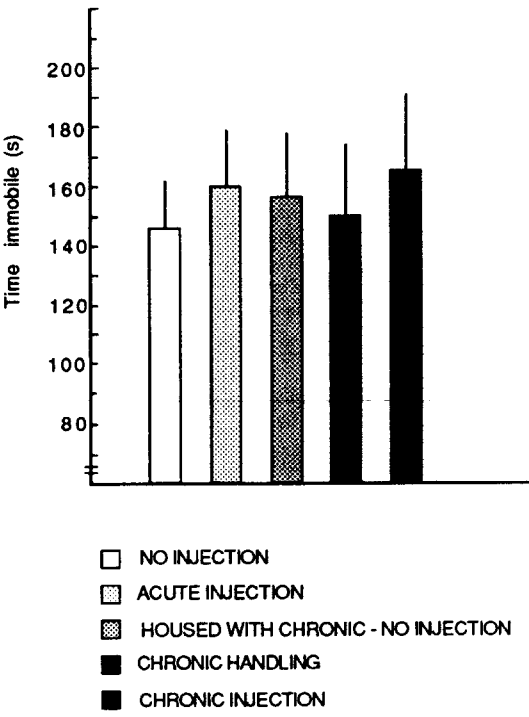

FIG. 1. Time spent immobile during an 8-min swim test in mice exposed to acute or chronic vehicle injections. Each bar represents a mean ± SEM of 14–24 mice.

Statistical Analysis

A BMDP statistical package was used to analyze the data. Results for the immobility in the swim test, the time spent in social interaction, and aggressive behavior in the resident-intruder test were analyzed using one- and two-way analysis of variance (ANOVA). Between-group comparisons were made using Fisher's least-significant difference test. All probabilities reported are two tailed.

RESULTS

Swim Test

Experiment 1. As can be seen from Fig. 1, none of the groups differed from each other in respect to the length of immobility time in the water.

Experiment 2. An acute vehicle injection did not significantly affect the length of time spent immobile in the water in control or fight-stressed mice (Fig. 2). Fight-stressed mice showed longer immobility times than controls, $F(1, 86) = 10.9, p < 0.001$.

Experiment 3. Immobility of mice isolated 7 days prior to the swim test (mean ± SEM; 170.8 ± 35.2 s) did not differ from immobility of group-housed animals (179.7 ± 50.0 s). A single vehicle injection failed to significantly affect behavior in the swim test of either isolated (144.9 ± 39.5 s) or group-housed mice (151.9 ± 33.7 s).

Resident-Intruder Test

There were no statistically significant differences in the time spent in social interaction, aggressive, or defensive behaviors between noninjected and injected mice (Table 1).

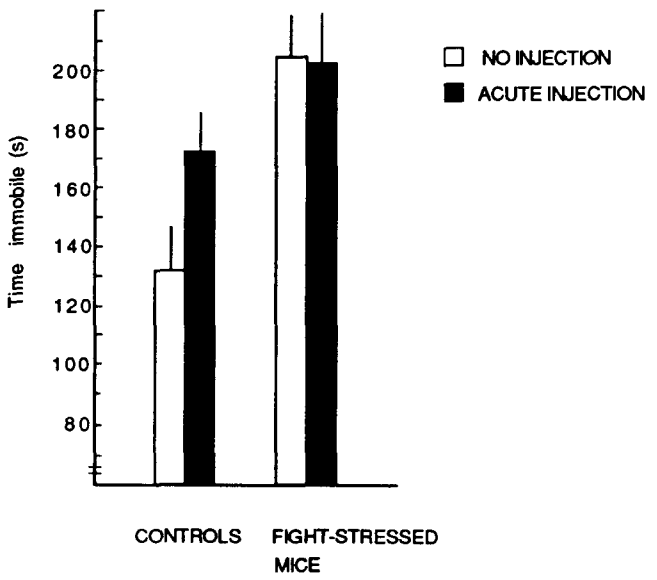

FIG. 2. Time spent immobile during an 8-min swim test in control and fight-stressed mice 30 min after a vehicle injection. Means ± SEM of 15-30 mice per group.

TABLE 1

TIME SPENT IN VARIOUS BEHAVIORS IN THE RESIDENT-INTRUDER TEST 30 MIN AFTER A VEHICLE INJECTION

Behavior	No Injection	Vehicle Injection
Social interaction (s)	72.1 ± 10.3	52.5 ± 7.7
Aggression (s)	26.8 ± 5.1	28.6 ± 6.5
Defensive (s)	2.6 ± 1.0	2.3 ± 0.7

Means ± SEM of 25 animals per group.

DISCUSSION

The present study showed that a vehicle injection does not alter behavior of male NIH Swiss mice in two animal models sensitive to the effects of stress; Porsolt's swim test of depressive behavior and resident-intruder paradigm of aggression. In earlier studies with the swim test, various stressors have been shown to lengthen immobility in the water (2,7,10). We found that the time spent immobile failed to be different in mice not exposed to handling prior to the test from mice treated with a single vehicle injection. Furthermore, mice given daily vehicle injections or that were handled daily over a 7-day period spent about the same length of time immobile in the swim test as mice left undisturbed.

We failed to detect any effect by a single injection on previously fight-stressed mice or on mice isolated for 1 week. The only difference noted appeared between nonstressed and chronically fight-stressed mice: The latter animals spent a significantly longer time immobile in the water. This finding confirms previous observations that stressors influence behavior in the swim test (10). The data further suggest that injections do not seem to be stressful to mice in the swim test.

Behavior in the resident-intruder paradigm was not altered by a vehicle injection. In earlier studies, elevated levels of aggression have been observed in animals that have been subjected to manipulations inducing, for example, pain and threat (14). Thus, the negative findings both in the swim test and in the resident-intruder paradigm seem to suggest that injections are not stressful to mice. However, earlier studies have unambiguously shown that even a single injection or handling induces physical changes indicative of stress in rodents. These manipulations increase plasma corticosterone levels (1) and CRF immunoreactivity in the hypothalamus (8), and stimulate hippocampal glucose utilization transiently via NMDA receptors (16). In addition, core temperature of rats have been found to be elevated after an acute vehicle injection (5). The present results may, therefore, propose that behavioral measures, such as immobility in the swim test and aggression in the resident-intruder paradigm, are less sensitive indicators of stress than biological measures, such as plasma corticosterone levels.

Although injection itself does not appear to alter behavior, previous studies have reported that chronic injections of vehicle interact with the behavioral effects of subsequently administered pharmacological compounds. In a study by Brett and Pratt (4), daily vehicle injections for 24 days abolished the anxiolytic effect of diazepam in the plus-maze of rats. Furthermore, Flemmer and Dilsaver (6) demonstrated that daily injections of saline for 14 days reversed the hypothermic ef-

fects of nicotine in rats. Therefore, injection and handling may interact with the behavioral and physical effects of various pharmacological compounds.

In conclusion, the swim test is sensitive to several antidepressant compounds and appears also to be sensitive to various stressors. The present findings indicate that mild stressors, such as injection or handling, do not significantly influence immobility in the swim test, even though they cause detectable physical changes. Furthermore, injection does not interact with the effects of other stressors or isolation in the swim test.

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