Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 449-453, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0104-P

Handling Alters Habituation and Response to Stimulus Change in the Holeboard

SANDRA E. FILE[1] AND EMMA FLUCK

Psychopharmacology Research Unit, UMDS Division of Pharmacology, Guy's Hospital, P.O. Box 3448, London SE1 9QH, UK

Received 4 January 1994

FILE S. E. AND E. FLUCK. *Handling alters habituation and response to stimulus change in the holeboard.* PHARMACOL BIOCHEM BEHAV **49**(3) 449-453, 1994.—The responses of rats that had been extensively handled for 18 days were compared in the holeboard with those of rats that had received handling for only 4 days before the test. The extensively handled group showed slower between-day habituation of exploratory head-dipping. They did not differ in the number of head-dips, but spent longer head-dipping, at holes with objects than at those without. The less-handled group did not differ in the time spent head-dipping, but made more head-dips at empty holes. Both groups reacted similarly to the removal of the objects on day 4. However, the extensively handled rats showed a greater response of increased head-dipping when a novel object was introduced on day 5. The groups did not differ in their locomotor activity, but the extensively handled group made more rears. The results are discussed with respect to the neurochemical changes that have been found after repeated handling.

Handling GABA 5-HT Stress Exploration Habituation

MENNINI et al. (17) reported that acute handling stress applied to naive animals resulted in lower benzodiazepine binding in cortical membranes, compared with the binding found in membranes of handling-habituated animals. The change in binding was due to a reduction in the number of benzodiazepine binding sites, with no change in affinity. Similar findings were reported by Andrews et al. (3), who found a gradual change in binding with duration of handling habituation from 2–21 days. Handling stress applied to naive animals also decreased $GABA_A$ receptor binding (4) and chloride flux (8), compared with rats habituated to handling for 15 days. Although these changes would all be consistent with an increased anxiogenic response in animals naive to handling, the changes in GABA uptake are in the opposite direction—that is, there was decreased GABA uptake in the hippocampus and frontal cortex of these handling-naive animals (14), it is possible that this decreased GABA uptake contributed to the down regulation of the $GABA_A$ receptors. Handling habituation also induces changes in the 5-HT system, and handled rats had higher 5-HT uptake in both frontal cortex and hippocampus, compared with rats naive to handling (14). Boix et al. (6) also found that extensive handling decreased 5-HT and increased 5-HIAA concentrations in the hippocampus. Stanford et al. (18) found that repeated intraperitoneal (IP) saline injections in rats down regulated cortical β-adrenoceptors.

Handling modifies behavioral responses in an animal test of anxiety. Thus, rats naive to handling had increased anxiogenic responses in the elevated plus-maze, compared with those habituated to handling for 21 days, but no differences in total activity (2). Handling-naive rats had enhanced sensitivity to the anxiolytic effects of the $GABA_B$ agonist baclofen, and the $5\text{-}HT_3$ receptor antagonist, zacopride, and reduced sensitivity to the anxiogenic effects of the $5\text{-}HT_{1A}$ receptor agonist, buspirone (2). Brett & Pratt (7) showed that handling for 28 vs. 5 days reduced the anxiolytic action of diazepam. Thus, an animal's history of handling not only changes its baseline level of responding in an animal test of anxiety, which could change the magnitude of a drug response, but may also change an animal's sensitivity to drugs. Similar results were found in a two-way active avoidance test: the extensively handled group made more avoidance responses than the less handled group, and as a result the effect of diazepam was reduced (5).

The effects of handling can also be detected in situations in which anxiety may play a more minor role. Lavigne et al. (16) found that the enhancement of the antinociceptive effects of morphine by cholecystokinin (CCK) antagonists was found only in rats naive to handling. Adamec et al. (1) found that the anxiogenic effect of corticotropin-releasing factor (CRF) was reduced in a group of surgically operated and handled

[1] To whom requests for reprints should be addressed.

rats. In addition, the rats that had received extensive handling associated with surgery made fewer head-dips and rears when tested in a holeboard than did handled animals that did not have operations. However, this experiment did not allow the separation of possible stressful effects of surgery itself from any effects due to adaptation to the stress of repeated handling. The purpose of the present experiment was therefore to compare exploratory head-dipping and motor activity in the holeboard in rats given brief prior handling (4 days) with those given extensive prior handling (18 days). Several components of the responses in the holeboard were studied: exploratory responses at empty holes were compared with those at holes under which objects were placed; between-day habituation was studied over 3 days; and the effects on head-dipping of the removal of objects on day 4 and of introducing a novel object on day 5 were examined.

METHOD

Animals

Male hooded Lister rats (Charles River, Margate, Kent), approximately 250 g at testing, were housed in groups of five with food and water freely available, in a room maintained at 22°C with lights on from 0700 to 1900 h.

Apparatus

The device used was a wooden box 60 × 60 × 35 cm with four holes, each 6.5 cm in diameter, equally spaced on the floor. Head dipping was measured by the interruption of infrared beams from cells located immediately beneath the edges of the holes. Locomotor activity and rearing were measured by the interruption of infrared beams from cells located in the walls of the box 4.5 and 12.5 cm from the floor. All scores were entered directly into a computer.

Procedure

The rats allocated to the extensively handled group (n = 33) first received 4 days of handling, during which they were picked up and weighed each day, followed by 14 days of handling and SC saline injections. The rats in the less-handled group (n = 30) received only 4 days of handling and weighing before the holeboard test. For the first comparison, the less-handled group (n = 20) arrived in the laboratory 5 days before testing; thus, like the more extensively handled group (n = 22), they were handled each day before the start of testing. They had, however, been in the animal house for a shorter period. Therefore, for the second comparison, both groups arrived at the same time; but those allocated to the less-handled group remained unhandled until 4 days before holeboard testing.

For the first 3 days of holeboard testing, two of the holes remained empty and two had the same objects placed under them on every day. On day 4, the objects were removed—that is, all holes were empty. On day 5, a novel object was placed under one of the holes that had been empty on all previous days. Each rat was placed in the holeboard for a 7.5-min trial on every day, and the apparatus was cleaned after each rat. The rats were tested in the same order (randomized for handling) on every day, between 0900 and 1300 h.

Statistics

The data were analyzed with split-plot analyses of variance with handling as the between-group factor and days of testing and holes as within-subject factors. Further comparisons were made with post-hoc Duncan's tests.

RESULTS

As there were no differences between the two groups of less-handled rats according to how long they had been in the animal house before the holeboard test, their data were combined for all the analyses.

Over the first 3 days there was a significant between-day habituation of both the number of head-dips and the time spent head-dipping ($F[2, 122]$ = 16.6 and 57.5, respectively; $p < 0.00001$). However, the habituation was significantly greater in the less-handled group, particularly between days 2 and 3 (handling × days, $F[2, 122]$ = 3.6 and 3.2 for number of head-dips and time, respectively; $p < 0.05$) (Fig. 1). The groups also differed over the first 3 days in the distribution of their responses at holes with and without objects (handling × holes, $F[1, 61]$ = 12.0, $p < 0.001$ for number of head-dips: $F[1, 61]$ = 13.8, $p < 0.0005$ for time head-dipping). Thus,

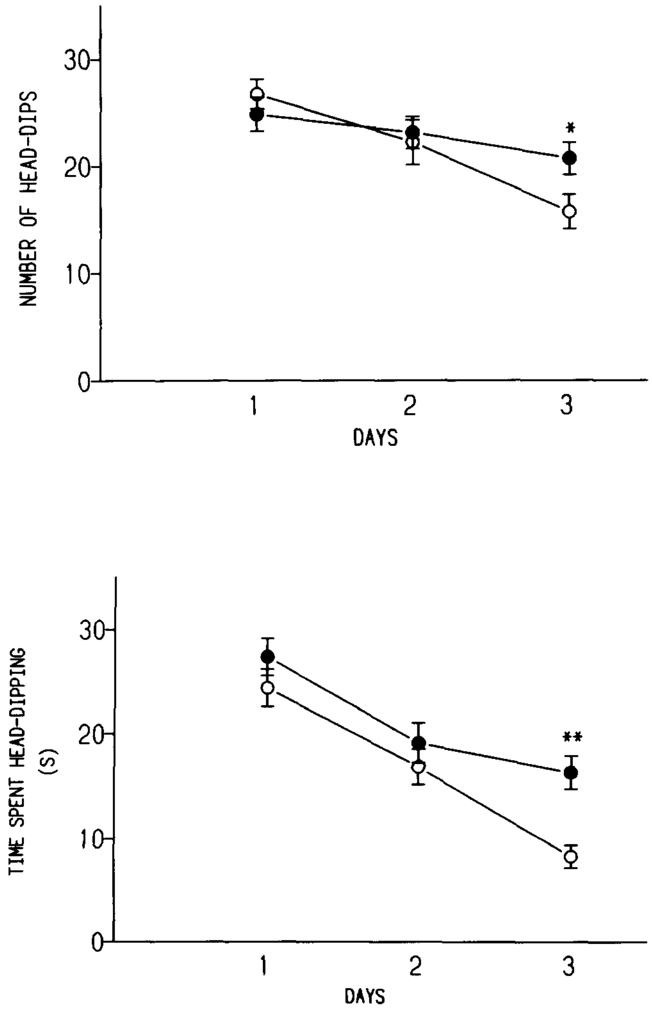

FIG. 1. Mean (±SEM) number of head-dips and time spent head-dipping over 3 successive days by rats handled for 4 (○—○) or 18 (●—●) days. **$p < 0.01$, post-hoc tests after analysis of variance; see text for statistical details.

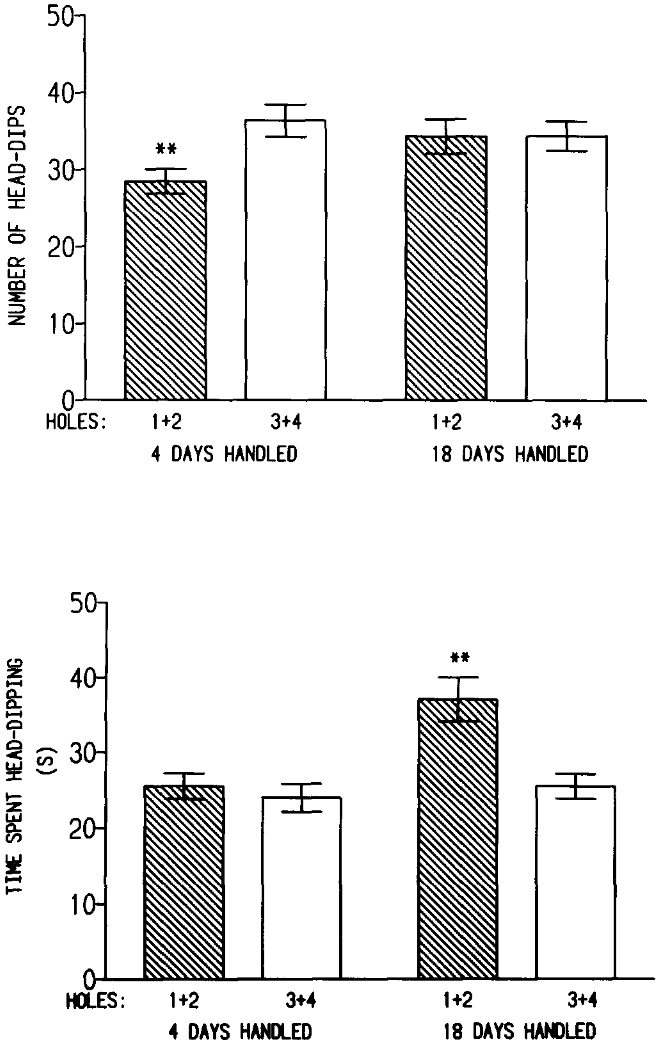

FIG. 2. Mean (±SEM) number of head-dips and time spent head-dipping at holes with objects (1 + 2, hatched bars) and those without (3 + 4, open bars) over the first 3 days by rats handled for 4 or 18 days. $^{**}p < 0.01$ compared with holes without objects, post-hoc tests after analysis of variance; see text for statistical details.

the extensively handled rats made the same number of head-dips at baited and unbaited holes, but spent longer head-dipping at the holes with objects; the less-handled rats made fewer head-dips at the holes with objects and differed very little in the time spent head-dipping at baited and unbaited holes (Fig. 2).

On day 4, the objects were removed and there was a significant days × holes interaction, because the rats showed a greater change in the time spent head-dipping at the holes that had been changed ($F[1, 60] = 3.8, p = 0.05$), but there was no difference between the two handling groups (data not shown).

On day 5, when a new object was introduced under one hole, there was a significant increase in head-dipping at this hole, compared with the one that had remained empty on all 5 days (days × holes, $F[1, 60] = 7.5, p < 0.01$ for number of head-dips; $F[1, 60] = 22.5, p < 0.00001$ for time head-dipping). This reaction to stimulus change was more marked in the extensively handled rats (handling × days × holes, $F[1, 60] = 7.3, p < 0.01$ for number of head-dips: $F[1, 60] = 7.3, p < 0.01$ for time spent head-dipping) (Fig. 3).

There was no significant difference in the locomotor activity of the two groups, but the extensively handled group made significantly more rears on all 5 days ($F[1, 60] = 22.4, p < 0.00001$) (Fig. 4).

DISCUSSION

Our extensively handled and injected rats showed increased time spent head-dipping and made more rears than the less-handled group. These results are in the opposite direction to those reported by Adamec et al. (1), suggesting that the lowered response in his animals resulted from the stress of surgery rather than the extent of handling, and that our changes were the result of an adaptation to the stress of handling.

Extensive handling made the rats rear more in the hole-board on all days and impaired between-day habituation, particularly from days 2 to 3. Impaired between-day habituation

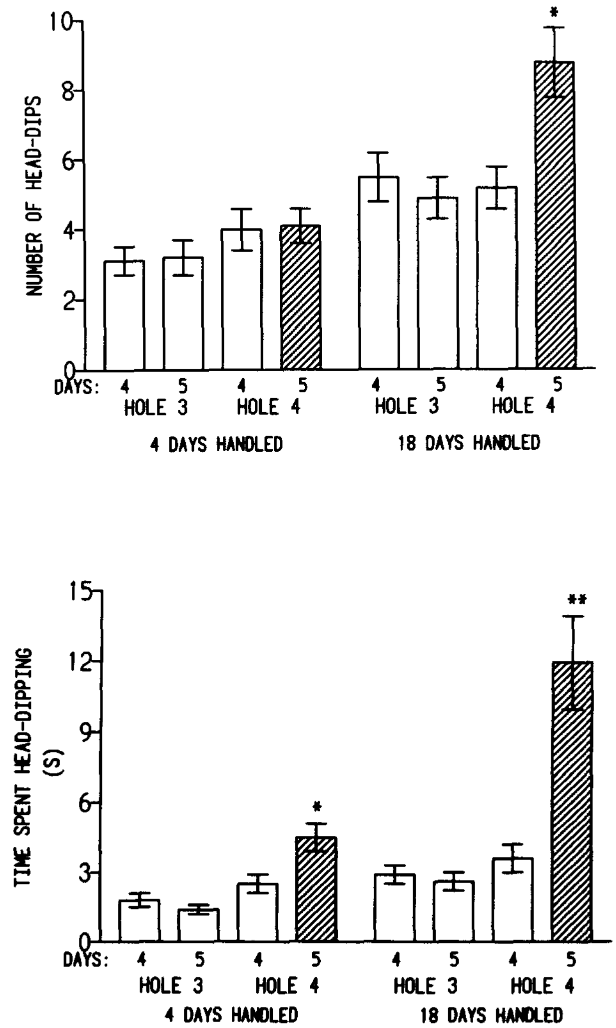

FIG. 3. Mean (±SEM) number of head-dips and time spent head-dipping at hole 3 (remained without objects on days 4 and 5) and hole 4 (object introduced on day 5). $^{*}p < 0.05$ $^{**}p < 0.01$ compared with day 4, post-hoc tests after analysis of variance; see text for statistical details.

has been found with chlordiazepoxide (10) and *N,N*-dimethyltryptamine (12), and thus the changes with handling may relate to the reported differences in the $GABA_A$ benzodiazepine and 5-HT systems. The impaired habituation does not seem to be due to an impairment of retention because there was a normal decrease in the time spent head-dipping between days 1 and 2, and the extensively handled group was as responsive to the removal of objects and more responsive to the introduction of a novel object than was the less-handled group. The normal habituation from days 1 to 2 also makes it unlikely that handling impaired the ability of the rats to classify a stimulus as irrelevant. A third possibility is that the increased time spent head-dipping on day 3 reflected a more detailed coding of the stimulus parameters; this could also explain the increased head-dipping on day 5, when a novel object was introduced. Although many different drug treatments reduce exploratory head-dipping (see reference 13 for review), only low doses of ethanol, chlordiazepoxide, and benzodiazepine receptor antagonists have been reported to increase it (10,15). There have been no reports of other treatments enhancing the response to stimulus change, although drugs acting on the $GABA_A$-benzodiazepine or 5-HT systems (e.g., ethanol and parachlorophenylalanine) have been reported to impair it (10,11). Thus, it is possible that the changes in head-dipping in the extensively handled rats are the result of the reported changes in their $GABA_A$-benzodiazepine or 5-HT systems, or both.

These results have important implications for behavioral and neurochemical studies in which drug treatment is given chronically. Some of the observed effects may not be the result of a change in the neurochemical action of chronic, as opposed to long-term, drug treatment, but may result from the neurochemical changes induced by extensive handling or the interaction between the two sets of changes. A similar conclusion was reached by Davis et al. (9), who found that some of the behavioral and neurochemical changes induced by repeated IP saline injections were prevented by chronic sibutramine treatment, whereas others were unaffected. In the present experiment the differences were found between animals handled for 4 days and those handled for 18 and receiving repeated injections. This suggests that there is continuing adaptation to handling, and that the neurochemical differences previously reported are also continuously developing and are not only found when comparisons are made with handling-naive groups.

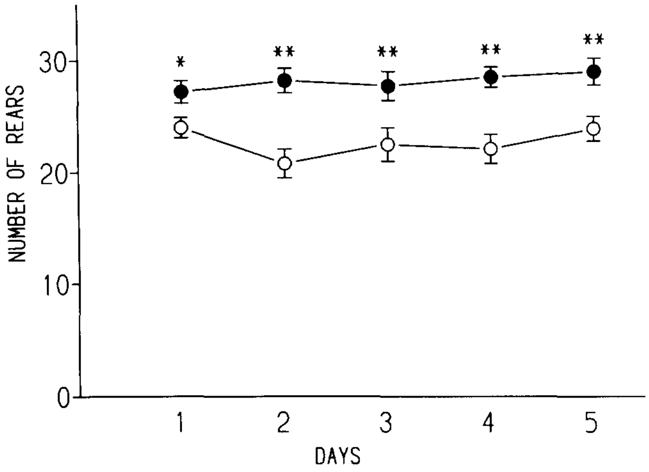

FIG. 4. Mean (± SEM) number of rears made over 5 successive test days by rats handled for 4 (○ – ○) or 18 (● – ●) days. $^*p < 0.05$, $^{**}p < 0.01$, post hoc tests after analysis of variance; see text for statistical details.

REFERENCES

1. Adamec, R. E.; Sayin, U.; Brown, A. The effects of corticotrophin releasing factor (CRF) and handling stress on behavior in the elevated plus-maze test of anxiety. J. Psychopharmacol. 5: 176–186; 1991.
2. Andrews, N.; File, S. E. Handling history of rats modifies behavioural effects of drugs in the elevated plus-maze test of anxiety. Eur. J. Pharmacol. 235:109–112; 1993.
3. Andrews, N.; Zharkovsky, A.; File, S. E. Acute handling stress downregulates benzodiazepine receptors: Reversal by diazepam. Eur. J. Pharmacol. 210:247–251; 1992.
4. Biggio, G.; Corda, M. G.; Concas, A.; Demontis, G.; Rosetti, Z.; Gessa, G. L. Rapid changes in GABA binding induced by stress in different areas of the rat brain. Brain Res. 229:363–369; 1981.
5. Boix, F.; Fernandez Teruel, A.; Tobena, A. The anxiolytic action of benzodiazepines is not present in handling-habituated rats. Pharmacol. Biochem. Behav. 31:541–546; 1988.
6. Boix, F.; Fernandez Teruel, A.; Escorihuela, R. M.; Tobena, A. Handling-habituation prevents the effects of diazepam and alprazolam on brain serotonin levels in rats. Behav. Brain Res. 36:209–215; 1990.
7. Brett, R. R.; Pratt, J. A. Chronic handling modifies the anxiolytic effect of diazepam in the elevated plus-maze. Eur. J. Pharmacol. 178:135–138; 1990.
8. Concas, A.; Serra, M.; Corda, M. G.; Biggio, G. Changes in ^{36}Cl flux and ^{35}S-TBPS binding induced by stress and GABAergic drugs. In: Biggio, G.; Costa, E., eds. Chloride channels and their modulation by neurotransmitters and drugs. New York: Raven Press; 1988:227–245.
9. Davis, S.; Heal, D. J.; Salmon, P.; Stanford, S. C. Is vehicle-injection an adequate control for evaluating drug effects? Clin. Neuropharmacol. 15(Suppl 1):400B; 1992.
10. File, S. E. A comparison of the effects of ethanol and chlordiazepoxide on exploration and on its habituation. Physiol. Psychol. 4:529–532; 1976.
11. File, S. E. Effects of parachlorophenylalanine and amphetamine on habituation of exploration. Pharmacol. Biochem. Behav. 6: 151–156; 1977.
12. File, S. E. Effects of *N,N*-dimethyltryptamine on behavioural habituation in the rat. Pharmacol. Biochem. Behav. 6:163–168; 1977.
13. File, S. E. Pharmacological manipulation of responses to novelty and their habituation. In: Cooper, S. J., ed. Theory in psychopharmacology, vol. 1. London: Academic Press; 1981:197–232.
14. File, S. E.; Andrews, N.; Zharkovsky, A. Handling habituation and chlordiazepoxide have different effects on GABA and 5-HT function in the frontal cortex and hippocampus. Eur. J. Pharmacol. 190:229–234.
15. File, S. E.; Pellow, S.; Jensen, L. H. Actions of the β-carboline ZK 93426 in an animal test of anxiety and the holeboard: Interactions with Ro 15-1788. J. Neural Trans. 65:103–114; 1986.
16. Lavigne, G. J.; Millington, W. R.; Mueller, G. P. The CCK-A and CCK-B receptor antagonists devazapide and L-365,260 enhance morphine antinociception only in nonacclimated rats

exposed to a novel environment. Neuropeptides 21:119–129; 1992.

17. Mennini, T.; Gobbi, M.; Perin, L.; Salmona, M. Rapid internalisation of benzodiazepine receptors in the rat cortex induced by handling. In: Biggio, G.; Costa, E., eds. Chloride channels and their modulation by neurotransmitters and drugs. New York: Raven Press; 1988:447–482.
18. Stanford, S. C.; Fillenz, M.; Ryan, E. The effect of repeated mild stress on cerebral cortical adrenoceptors and noradrenaline synthesis in the rat. Neurosci. Lett. 45:163–167; 1984