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Cyclosporine A Affects Open Field Behavior in DA Rats

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VON HÖRSTEN, S. M. S. EXTON, J. VÖGE, J. WESTERMANN, M. SCHULT, E. NAGEL, R. E. SCHMIDT AND M. SCHEDLOWSKI. *Cyclosporine A affects open field behavior in DA rats.* PHARMACOL BIOCHEM BEHAV **60**(1) 71–76, 1998.—Since the introduction of Cyclosporine A (CsA) for immunosuppression in solid-organ transplantation, the rate of allograft rejection has decreased substantially. However, treatment with CsA induces neuropsychological complications in patients, including affective disorders such as anxiety, disorientation, depression, aggression, paranoia, and apathy. These CsA-induced affective side effects cannot be extensively studied in humans. Therefore, this study investigates the effects of intraperitoneal CsA (20 mg/kg) injections on the open-field behavior of male Dark Agouti (DA) rats 1, 6, 12, and 23 h after drug administration on 3 consecutive days. CsA induced an increase in emotionality in DA rats 6 h after injection, reflected by decreased ambulatory activity in the open field and increased defecation. In addition, a decrease in rearing activity was observed 12 h after CsA administration. These behavioral alterations are discussed in the view of changes in cytokine profiles induced by CsA. © 1998 Elsevier Science Inc.

Cyclosporine A Open field behavior Emotionality Rearing Activity Rat

CYCLOSPORINE A (CsA) is used as a first line treatment for preventing allograft rejection (24). CsA is a lipophilic cyclic polypeptide that produces calcium-dependent, specific, reversible inhibition of transcription of interleukin-2 (IL-2) and several other cytokines (17,19). However, clinical immunosuppressive regimens using CsA induce changes in the mental state of patients (32). In 20% of recipients of kidney (26) and liver (10) transplants, CsA administration causes neuropsychological side effects such as tremor, headache, depression, anxiety, confusion, and somnolence. Studies in rodents are therefore necessary to further investigate CsA-induced affective disorders. However, the effects of CsA treatment on the emotionality and exploratory activity of rats has not been investigated in detail.

Thus, the present experiment was performed to analyze CsA-induced effects on behavioral activity and emotionality in male DA rats using the open field (OF) test. The OF test was chosen because it is sensitive to pharmacological manipulations (40). Under constant, clearly defined conditions the OF test has high reliability and validity for the measurement of activity, emotionality (12), and anxiety-related behavior towards a novel environment (40). Defecation and various parameters of motor activity are the major indices of emotional reactivity (3,12,40). The reliability and validity of defecation and initial degree of ambulation are well established (1,3,18), and the validity of defecation as the prime index of emotionality has been confirmed (42). In this study behavioral parameters were analyzed 1, 6, 12, and 23 h after intraperitoneal (IP) injection of either CsA (20 mg/kg) or vehicle in an OF test for 3 min, on 3 consecutive days. The results demonstrate that CsA treatment induces transient alterations in ambulatory activity, defecation, and rearing activity in DA rats.

METHOD

Animals

Eighty male Dark Agouti (DA) rats aged 12 ± 2 weeks were obtained from the Central Animal Laboratory, Medical

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School of Hannover (Germany). A 12 L:12 D cycle was maintained throughout the experiment, with lights off at 0700 h. Animals were individually housed in standard plastic-based laboratory cages (40 × 26 × 15cm). Standard lab chow (Altromin) and tap water were available ad lib. Cages were kept in an air-conditioned, sound-proofed room, at an ambient temperature of 24.0 ± 0.5°C. To habituate animals to the presence of the experimenter, the change of bedding once a week and weighing of the animals was always carried out by the same experimenter. Experiments were conducted in accordance with the ethical regulations for animal experimentation in Lower Saxony, Germany.

Immunosuppression

Cyclosporine A (SANDIMMUNE®, Sandoz AG, Nürnberg, Germany) was injected at a dosage of 20 mg/kg. For IP injection of the verum compound, 100 mg CsA was dissolved in 40 mg ethanol plus 810 mg Miglyol oil. For the IP injection of the vehicle control substance, 40 mg ethanol was mixed with 810 mg Miglyol without adding CsA. Phosphate-buffered saline was used for the appropiate dilution of verum and vehicle. This dosage was chosen in line with other immunobehavioral experiments where the same dosage was effective in producing conditioned immunosuppression (20,27).

Apparatus

The OF has previously been described in detail (23). Briefly, the OF test consists of the measurement of behavior elicited by placing the animal in a novel open space from which escape was prevented by a surrounding wall. This space was a rectangular box made of gray plastic (125 × 125 cm). The floor consisted of gray plastic with a red grid dividing the OF into 25 squares (25 × 25 cm). The test apparatus was situated in a separate sound-proof room next to the animals housing room. A masking noise of approximately 20 dB was provided by the air-conditioning system. Illumination was provided by four red photo laboratory light bulbs placed 100 cm above the center of the field.

Procedure

Within 2 weeks prior to the start of the experiment all animals were habituated to experimental handling (transport of the cage, holding by hands, etc.) and the procedure of drug application by three sham IP injections.

The experiment comprised four groups of 20 rats (10 vehicle controls and 10 CsA-treated experimental animals) tested 1, 6, 12, and 23 h after IP CsA/vehicle administration in the OF, respectively. These time points were chosen because the CsA plasma level peaks 5–6 h after IP CsA application and is metabolized 24 h after administration (30).

To check for habituation effects and possible drug effects on repeated test exposure, rats of both groups were subjected to three OF tests on 3 consecutive days (one test per day) for 3 min each. The first trial of the OF test is suggested as the primary unconditioned indicator of emotionality in laboratory investigations of rats (34). Furthermore, the use of repeated tests incorporates all prior experience of the test situation as an additional influencing factor (40), probably caused by habituation and learning. Nevertheless, repeated tests have proved to increase reliability of the test (42).

CsA administration took place on each test day at 0700 h (beginning of the dark/active period), when the lights went off. Testing was regularly performed at 0800 h (1 h group), 1300 h (6 h group), 1900 h (12 h group), and at 0600 h the next morning (23 h group). Each rat was transported to the testing room using the home cage. The experimenter was blind to the treatment of the animals. The rat was placed in the same square facing the center square, and its behavior was observed continuously for 3 min. "Activity" (number of grid lines crossed, ambulation) and "exploration" (number of inner grid lines crossed, investigation of the novel object in the third test) was recorded by following the path of the animal with a pencil on a protocol paper. "Rearing" (vertical locomotor activity), "grooming" (rubbing its nose with its forepaws, preening), and "defecation" (number of boli) were recorded in the same protocol. In the third OF test a rectangular partition (25 × 25 × 50 cm) made of gray plastic with one open side, not visible for the subject from its starting point, was placed on the center square of the field. The number of animals that reached the partition and the number of animals that went inside the partition were recorded. After 3 min of testing, animals were returned to the home cage and transported to the holding room. The OF was carefully cleaned between OF trials using tap water. The OF tests and the transport of animals were always performed by the same experimenter.

Statistical Analysis

All behavioral data were analyzed by a three-way [treatment × test time × test day (repeated measurement)] analysis of variance (ANOVA) for repeated measures. Post hoc simple effects analyses were implemented, with Fisher adjustments made to avoid experimenterwise error. Because three-way ANOVA showed a nonsignificant main factor effect of "test day," results from the two-way analysis (treatment × test time) are presented.

RESULTS

Three-way ANOVA (treatment × test time × test day) did not reveal a significant effect for the main factor "test day," indicating that repeated testing on 3 consecutive days did not induce habituation.

The OF parameters "activity," "exploration," "rearing," and "defecation" in the three consecutive tests in CsA-treated and vehicle control rats, 1, 6, 12, and 23 h after injection are shown in Fig. 1.

Activity

The horizontal ambulatory activity of experimental rats was significantly decreased in the second OF test 6 h after CsA administration, $F(3, 72) = 2.7$, $p \leq 0.05$; Fig. 1. A similar effect was observed in the first and third test. However, results did not reach statistical significance.

Exploration

Experimental animals showed a decrease in the number of inner lines crossed during the first OF test 6 h after CsA administration, $F(3, 72) = 2.86$, $p \leq 0.05$; Fig. 1. No difference was found between the number of animals that reached the partition or went inside the partition in the third test.

Rearing

The rearing activity of CsA-treated rats significantly decreased in the second OF tests, $F(3, 72) = 9.08$, $p \leq 0.0001$;

Fig. 1. Post hoc analysis revealed this effect to occur 12 h after CsA administration in all three tests. Furthermore, in the second OF test the rearing activity was increased 1 and 23 h after CsA treatment.

Defecation

The number of fecal boli of CsA-treated rats increased in the first OF test, $F(3, 72) = 2.79$, $p \leq 0.05$, Fig. 1. Post hoc analysis revealed this effect to occur 6 h after treatment in all three tests.

Grooming

The incidence of grooming behavior in CsA-treated rats did not differ from vehicle controls (data not shown).

Total Activity in Three Tests

Figure 2 summarizes the behavioral indices of locomotor activity, rearing, exploration, and defecation as totals from three consecutive tests. ANOVA revealed significant group × time interactions in "exploration," $F(3, 72) = 2.78$, $p \leq 0.05$, "rearing," $F(3, 72) = 4.69$, $p \leq 0.01$, and "defecation," $F(3, 72) = 3.33$, $p \leq 0.05$. The ambulatory and the rearing activity of control animals displayed the highest values in the middle of the active (dark) period (6 and 12 h after injection of vehicle). The incidence of defecation in control animals remained constant within the observation period. In contrast, CsA-treated animals showed the highest ambulation, rearing, and exploration activity 24 h after CsA administration. In addition, defecation increased 6 and 12 h after CsA treatment.

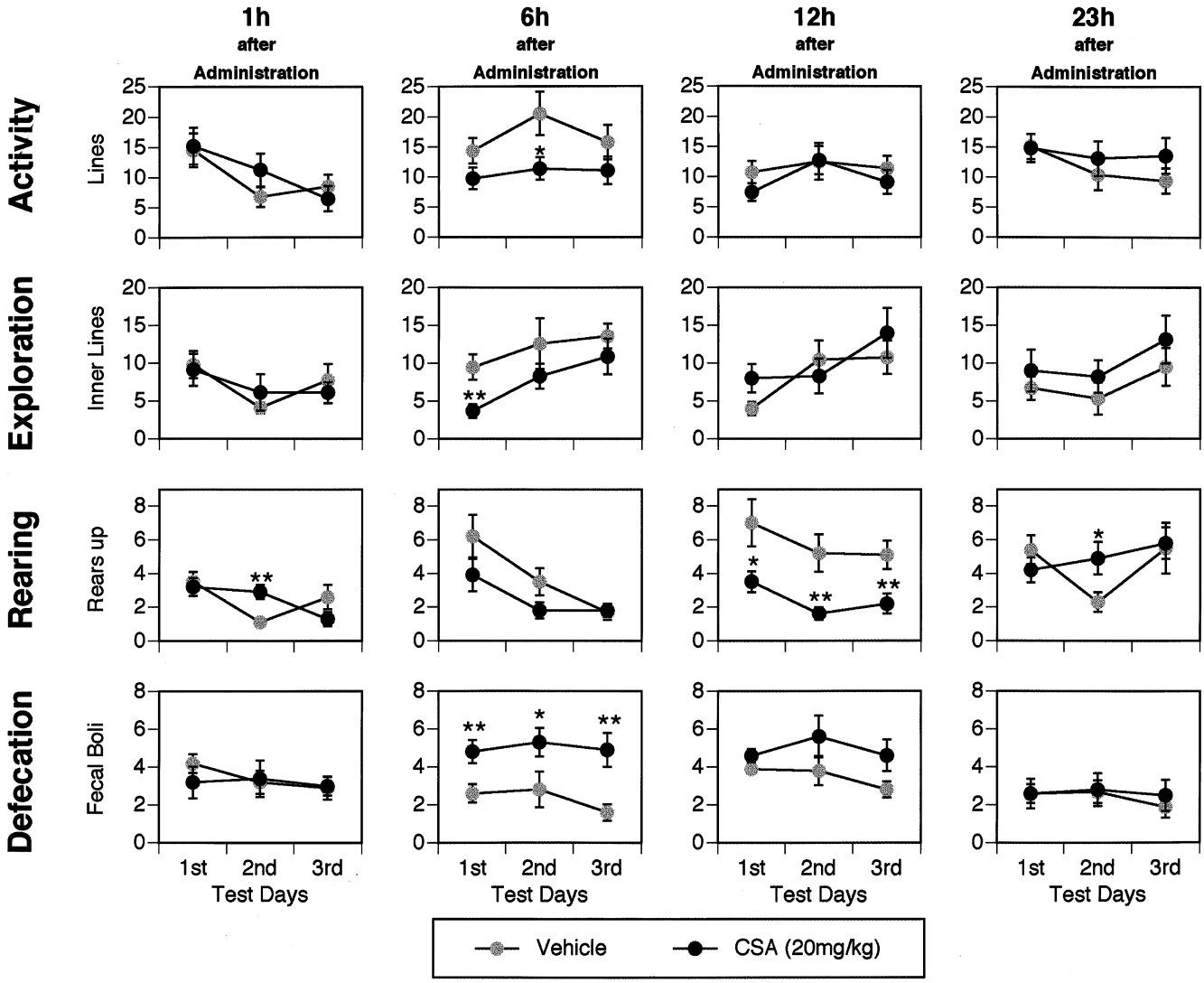

FIG. 1. Effect of CsA/vehicle treatment on behavioral parameters (activity, exploration, rearing, defecation) of DA rats in open-field tests 1, 6, 12, and 23 h after application on 3 consecutive days (treatments × test day across test time). Data are represented as mean ± SE. Significant post hoc effects are depicted by $*p \leq 0.05$, and $**p \leq 0.01$.

DISCUSSION

Treatment of patients with the immunosuppressive drug CsA produces neuropsychological side effects including alterations in emotional responses (25). The present study analyzed the effects of single IP injections of CsA on the OF behavior of DA rats in three repeated tests, which has been shown to be a reliable method for testing emotionality in rodents (1,3,18,40,42). The present results demonstrate that ambulatory activity decreased, whereas the incidence of defecation increased 6 h after CsA administration, reflecting an increased emotionality in CsA-treated rats (12). However, rearing behavior decreased 12 h after drug administration.

In rats, the bioavailability of CsA in the serum peaks at approximately 5 h after IP administration (30). Thus, the increased bioavailability 6 h after CsA injections may produce the increase in emotionality. The decreased rearing activity 12 h after drug administration, however, may be due to a delayed indirect drug action. The fact that CsA administration does have behavioral effects is supported by other reports, where

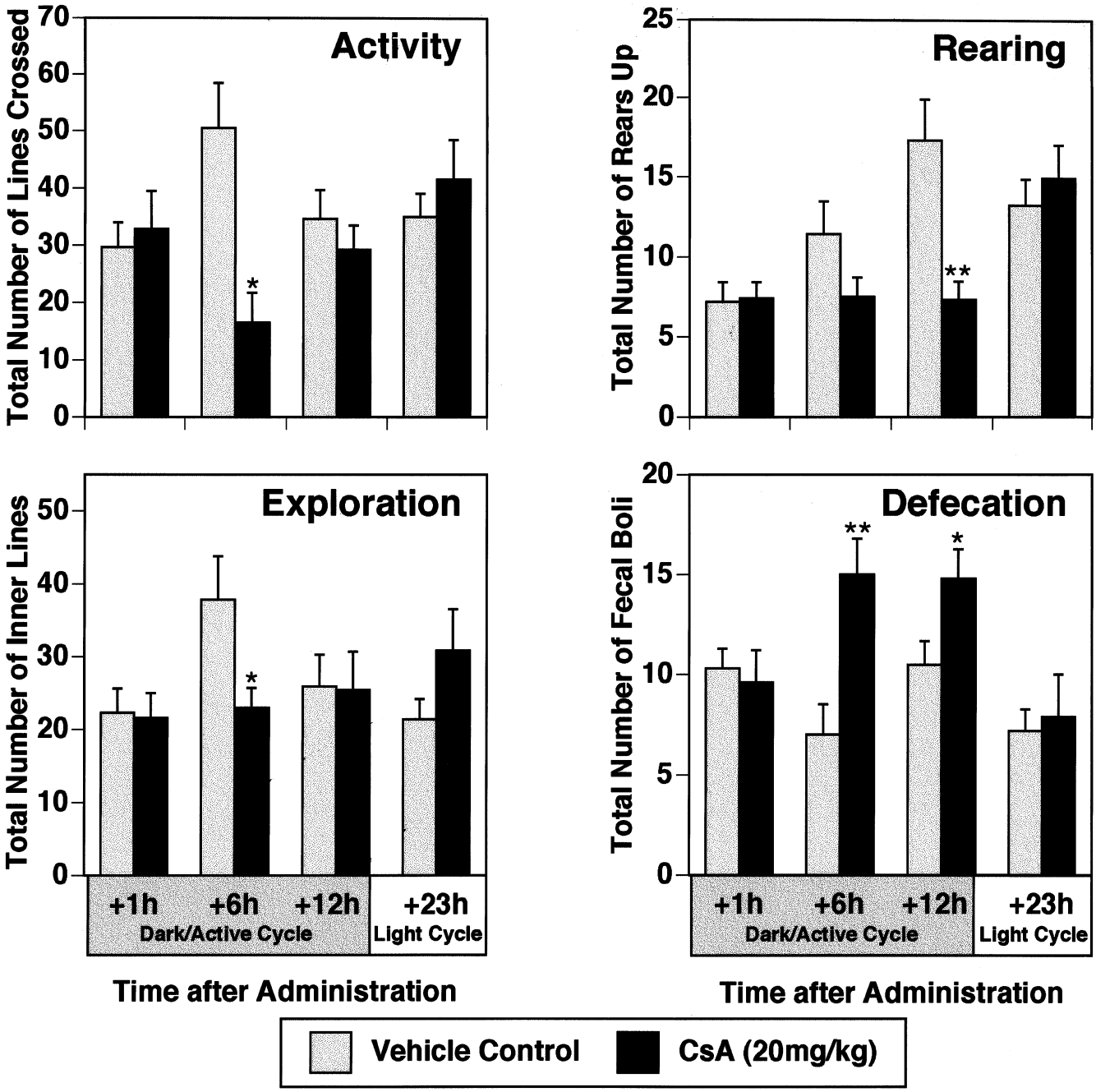

FIG. 2. Effect of CsA/vehicle treatment on total behavioral activity from three OF tests (total of treatments × test time). Data are represented as mean ± SE. Significant post hoc effects are depicted by $*p \leq 0.05$), and $**p \leq 0.01$.

CsA suppressed morphine-induced place preference (38), walking latency, and alley escape behavior (16), and increased nocturnal spontaneous and amphetamine-induced locomotor behavior (7) in rats. However, the current data have to be confirmed through other anxiety-related behavioral tests. In addition, the decrease in activity combined with a reduced explorative behavior in rats might reflect sedation rather than an increased emotionality. Therefore, in future investigations lower doses of CsA should be used to test whether a reduced exploration can also be observed without a reduction of locomotor activity.

The mechanisms of CsA-induced behavioral alterations are unknown. CsA has been shown to affect thermoregulation (9). Thus, drug-treated animals might display decreased activity because they become hypothermic or CsA treatment induces sickness behavior (6).

Another explanation for the CsA-induced behavioral effects is provided with the CsA blockade of cytokine upregulation, in particular IL-2 (14,15,19). Because CsA is known not to cross the intact blood–brain barrier (17,24), the behavioral effects of CsA are likely to involve the perception of altered cytokine levels by the central nervous system as it was demonstrated for IL-1 (5,28). The nervous system and immune system interact via afferent and efferent pathways (31). Neurotransmitters and neuropeptides affect lymphocyte migration and functions (35,41), and cytokines such as IL-1 and IL-2 modulate neural and neuroendocrine functions (5,21). In particular, it has been shown that proinflammatory cytokines such as IL-1, which are released at the periphery by activated accessory immune cells, act on the brain via a neural pathway involving activation of peripheral sensory nerves (8,28,29). In addition, IL-2 receptors are expressed in the hippocampus, which is a major center of emotional integration (21). Furthermore, IL-2 adminstration induces behavioral sedation and changes in the hypothalamic and pituitary activity in rats (4,11,22,36,43). These findings in rats may parallel observations in humans, where treatment of patients with IL-2 (13) or CsA (33,39) results in neuropsychiatric dysfunctions. However, this hypothesis needs to be confirmed not only by showing a hypothalamic response to peripheral CsA, but also by demonstrating the perception of CsA by the autonomic nervous system, and by in vivo effects of IL-2 on emotionality in rodents (37).

In summary, this study demonstrates CsA-induced behavioral alterations in DA rats in OF tests. The emotionality of DA rats increased 6 h after CsA administration and the rearing activity decreased 12 h after CsA application. These behavioral effects may be due to altered cytokine profiles induced by CsA. In future studies the direct behavioral effects of IL-2, together with the underlying mechanisms of these effects, need to be analyzed.

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