Chronic restraint stress impairs acquisition and retention of spatial memory task in rats

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Chronic restraint stress causes dendritic atrophy of CA3 pyramidal neurons of the hippocampus. In this study, we assessed the functional consequences of dendritic atrophy on the acquisition and retention of spatial memory, using a T-maze task. 45-day-old male Wistar rats, subjected to 6 h of daily restraint stress over a period of 21 days, were tested for left–right discrimination task for food reward in a T-maze. We found a significant ($P < 0.001$) deficit in both acquisition and retention of the task in stressed rats compared to controls. To rule out the possibility that gastric ulcers, induced by stress, could work as a deterrent for rats to seek the food reward, rats were also treated with an antacid. Even though the antacid treatment prevented the stress-induced ulcer formation, the learning and memory deficits were not prevented. These results demonstrate that chronic restraint stress impairs spatial learning and memory in rats.

THE role of hippocampus in mediating cognitive functions such as learning and memory is well established. The hippocampus is highly susceptible for various endogenous and exogenous insults, including stress[1–4]. Chronic stress causes neuroanatomical changes in the hippocampus, such as atrophy of apical dendrites of CA3 pyramidal neurons[2,3,5], increase in dendritic spines and excrescences[3] and reduces corticosteroid receptors[6]. Furthermore, the atrophy of CA3 dendrites caused by restraint stress is not permanent. It can return to the pre-stress condition following rehabilitation for a period of 45 days after the last stress session[2]. However, the structural changes (throughout the hippocampal pyramidal neurons) could be permanent, when the stress is severe and sufficiently long-lasting[2,7]. Long-term treatment of rats with high levels of corticosteroids also resulted in dendritic atrophy and pyramidal cell loss[8,9]. Recently, we have shown that blocking of excitatory glutamatergic inputs to the hippocampus by bilateral entorhinal cortex lesions attenuates the stress-induced atrophy of dendrites in CA3 neurons[10]. These studies suggest that the hippocampal changes associated with stress are mediated, at least in part, by adrenal steroids and excitatory amino acids.

The hippocampus has been implicated as a critical structure for various aspects of learning and memory, particularly for solving tasks which require spatial memory[11]. The hippocampus is a critical integrative centre involved in the regulation of exploratory activities and in incorporating spatial information[12] and T-maze tests enable the animals to learn spatial information[13]. Lesions of the CA3 region of the hippocampus have been reported to impair learning through spatial memory disturbance[14].

Involvement of CA3 region in memory is further demonstrated by using nootrophic drugs that affect the long-term potentiation (LTP) in mossy fibre-CA3 system[15]. In addition, single as well as repeated corticosterone injections inhibit LTP in the hippocampus[16]. Rats which had higher basal levels of corticosterone throughout their life span or at old age display increased hippocampal cell loss[17]. However, the effect of chronic restraint stress on the spatial learning and memory in T-maze tasks has not been investigated. Accordingly, we have evaluated the effect of chronic restraint stress on the left–right discrimination task for food reward in a T-maze, since the T-maze tasks form a powerful spatial recognition memory test[18].

Male Wistar rats (45-days-old) weighing 100–120 g (obtained from Central Animal Research Facility at National Institute of Mental Health and Neurosciences, Bangalore, India) were divided into four groups; (a) normal control (NC), (b) stressed (ST): these rats were restrained in a wire mesh restrainer 6 h/day for 21 days (for details see our earlier reports[2,3,10]), (c) normal control rats treated with antacid (NA), and (d) stressed rats treated with antacid (SA). Each group consisted of six rats. NA and SA groups of rats received antacid (50 mg/kg, b.w. orally) for 21 days, to rule out the effect of gastric ulcers acting as a deterrent for rats to seek the food reward in behavioural tests.

All groups of rats were reared in a 12 h light–dark cycle and were housed individually in polypropylene cages with *ad-lib* food and water. Experiments were conducted in strict accordance with the NIH guidelines (NIH Publication No. 86-23, revised 1985) and were also approved by the local ethical committee.

After 21 days, all groups of rats were subjected to the left–right discrimination test in a T-maze. In this test, rats have to discriminate either left or right arm of the T-maze in order to get the food reward. The T-maze consisted of a start box (12 × 12 cm), stem (35 × 12 cm), choice area (15 × 12 cm) and two arms (35 × 12 cm); each arm had a goal area (15 × 12 cm) containing a food well. The side walls were of 40 cm in height[19]. The stem and the start box were separated by a sliding door, and a cloth curtain separated the arm and goal areas so that the food well from the choice area is not visible to the rat. 16 W bulbs illuminated the start box, choice and goal areas. The T-maze was kept in a dimly lit, sound attenuated room. Immediately after the last session of stress, rats were sub-

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jected to partial food deprivation and behavioural testing was carried out in three phases.

During the orientation and training session, rats were placed in the start box for 30 s, the sliding door was opened and rats were allowed to explore the T-maze for 30 min and when they reached the goal area, 2–3 food pellets of 10 mg each (semisynthetic 18% CW balanced diet) were provided. In the next 10 trials (with an inter-trial interval of 30 s), rats were trained to reach either the left arm (left-rewarded group) or the right arm (right-rewarded group) and they were rewarded with one food pellet.

The learning (acquisition) test was similar to that of training session and was conducted from four to six days after the end of the stress period. In each session of 10 trials, the number of errors, i.e. entry into the non-rewarded arm (right arm for left-rewarded group and left arm for right-rewarded group) was recorded. Since there were no significant differences between the performance of left- and right-rewarded groups of rats, the data from both these groups were pooled and analysed together.

Two days following the last learning session (i.e. post-stress day 8), memory retention test was carried out. Rats were given a single session of 30 trials and the number of errors committed by each rat was recorded. At the end of behavioural experiments, all groups of rats were killed under deep anaesthesia (pentabarbitone sodium, 50 mg/kg, b.w.); the adrenal wet weights (both left and right adrenals) and the presence or absence of gastric ulcers was determined.

The behavioural data were statistically analysed by two-way ANOVA with repeated measures on one-factor[20]. The data on the mean number of errors in both acquisition and retention tests, and adrenal weights were subjected to one-way ANOVA, followed by least significant difference (LSD) post-hoc test for inter-group comparisons[21]. The results are expressed as mean ± SD and values of $P < 0.05$ were considered statistically significant.

Figure 1 illustrates the gastric mucosa from different groups of rats. Stressed group of rats had gastric ulcers, which appeared as small streaks with multiple foldings and patches on the gastric mucosa (Figure 1 *b*) compared to control (Figure 1 *a*). However, antacid treatment prevented the ulcer formation in stressed rats as indicated by the absence of streaks, foldings and patches in the gastric mucosa (Figure 1 *d*).

Figure 2 shows the mean adrenal wet weights of different groups of rats. The adrenal weights were significantly higher in ST and SA groups of rats compared to NC and NA groups of rats ($F_{3,20} = 26.19$, $P < 0.001$). This indicates the stress-induced hypertrophy of adrenal glands. However, the antacid treatment did not have any effect on adrenal weights (Figure 2).

The acquisition and retention data subjected to two-factor ANOVA with repeated measures revealed a significant effect between groups ($F_{3,20} = 23.9$, $P < 0.001$ and

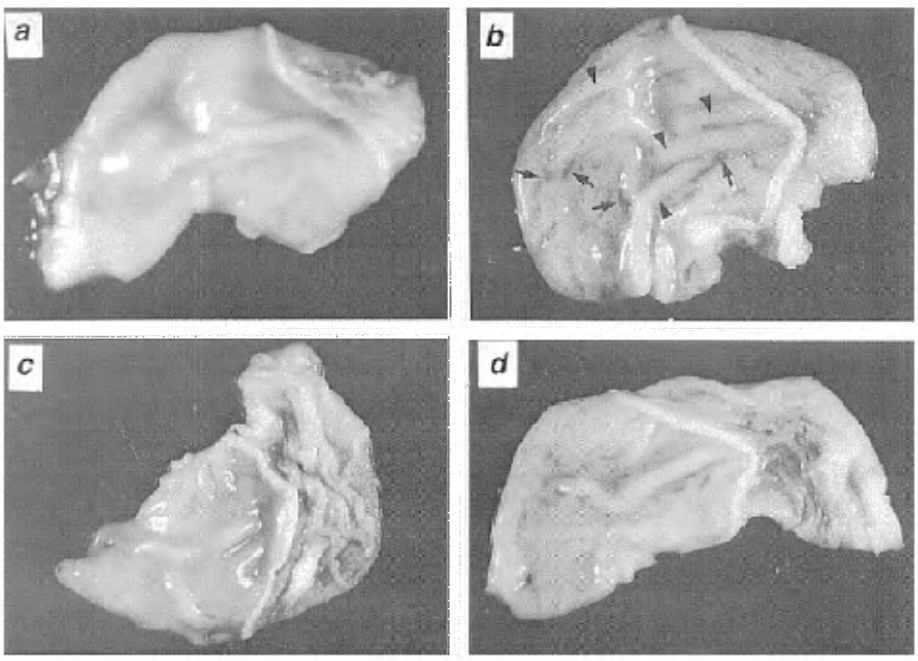

Figure 1. Representative photomicrographs of gastric mucosa from ***a***, normal control (NC); ***b***, stressed (ST); ***c***, NC rats treated with antacid (NA); and ***d***, ST rats treated with antacid (SA) groups. Note the presence of numerous ulcer spots (indicated by arrows) and more number of foldings (indicated by arrowheads) in ***b*** compared to ***a*** and ***c***. These features were absent in ***d***.

$F_{3,20} = 18.67$, $P < 0.001$), trials ($F_{9,180} = 2.79$, $P < 0.01$ and $F_{29,580} = 2.36$, $P < 0.001$) and interaction ($F_{27,180} = 1.67$, $P < 0.05$ and $F_{87,580} = 1.63$, $P < 0.01$). The mean number of errors committed per session by ST and SA groups of rats was significantly higher in the acquisition (Figure 3 *a*; $F_{3,20} = 31.84$, $P < 0.001$) as well as in the retention (Figure 3 *b*; $F_{3,20} = 19.39$, $P < 0.001$) compared to NC and NA groups of rats. Although the antacid treatment prevented the ulcer formation in the SA group of rats, it did not produce any significant improvement in their performance in acquisition and retention tests.

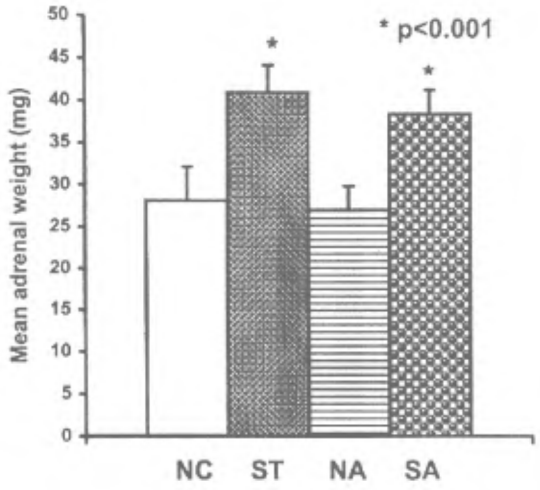

Figure 2. Adrenal wet weights in NC, ST, NA and SA groups of rats (mean ± SD, for abbreviations refer to Figure 1). Note a significant increase in adrenal weights in ST and SA groups compared to NC and NA groups, respectively (*$P < 0.001$; one-way ANOVA followed by LSD test).

These experiments indicate that 21 days of restraint stress results in an impaired acquisition and retention of spatial learning and memory as assessed by performance in T-maze tasks. These findings are in agreement with previous studies showing an impaired performance on two different types of spatial memory tasks in stressed rats[22,23]. In addition, Dominique *et al.*[24] reported recently that the glucocorticoids and foot shock stress-induced impairment in the retrieval of long-term spatial memory in a water maze. On the basis of our data[2,3] and previous data on the effects of chronic stress on hippocampal morphology, we suggest that atrophy of CA3 neurons caused by chronic stress may be responsible for spatial learning/memory impairment.

Chronic restraint stress-induced ulcerogenesis in the gastric muscosa may be a brain-driven event[25], because the hippocampal and entorhinal cortex lesions are known to aggravate stress ulceration in rats[26]. How these structures influence the formation of gastric ulcers is unclear, but their connections with hypothalamic areas, via relays in the amygdala may bring about this effect[27]. Some studies suggest that the central amygdala modulates the degree of stress ulceration[28]. Recent studies have shown that high-frequency electrical stimulation near the pyramidal cells of ventral CA1 area produced LTP in the central amygdala and it attenuated gastric stress ulcers[29]. These findings implicate a pathway from ventral hippocampus to the central amygdala, which may be involved in the modulation of stress-induced ulcer development. Thus, the amygdala and the hippocampal formation, apparently, modulate the degree to which stressful experiences produce pathological changes in the gastrointestinal system[25]. It is possible that stress-induced atrophy of pyramidal

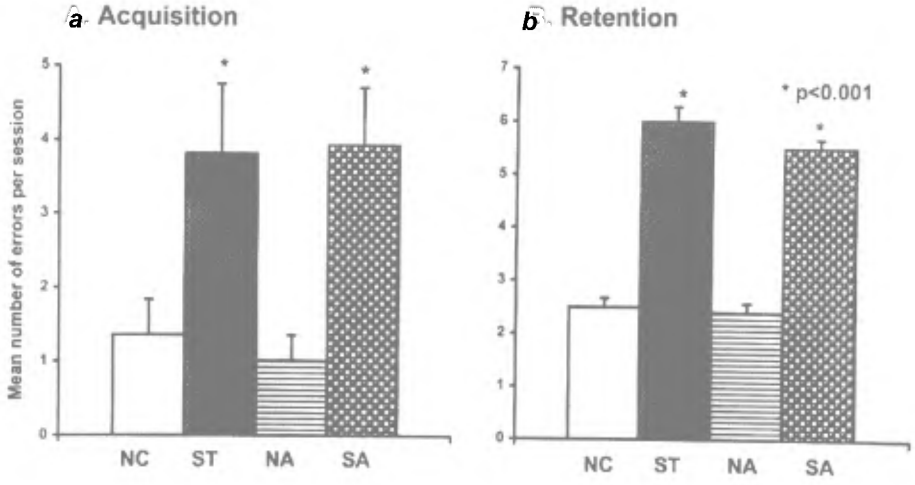

Figure 3. Number of errors per session in the acquisition (***a***) and retention (***b***) tests in the left-right discrimination task in T-maze from NC, ST, NA and SA groups of rats (mean ± SD, for abbreviations refer to Figure 1). Note more number of errors made in ST and SA group compared to NC and NA groups of rats in both acquisition and retention tests (*$P < 0.001$; one-way ANOVA followed by LSD test).

neurons in the hippocampus may contribute to the ulcer formation.

The impaired performance of stressed rats in the food-reward based spatial-memory task is not due to the presence of gastric ulcers, produced by stress. Antacid treatment while preventing the ulcer formation did not have any beneficial effect on the performance of stressed rats in the above task. The selective atrophy of distal dendrites following stress[2,3] was accompanied by an increase in the number of spines and excrescences[3] in CA3 pyramidal neurons of the hippocampus. However, compensatory mechanisms such as an increase in the spine density to overcome the impairment of neuronal function as a result of dendritic atrophy, may not be sufficient to prevent the memory deficit caused by restraint stress.

Sunanda *et al.*[30] have shown that stress increases the levels of glutamate and its release[31] in the hippocampus. Stress-induced impairment in radial-arm maze performance was blocked by phenytoin, a drug that interferes with glutamate release and transmission[23] and it also prevents the atrophy of CA3 dendrites[32]. We have also observed that blocking of excitatory glutamatergic inputs to the hippocampus by bilateral lesions of entorhinal cortex attenuated the stress-induced dendritic atrophy in CA3 neurons of the hippocampus[10].

Brain regions other than the hippocampus may have been affected by chronic restraint stress paradigm and could have contributed to the memory impairment, since corticosteroid receptors are present ubiquitously in the brain[33] and consequently, every region will have the potential to be affected by chronic stress. It will be interesting to see whether chronic restraint stress also induces morphological changes in regions other than the hippocampus.

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