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Short Communication

Repeated stress causes reversible impairments of spatial memory performance

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Abstract

Restraint stress, 6 h/day for 21 days, caused an impairment, during acquisition, of the performance of a spatial memory task, the eight-arm radial maze. The impairment was reversible, temporally limited and blocked by phenytoin, a blocker of excitatory amino acid action, or tianeptine, an antidepressant, which lowers extracellular serotonin. These effects on behavior parallel the reversible stress-induced atrophy of dendrites of hippocampal CA3 neurons that are also blocked by the drugs.

Key words: Spatial memory; Radial arm maze; Hippocampus; Stress; Tianeptine; Phenytoin

Evidence is increasing that chronic exposure to corticosterone (CORT) contributes to neuronal loss in the brain. Furthermore, these actions of CORT may contribute to aging of the brain. For example, a relatively short period of restraint stress in rats, 21 days, or injection of CORT for 21 days, results in decreased dendritic branch points and total dendritic length of apical dendrites of pyramidal cells in the CA3 region of the hippocampus [15,18]. Extended daily administration of CORT for 12 wk, or prolonged and severe stress, results in cell loss in this population [10,14]. Neurochemical and electrophysiological alterations of neurons have also been shown after repeated stress or CORT administration [8].

While deleterious effects of excess CORT on the brain, especially the hippocampus, have been shown, there is little direct evidence to indicate whether brain function itself is compromised after exposure to excess CORT. Since the hippocampus is important for many aspects of learning and memory, such as spatial maze learning [2], it might be expected that chronic stress would be associated with impairments in these functions. Meaney and colleagues [3,9] have shown that rats who have higher basal and stress levels of CORT during their lifespan or at old age display enhanced hippocampal cell loss and spatial memory deficits; however, whether stress or CORT administration directly affects learning and/or memory function has not been examined.

In this study, we examined whether chronic stress affects spatial memory performance in rats. We utilized the paradigm of Watanabe et al. [15], in which rats are restrained daily for 21 days because this procedure is known to cause atrophy of apical dendrites of CA3 pyramidal neurons [15–17]. In addition, we tested whether phenytoin (Dilantin), an anti-epileptic, or tianeptine, a novel antidepressant, attenuate stress effects on spatial memory performance. These drugs block stress-dependent atrophy of CA3 pyramidal neurons [16,17].

Male Sprague–Dawley rats (~ 2.5 months old on arrival; Harlan Sprague–Dawley, Indianapolis, IN) were housed under a reversed light–dark cycle (lights off 07:00–19:00) and in accordance with the NIH Guide for Care and Use of Animals. Controls remained in their cages except for weighing while restrained rats were placed in Plexiglas restrainers in their home cages for 6 h/day (~ 10:00–16:00) for 21 days. Experiments were run with two or three cohorts of 10–15 rats containing control and stressed rats. In some experiments, rats received phenytoin (80 mg/kg s.c. in propylene glycol; Sigma, St Louis, MO) or tianeptine (15

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mg/kg s.c. in propylene glycol; Servier, France). Injections were administered before placing the rats in restrainers. The phenytoin dose is within the range used by others to inhibit ischemic damage (see Watanabe et al. [16]) and it produced no observable effect on the behavior or learning ability of the animals. The tianeptine dose is within the range to produce effects on neurochemical, neuroendocrine and behavioral responses [17], and tianeptine also did not affect overt behavior or learning ability of the rats.

Spatial memory was assessed using the eight-arm radial maze [4,5]. Rats (control and stressed) were put on dietary restriction, receiving two or three pellets of food/day, immediately after the last period of restraint. Within 3–4 days, they reached 85% of their normal body weight. Training on the maze was carried out on days 3–8 poststress (In one experiment, rats were stressed for 21 days but dietary restriction and training did not begin until 18 days after the last restraint session.) The nine training trials gradually shape the rats to go to the ends of the arms for a food reinforcement, a peanut. By the last two training trials, the task was identical with regular trials: one-half of a peanut is placed in food receptacles at the end of each of the eight arms, and rats are given a maximum of 8 min to visit all eight arms and eat the eight peanuts. A visit to an arm is scored if the subject traverses three-fourths of the arm's length, if the arm is entered but food not eaten, or if the arm is entered and food eaten. Re-entries into arms previously visited are counted as mistakes. Subjects received four of these regular trials on days 9–11 poststress. On days 13–16 poststress, trials with a delay were given. After the rat had made four choices, he was removed from the maze, put back in his home cage and then, after a designated period, returned to the maze to finish his last four choices. A practice delay of 10 min was given, then two 1-h, two 2-h and finally two 3-h delays were given.

Performance was scored by the number of correct choices in the first eight visits and by the visit where the first mistake occurred. Data was analysed by two-way ANOVA (group vs. trials) with repeated measures on trials.

Performance of rats on the radial maze after 21 days of restraint for 6 h/day is shown in Fig. 1 (Expt. 1). Stress was associated with small but significant decrements in performance. Stressed rats made their first mistake earlier than control rats, approximately their sixth choice, whereas controls made their first mistake on their seventh choice ($P < 0.01$). The number of correct choices in the first eight visits was lower in the stressed rats, 6.7 ± 0.15 vs. 7.1 ± 0.13 in control rats ($P < 0.05$). The impaired performance was not permanent since in trials with delays, which were conducted for 5 days after the regular trials, the stressed rats were no longer impaired (data not shown). In delay trials,

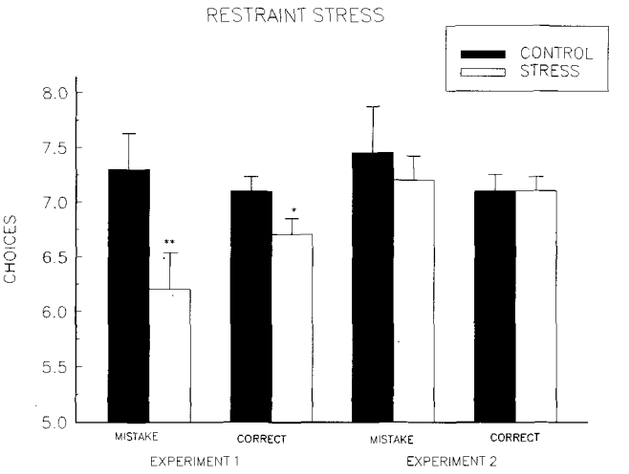

Fig. 1. Effect of restraint stress on radial arm maze performance. Choice where first mistake occurred (mistake) and number of correct choices in first eight visits (correct) are plotted for control (solid bars) and stressed (open bars) rats. Entries are average ± S.E.M. for four trials. In Expt. 1, 13 control and 14 stressed rats were trained and evaluated immediately after restraint sessions. In Expt. 2, six control and nine stressed rats were evaluated 18 days after last restraint session. See text for further details of experiments. Data analysed by two-way repeated measures ANOVA (group × trials). In Expt. 1, there was a significant group effect for mistake ($F_{1,25} = 5.31$, $P < 0.03$) and for correct ($F_{1,25} = 3.90$, $P < 0.05$) but no significant trials or interaction effects. In Expt. 2, there were no significant effects.

rats were removed from the maze after their fourth choice and returned to their home cages. After a specified time, they were returned to the maze to finish their last four arms. Both stressed and control groups scored ~ 6 for number of correct choices in first eight visits for delay intervals of 1–3 h.

The temporal dependency of stress-related impairments in performance is shown in Fig. 1 (Expt. 2). A second group of rats underwent 21 days of daily restraint stress but training on the maze was delayed until 18 days after the last restraint session. There were no differences in performance between the control and stressed rats. Both groups made their first error at approximately the seventh visit and averaged approximately seven correct choices in the first eight visits.

We next examined whether phenytoin, which interferes with excitatory amino acid release and transmission [16], would block effects of stress on spatial memory performance. Phenytoin was administered daily to control as well as restrained rats at the beginning of each of the 21 restraint sessions. As shown in Table 1, phenytoin-treated stressed rats performed identically to phenytoin-treated control rats. Moreover, control rats given phenytoin were not impaired relative to control rats in Expt. 1. In a separate experiment, the ability of tianeptine, an antidepressant, which enhances serotonin (5HT) uptake [17], to block stress-dependent

Table 1
Effect of phenytoin or tianeptine administration on radial arm maze performance of control and stressed rats

Group	Correct in first eight choices	Choice with first mistake
Control + phenytoin (14)	6.9 ± 0.09	6.9 ± 0.19
Stress + phenytoin (14)	6.8 ± 0.15	7.0 ± 0.21
Control + tianeptine (7)	6.9 ± 0.15	7.0 ± 0.34
Stress + tianeptine (7)	6.8 ± 0.09	6.7 ± 0.27

Entries are from two separate experiments (phenytoin and tianeptine) and are mean ± S.E.M. for four radial arm maze trials with indicated subject numbers *(n)*. Stressed rats received 6 h of restraint for 21 days. Drug injections were given just before restraint. See text for further details of experiments. Data analysed by two-way repeated measures ANOVA (group × trials) and no significant differences were found.

effects on performance was also examined (Table 1). Like phenytoin, daily administration of tianeptine to stressed rats just before the restraint session resulted in behavior which was not different from tianeptine-treated control rats. Nor did tianeptine treatment impair the acquisition of control rats.

These experiments indicate that 21 days of daily restraint stress is associated with impaired acquisition and performance of a spatial memory task, the eight-arm radial maze, and suggest that the hippocampal atrophy present after this stress regimen [15] has a functional consequence. Performance impairments do not prevent learning since, with continued testing, the stressed rats performed as well as the controls. In addition, the period of impaired acquisition is temporally limited since rats who were stressed but not evaluated until 18 days after the stress were not impaired in their performance. These behavioral results suggest that the stress-dependent dendritic changes may be reversible; indeed, this appears to be the case (A.M. Magarinos and B.S. McEwen, unpubl. obs.).

The stress-dependent impairments are small in magnitude but it should be emphasized that the trials were conducted 9–12 days after the last restraint session. The delay in testing is because of the time necessary for the rats to lose sufficient weight to be motivated to do the task and to be trained on the task. A spatial memory task, which could be applied closer to the end of the stress period, might show larger deficits in the stressed rats. Aged rats are also impaired in spatial memory tasks, scoring 6–6.5 for number of correct choices in first eight visits compared with 6.7 for the stressed rats in this study ([4]; V. Luine and M. Rodriguez, unpubl. data). Thus, stress-induced impairments are qualitatively similar but somewhat smaller than age-dependent impairments.

Numerous studies have shown the importance of the hippocampus in spatial memory and have found decrements in performance after lesions specific to either CA1 or the dentate gyrus [1,13]. These results suggest that damage to CA3 is also sufficient to affect maze performance. Other studies have indirectly implicated impaired memory function to stress- or CORT-dependent damage of the hippocampus. Aged rats, who showed impaired Morris water maze performance (a spatial memory task) as compared with young rats, had higher basal CORT levels, prolonged stress levels of CORT and significantly greater hippocampal pyramidal cell loss than other aged rats who were not impaired on the Morris task [3]. In addition, daily "handling" of neonatal rats results in adults who show lower CORT levels in response to stress and a more efficient shut off of CORT secretion after stress. More importantly, these rats show less hippocampal cell loss and better Morris maze performance than controls [9]. In addition to their involvement in loss of hippocampal pyramidal neurons [11], glucocorticoids are also implicated in the atrophy of apical dendrites of CA3 pyramidal neurons [6,18]. Thus, while briefer periods of stress cause reversible atrophy of CA3 neurons and reversible impairments in spatial memory acquisition, the long-term exposure to glucocorticoids and severe stress appears to permanently damage the hippocampus and permanently impair cognitive function.

The ability of phenytoin or tianeptine to block the stress-induced atrophy of CA3 dendrites [16,17] and to attenuate stress-dependent impairments in spatial memory performance suggests that both excitatory amino acids (EAA) and 5-HT may be involved in the degenerative process along with glucocorticoids. EAAs have been linked to brain damage and glucocorticoids have been shown to exacerbate this damage [8,11,12]. Within the hippocampus, CA3 neurons receive mossy fiber innervation containing EAA onto their apical dendrites from the dentate gyrus area [8,18]. The novel antidepressant, tianeptine, which facilitates 5HT uptake and decreases extracellular 5HT levels [3,9], also blocks stress-dependent declines in spatial memory performance. It was previously reported that tianeptine blocks stress- and CORT-dependent atrophy of CA3 dendrites but does not alter basal or stress-induced levels of CORT or thymus weight [17]. Thus, tianeptine effects on behavior are not due to a suppressive effect on the adrenocortical stress response or to an antiglucocorticoid action. In addition, we have found that chronic ingestion of CORT is associated with elevated levels of 5-HT in the hippocampus [5] and that stress alters hippocampal 5HT receptors [7]. Thus, enhanced 5HT activity may contribute to the production of dendritic atrophy, or, alternatively, 5-HT may interact with EAAs to produce hippocampal neuronal atrophy. These speculations suggest that future research should directly assess the contributions of 5HT and/or EAAs to hippocampal dendritic atrophy and/or to decrements in spatial memory performance.

In conclusion, chronic restraint stress is associated

with a reversible impairment of the acquisition and performance of a spatial memory task in rats. Thus, the potentially damaging properties on the brain of glucocorticoids, which have been shown anatomically, chemically and electrophysiologically, have now been extended to an important function of the brain, learning and memory.

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