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ORIGINAL PAPER

Self-Stimulation Rewarding Experience Restores Stress-Induced CA3 Dendritic Atrophy, Spatial Memory Deficits and Alterations in the Levels of Neurotransmitters in the Hippocampus

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Abstract Chronic restraint stress causes spatial learning and memory deficits, dendritic atrophy of the hippocampal pyramidal neurons and alterations in the levels of neurotransmitters in the hippocampus. In contrast, intracranial self-stimulation (ICSS) rewarding behavioral experience is known to increase dendritic arborization, spine and synaptic density, and increase neurotransmitter levels in the hippocampus. In addition, ICSS facilitates operant and spatial learning, and ameliorates fornix-lesion induced behavioral deficits. Although the effects of stress and ICSS are documented, it is not known whether ICSS following stress would ameliorate the stress-induced deficits. Accordingly, the present study was aimed to evaluate the role of ICSS on stress-induced changes in hippocampal morphology, neurochemistry, and behavioral performance in the T-maze. Experiments were conducted on adult male Wistar rats, which were randomly divided into four groups; normal control, stress (ST), self-stimulation (SS), and stress + self-stimulation (ST + SS). Stress group of rats were subjected to restraint stress for 6 h daily over 21 days, SS group animals were subjected to SS from ventral tegmental area for 10 days and ST + SS rats were subjected to restraint stress for 21 days followed by 10 days of SS. Interestingly, our results show that stress-induced behavioral deficits, dendritic atrophy, and decreased levels of neurotransmitters were completely reversed following 10 days of SS experience. We propose that SS rewarding behavioral experience ameliorates the stress-induced cognitive deficits by inducing structural and biochemical changes in the hippocampus.

Keywords Chronic restraint stress · Biogenic amines · Hippocampus · Spatial learning · Dendritic atrophy · Acetylcholinesterase activity · T-maze · Rewarded alternation task

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Introduction

Severe and prolonged stress precipitates affective disorders and causes impairment in learning and memory. Earlier, we have demonstrated that 21 days of restraint stress impairs acquisition of T-maze [1] and radial arm maze tasks [2, 3]. Furthermore, stress-induced impairment in learning is shown in other paradigms like the Y-maze [4], Barnes maze [5] and Morris water maze [6]. In addition to stress, enhanced glucocorticoids (GCs) have been shown to produce learning deficits [7]. It has also been demonstrated in animals, that excessive corticosterone can impair spatial learning [5, 8]. Further, the cognitive impairment and the hippocampal degeneration associated with chronic stress are thought to be at least in part due to the elevated levels of GCs since blockade of GC receptors or GC synthesis prevents stress-induced dendritic atrophy and cognitive deficits [8–11].

Among the cellular changes that could underlie the stress-induced behavioral dysfunction, alteration in the morphology of hippocampal CA3 neurons is the most prominent [12–15]. Studies from our laboratory have shown that 21 days of chronic restraint stress causes atrophy of apical dendrites [13] and alterations in the number of dendritic spines and excrescences of CA3 pyramidal neurons of the hippocampus [16]. The stress-induced structural changes and cognitive dysfunctions are mediated by tissue

plasminogen activator and plasminogen [17]. Furthermore, the atrophy of CA3 dendrites can be reversed following rehabilitation for a period of 45 days after the last session of stress [13]. However, when the stress is severe and sufficiently long lasting, the structural changes in the hippocampal pyramidal neurons could not be reversed [13]. Neurochemically, such dendritic remodeling in CA3 pyramidal neurons is mediated by mechanisms that involve high levels of GC secretion and activation of excitatory amino acid release [12, 14]. Further, cholinergic dysfunction and decreased levels of biogenic amines in the hippocampus [18] have been shown following restraint stress.

Intracranial electrical self-stimulation (ICSS) is an intensely rewarding behavioral experience, more influential than feeding or sexual behavior [19]. In contrast to the effects of stress, ICSS increases the dendritic arborization [20–22], spine and synaptic density in CA3 pyramidal neurons [23–25] and enhances the levels of noradrenaline (NA), dopamine (DA), glutamate, and acetylcholinesterase (AChE) activity in the hippocampus [26]. In addition, ICSS experience facilitates the acquisition and performance in operant and spatial learning tasks and ameliorates the fornix lesion induced spatial learning deficits [27, 28].

Although the effects of stress and ICSS are documented, it is not known whether ICSS treatment following stress ameliorates the stress-induced deficits. Accordingly, in the present study, we have evaluated the effect of SS rewarding experience on stress-induced behavioral, morphological and neurochemical deficits.

Experimental procedures

Subjects

Adult male Wistar rats (200–250 g; 2–2.5 months old) obtained from Central Animal Research Facility, NIMHANS, Bangalore were used in the study. Rats were housed three per cage in polypropylene cages (22.5 × 35.5 × 15 cm) in a temperature (25 ± 2°C), humidity (50–55%) and light-controlled (12 h-light-dark cycle) environment, with food and water ad libitum except during the periods of stress. The experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised 1996) and Institutional animals ethics committee approved the experimental protocols. All efforts were made to minimize both the suffering and the number of animals used.

Groups

Rats were randomly divided into four groups, consisting of six rats each; (a) normal control (NC): this group of rats remained undisturbed in their home cages except during change of bedding, (b) stress (ST): rats were restrained in a wire-mesh restrainer for a period of 6 h per day (10:00–16:00 h), for 21 days as described earlier [1–3, 14] followed by a 17 days stress-free period, (c) self-stimulation (SS) and (d) stress + self-stimulation (ST + SS) groups. Rats belonging to SS and ST + SS groups were stereotaxically implanted with bipolar electrodes bilaterally in the substantia nigra-ventral tegmental area (SN-VTA).

Electrode implantation and testing to obtain self-stimulation behavior

The electrode implantation, behavioral testing and training for SS were done as described earlier [20–29]. SS and ST + SS groups of animals were stereotaxically implanted with epoxylite-coated bipolar nichrome electrodes (28 s.g.w) bilaterally in the SN-VTA. The stereotaxic coordinates were, antero-posterior (AP): –4.8 to –6.5 mm; medio-lateral (ML): 1.0–1.2 mm and dorso-ventral (DV): 8.3–8.5 mm (Paxinos and Watson rat atlas; [30]). After 5–7 days of post-surgical recovery, rats of SS and ST + SS groups were trained for pedal pressing to obtain electrical stimulation in a modified Skinner's operant chamber [29, 31].

The modified Skinner's chamber was a locally fabricated operant chamber made of Plexiglas (inner dimensions: 22 × 22 × 24 cm) that had a stainless steel pedal (4 cm wide with 2 cm projection length) positioned on one wall of the chamber, 6.5 cm above the grid floor. Through a micro switch, the pedal was connected to a pulse programmer, which delivered the desired pulses into the brain through the implanted electrodes. Each pedal press delivered a stimulus train of square waves for a duration of 0.25 s. The current intensity was adjusted in the range of 25–75 μA for each electrode site in such a way as to elicit maximum pedal press responses under fixed stimulus frequency. The number of pedal press responses per session was recorded in an automated digital counter.

Training procedures of pedal press for self-stimulation

After 5–7 days of post-surgical recovery, the rats were shaped and screened to press the pedal for ICSS. Data from reliable and sustainable rats having self-stimulation (SS) responses from the SN-VTA site on a continuous reinforcement schedule (CRF 1:1) were taken for analysis. The implanted rat was connected to the stimulator (pulse generator) and placed in the modified Skinner operant chamber. The experimenter administered low current intensity stimulations and the behavior of the rat was observed. Once the rat showed signs of getting the

electrical stimulus, the current intensity was increased progressively in steps. With these stimulations, rats start exploring the sources of stimulation; sniffing near the pedal, running toward the pedal and the vibration of the whiskers. A rat that is positive approaches the pedal. In order to enhance the chances that it repeats coming to the pedal, the number of successive stimulations are increased and such stimulations were restricted only when the animal approached the pedal to manipulate it for getting electrical stimulations. During this process the rat bumps over the pedal with forelimbs. With minor alterations by either increasing or decreasing the current intensity, the animal is made to press the pedal repeatedly to get the stimulation by itself. For few minutes it was left to improve its pedal press performance [20–26].

The SS and ST + SS groups of animals were allowed 15 min of daily SS from each site for a period of 10 days. The operant chamber was cleaned with alcohol after each session. There was no significant difference in the pedal press responses between SS and ST + SS groups. The mean (±SD) number of pedal press responses in SS and ST + SS groups for a 15 min session was 1,498 ± 122 and 1,592 ± 55, respectively. The placement of bipolar electrodes in SN-VTA, billaterally in SS experienced rats ($n = 12$) was mapped in 40 μm thick coronal sections of the brain stained with cresyl violet. The electrodes were localized from –4.8 to –6.3 mm with reference to bregma (Fig. 1), according to the Paxinos and Watson rat atlas [30].

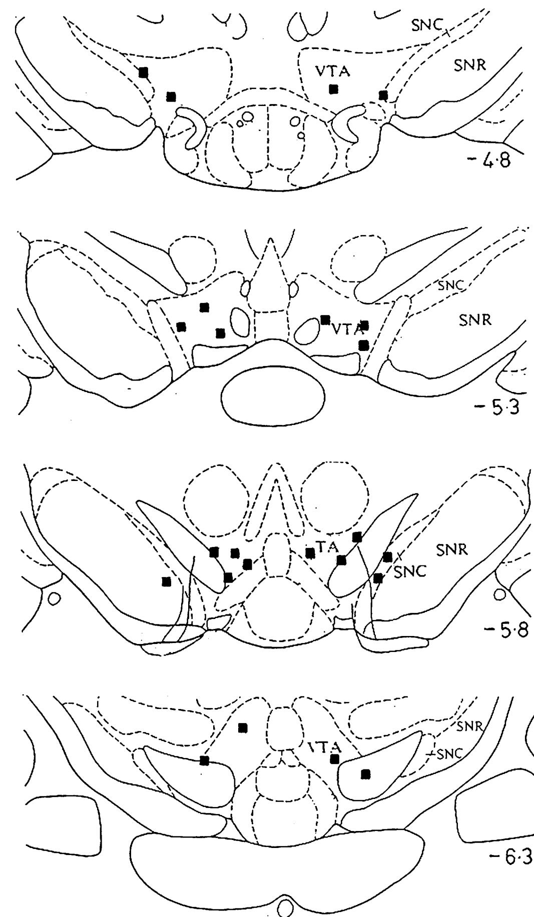

Fig. 1 Mapping of electrode placements in SN-VTA, bilaterally in SS experienced rats ($n = 12$). Each *square* represents the location of bipolar electrodes and the electrodes were localized from –4.8 to –6.3 mm with reference to Bregma according to the Paxinos and Watson rat atlas. *SNC* Substantia nigra pars compacta, *SNR* substantial nigra pars reticulata, *VTA* ventral tegmental area

Quantification of dendritic arborization of CA3 pyramidal neurons of the hippocampus

The dendritic morphology of the CA3 pyramidal neurons was assessed using the rapid Golgi staining technique [13, 32]. After completion of the experiments, all groups of rats were deeply anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) and decapitated quickly. The brain was exposed by an incision along the midline of the skull and a small amount of fixative was poured on the exposed brain immediately. The brain was removed quickly and blocks of tissue containing the hippocampus were dissected and transferred to 25 ml of the fixative (5 g potassium dichromate, 5 g chloral hydrate, 8 ml glutaraldehyde, 6 ml formaldehyde, 6–10 drops dimethyl sulfoxide in 100 ml of distilled water) in an amber colored bottle [20, 33].

After about 5 days of fixation, tissues were silver impregnated with 0.75% silver nitrate ($AgNO_3$) solution for 48 h. After thorough dehydration in absolute alcohol for 10 min, 120 μm thick transverse sections were obtained from the dorsal hippocampus, using a rotary microtome (Jung RM 2055, Leica, Mannheim, Germany). Sections were collected serially, dehydrated in absolute alcohol, cleared in xylene and coverslipped. Slides were coded prior to quantitative analysis and the code was broken only after the analysis was completed. The Golgi-impregnated neurons chosen for analysis had to satisfy the following criteria: (1) presence of untruncated dendrites; (2) consistent and dark impregnation along the entire extent of all of the dendrites and (3) relative isolation from neighboring impregnated neurons to avoid interference with analysis.

For morphological quantification of dendritic arbors, ten short-shaft pyramidal neurons from each animal were analyzed from the CA3 area of the dorsal hippocampus [34]. Camera lucida tracings (500×) of selected neurons were obtained using Leitz microscope and using the center of the soma as the reference point, branch points and dendritic intersections were measured as a function of radial distance from the soma, by adding up all values in each successive concentric segment (Sholl's analysis;

segment diameter: 50 μm) [13]. Alterations in the apical dendritic morphology were quantified in terms of dendritic branching points and intersections at various segments and total number of branching points.

Assay of acetylcholinesterase activity

Acetylcholinesterase activity was measured by modified Ellman's method [35]. After the stress and SS protocols, the hippocampus was quickly dissected out and homogenized in 0.1 M phosphate buffer, pH 8. The reaction mixture consisted of 2.6 ml of phosphate buffer (0.1 M, pH 8.0), 0.4 ml aliquot of homogenate, and 0.1 ml of 0.01 M dithiobisnitrobenzoic acid (DTNB). After the addition of the substrate acetylthiocholine iodide (0.075 M), change in the absorbance was noted every 2 min for 10 min at 412 nm using a spectrophotometer. The activity was expressed as micromoles hydrolyzed per min per gram of tissue [2, 18, 26, 36].

Estimation of levels of biogenic amines

After the completion of experimental protocols, rats from all groups were sacrificed, the hippocampus was dissected out and homogenized in 2 ml of 15 mM sodium acetate buffer with 20% methanol and centrifuged at 1,850 *g* for 30 min at 4°C. The supernatant was filtered through a 0.2 μm pore size cellulose acetate filter (Sartorius, Goettingen, Germany) and was stored at –80°C till further analysis. Stock solutions of standards namely, NA, DA, and 5-HT were made at a concentration of 1 mg/ml and diluted to obtain a concentration of 10 pg/μl of injection volume. The analysis was done using isocratic ion-pair HPLC (Shimadzu Co., Kyoto, Japan) with electrochemical detection (Bio-Rad, Hercules, CA, USA). The mobile phase consisted of sodium acetate (20 mM), heptane sulfonic acid (5 mM), EDTA (0.1 mM), and dibutylamine (0.04%) mixed with methanol (5.4%). The electrochemical conditions maintained included an applied potential of 650 mV and sensitivity of 2 nA/V. NA, DA, and 5-HT were separated on a reverse phase Nucleosil C-18 analytical column (15 × 0.46 cm; 3 μm particle size; Supelco, St. Louis, MO, USA) with a flow rate of 0.9 ml/min and injection volume of 20 μl. Chromatography data were processed and chromatograms were analyzed with Winchrom data station (Indtech Instruments, Mumbai, India) [37]. The peaks in the samples were identified by comparing their retention time with that of the standard solution and quantified by comparing its peak area with that of the standards and the concentration of the biogenic amines was determined and expressed as ng/g tissue wet weight [2, 3].

Assessment of behavior in T-maze rewarded alternation task

All groups of rats were subjected to rewarded alternation task in T-maze to assess their spatial learning abilities [1, 28, 38]. In this test, rats have to discriminate between the left and right arm of the T-maze and remember their choice in the previous trial in order to obtain 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 sidewalls were of 40 cm in height [39]. The stem and the start box were separated by a sliding door, and a curtain separated the arm and goal areas so that the food well from the choice area is not visible to the rat. About 16 W bulbs illuminated the start box, choice, and goal areas. The maze was kept in a dimly lit and sound attenuated room.

Prior to the training, the animals were kept on a restricted diet and body weight was maintained at 85% of their free feeding weight, with water available ad libitum. Before each rat was placed in the T-maze, it was thoroughly cleaned with 70% alcohol to remove any possible olfactory cues. The rats were first acclimatized to the T-maze before the training sessions by placing the rat in the start box and the sliding door opened after 30 s. The rats were then allowed to explore the T-maze for 30 min during which, if they reached the goal area, they were rewarded with two to three food pellets of 10 mg each. In the next ten trials (with an inter-trial interval of 30 s), rats were trained to reach the goal area of either of the arms, alternatively. We followed the rewarded alternation paradigm of the T-maze, which has been shown to be sensitive in detecting hippocampal dysfunction [40]. In the rewarded alternation task, either the left or right arm of the T-maze was baited depending on the rat's choice in the previous trial (i.e., if the rat chose the right arm, the left arm was baited in the next trial and vice versa). The learning (acquisition) test was similar to that of training session and was conducted for 8 days. In each session of ten trials, the number of errors, i.e., entry into the non-rewarded arm was recorded. The rats were considered to have performed the task successfully only when they made eight correct choices out of ten-trials/session (criterion of acquisition). The number of correct choices per session and the time taken to reach the goal area (latency) were recorded.

Statistical analyses

The data of mean number of alternations and the time taken to reach the goal area (latency) in rewarded alternation task were analyzed by two-factor ANOVA with repeated measures on one-factor followed by Newman Keul's post hoc

test. The data on sessions to reach the criterion (acquisition), total number of dendritic branching points and levels of neurotransmitters were subjected to one-way ANOVA followed by Tukey's post hoc test. The segmental-wise data of dendritic branching points and intersections were analyzed by two-way ANOVA followed by Tukey's post hoc test. The data is expressed as mean ± SEM and values of $p < 0.05$ were considered statistically significant.

Results

Role of ICSS on stress-induced dendritic atrophy in CA3 hippocampal neurons

Dendritic branching points

Analysis of apical dendrites data by two-way ANOVA revealed a significant difference between groups ($F_{3, 169} = 44.13$, $p < 0.001$). The number of branching points was significantly ($p < 0.001$) decreased in all the segments in ST group of rats when compared to controls. The number of branching points was increased significantly in SS group of rats ($p < 0.001$). Interestingly, stress-induced decrease in dendritic branching points was restored to control levels following 10 days of SS ($p < 0.001$ vs. stress; Figs. 2, 3, and 4A, C).

Dendritic intersections

The data on intersections when subjected to ANOVA, showed a significant difference between groups ($F_{3, 198} = 65.39$, $p < 0.001$). The segmental analysis of dendritic intersections revealed a decrease following stress and an increase following SS ($p < 0.001$; Fig. 4B). The stress-induced decrease in the number of intersections was restored to normal in ST + SS group of rats ($p < 0.001$; Fig. 4B).

Role of ICSS on stress-induced decrease in AChE activity and levels of biogenic amines in the hippocampus

AChE activity

One-way ANOVA revealed a significant effect of the groups on the AChE activity ($F_{3, 21} = 31.69$, $p < 0.001$). The AChE activity in ST group of rats was significantly decreased in the hippocampus ($p < 0.01$; Fig. 5A). Interestingly, stress induced decrease in AChE activity was completely reversed following SS experience ($p < 0.05$). In addition, AChE activity in SS experienced rats was higher ($p < 0.001$) compared to all other groups (Fig. 5A).

Levels of biogenic amines

There was a significant effect of the groups on the levels of NA ($F_{3, 22} = 35.22$, $p < 0.001$), DA ($F_{3, 25} = 31.69$, $p < 0.001$) and 5-HT ($F_{3, 25} = 67.09$, $p < 0.001$). The concentrations of NA, DA, and 5-HT were decreased in the (ST) rats ($p < 0.001$) compared to controls. SS group of rats showed a significant increase in NA ($p < 0.001$), DA ($p < 0.001$) and 5-HT ($p < 0.001$) levels compared to ST and the control groups (Fig. 5B–D). The decreased levels of amines seen in ST group were restored to normal levels in ST + SS animals ($p < 0.001$) (Fig. 5).

Role of ICSS on stress-induced cognitive deficits

In the T-maze rewarded alternation task, ST group of rats reached the criterion in 6–7 sessions, whereas the NC and ST + SS groups took 4–5 sessions. Strikingly, the SS group required only 2–3 sessions to reach the criterion (Fig. 6A). The delay in the acquisition of spatial task by the ST group

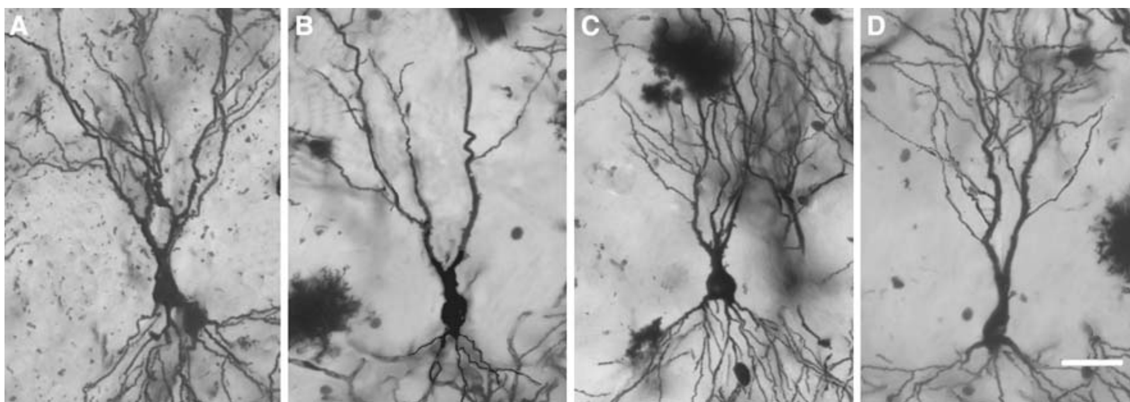

Fig. 2 Representative photomicrographs of short-shaft CA3 pyramidal neurons of the hippocampus from normal control (**A**), stress (**B**), self-stimulation (**C**), and stressed animals exposed to SS (**D**). *Scale bar* in *D* = 100 µm and applies to all the neurons

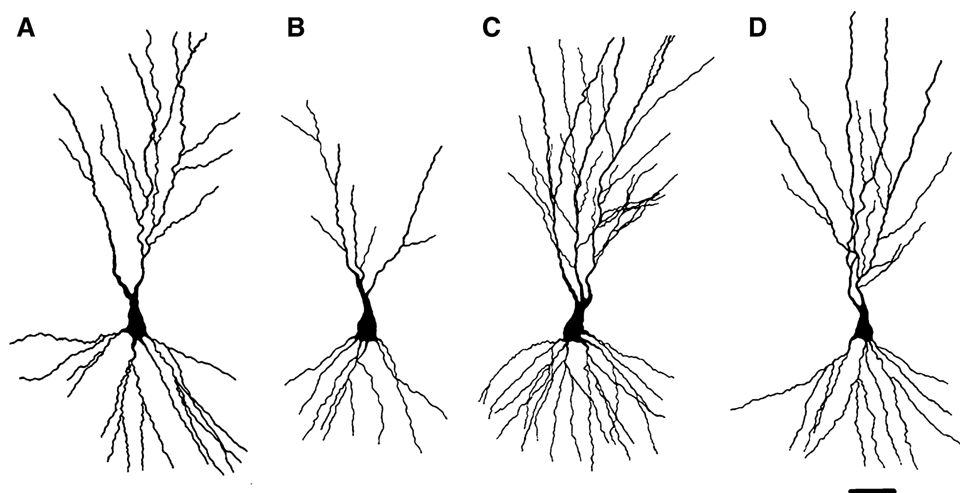

Fig. 3 Representative camera lucida tracings of short-shaft CA3 pyramidal neurons of the hippocampus from normal control (**A**), stress (**B**), self-stimulation (**C**), and stressed animals exposed to SS (**D**) groups of rats. Note a decrease in the number of dendrites in stress (**B**) compared to control (**A**) and an increase of dendritic arbors in **C**. Interestingly, self-stimulation experience reversed the stress-induced reduction in the number of dendrites (**D**). *Scale bar* in *D* = 50 μm and applies to all the neurons

was statistically significant ($F_{3,\ 25} = 4.03$; $p < 0.001$) compared to other groups of rats and was restored in the ST + SS group ($p < 0.001$). ANOVA on the number of correct choices in each session revealed a difference between groups ($F_{3,\ 25} = 21.76$; $p < 0.001$) and sessions ($F_{7,\ 175} = 12.68$; $p < 0.001$) (Fig. 6B). In addition, stressed animals showed an increased latency (time taken to reach the goal area from the start box) when compared to other groups. The latency data also revealed a significant difference between groups ($F_{3,\ 25} = 13.79$; $p < 0.001$) as well as sessions ($F_{7,175} = 11.58$; $p < 0.001$) (Fig. 6C). SS after stress restored both the number of correct choices/session (Fig. 6B) and the time taken to reach the goal area (Fig. 6C).

Discussion

The main finding in the current study is that stress impairs learning in a T-maze task and is accompanied by dendritic atrophy and neurochemical changes in the hippocampus. Further, the stress-induced cognitive deficits were reversed by ICSS accompanied by the reversal of CA3 dendritic atrophy and restoration of levels of biogenic amines and AChE activity in the hippocampus.

Reversal of stress-induced CA3 dendritic atrophy by self-stimulation

The morphological data in the current study suggest that chronic stress induces a significant dendritic atrophy while self-stimulation experience from SN-VTA for 10 days increases dendritic arborization of hippocampal CA3 neurons. Interestingly, when stressed rats were subjected to SS experience, they show a reversal of dendritic atrophy.

The observed stress-induced dendritic atrophy is in agreement with previous studies [13, 16, 41]. We also found that SS can produce dendritic hypertrophy, which is in line with the previous findings from our laboratory. It was shown earlier that self-stimulation rewarding experience for 10 days could result in an increase in the dendritic branching and dendritic length in CA3 hippocampal pyramidal neurons [20–23, 29].

The effect of sustained elevation of circulating GCs on the morphology and survival of neurons in the hippocampus has been evaluated [42, 43]. These studies have shown that GCs have deleterious effects on pyramidal neurons in the CA3 region. Uno et al. [44] have showed pronounced neuronal degeneration in the CA3 region of the hippocampus in monkeys, when subjected to prolonged social stress. Watanabe et al. [41] reported a significant decrease in the apical branching points and dendritic length in CA3 neurons of the hippocampus of animals subjected to restraint stress. An earlier study from our laboratory has shown that chronic restraint stress of 6 h a day for 21 days enhanced the number of dendritic spines and excrescences in CA3 pyramidal neurons of the hippocampus [16]. Electron microscopy study has shown that chronic stress alters the synaptic terminal structure in the hippocampus [45]. Our current findings together with all these reports reiterate that stress is associated with marked morphological changes in the hippocampus.

On the contrary, SS rewarding experience increases the numerical density of dendritic spines in both apical and basal dendrites in CA3 hippocampal pyramidal neurons [24, 25]. An increase in the numerical density of spines and thorny excrescences in apical dendrites of CA3 neurons of the hippocampus [23] was also reported. Furthermore, SS increased the concentration of noradrenaline, dopamine, glutamate, and AChE activity in both the hippocampus and motor cortex [26]. Long-lasting structural changes in the

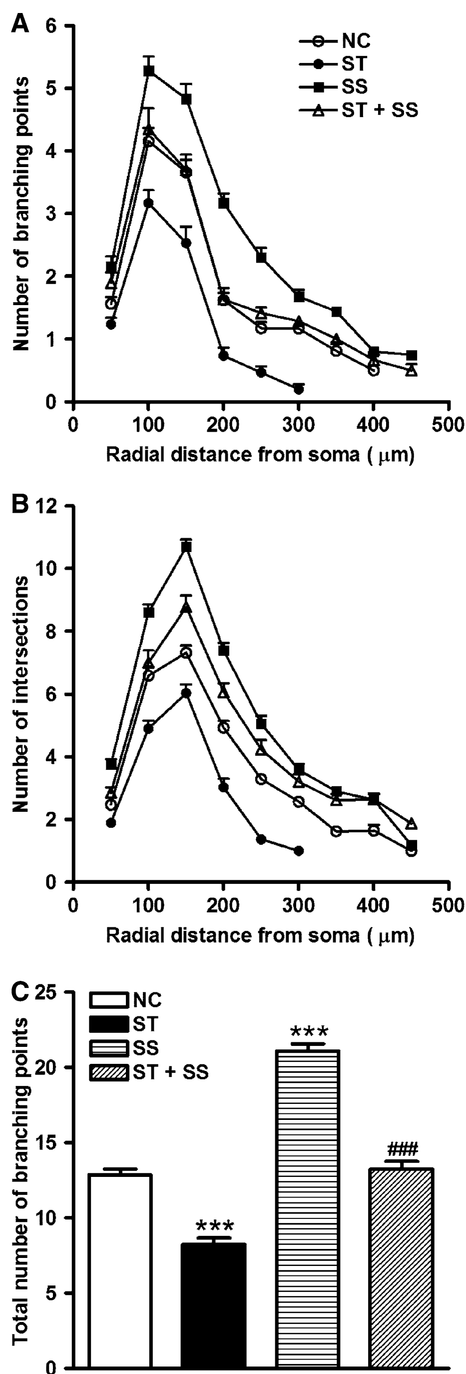

Fig. 4 Reversal of stress-induced CA3 dendritic atrophy by SS. Segmental distribution of apical dendritic branching points (**A**), intersections (**B**) and total number of branching points (**C**) of CA3 pyramidal neurons of the hippocampus from normal control (*NC*), stress (*ST*), self-stimulation (*SS*) and stressed animals exposed to SS (ST + SS) groups of rats. Note a decrease in the number of branching points and intersections in stress compared to control. Self-stimulation experience reversed the stress-induced dendritic atrophy. $^{***}p < 0.001$ vs. NC; $^{###}p < 0.001$ vs. ST, One-way ANOVA followed by Tukey's post hoc test. Values expressed as mean ± SEM

hippocampal and motor cortical neurons was observed following self-stimulation experience in adult rats [22]. The above studies clearly suggest that SS experience induces sustainable structural changes in hippocampal neurons. Accordingly, self-stimulation induced robust plasticity might be responsible for reversal of stress-induced dendritic atrophy of CA3 pyramidal neurons of the hippocampus.

Contrary to the current findings, an earlier study demonstrates recovery of dendritic morphology after 10 or 20 days of cessation of stress [46]. It is not entirely clear what accounts for these differences. We used Wistar rats as opposed to Sprague Dawley rats used in that study and we observed a marked dendritic atrophy in the stressed animals [13] compared to the earlier study [46]. It is possible that these factors such as the strain of the animal and the extent of dendritic atrophy contribute to the differences. Further, it may be argued that spontaneous recovery during the periods of SS to stressed rats resulted in the reversal of dendritic atrophy. As a control for this, the stress group of rats in the current study were allowed a stress-free recovery period of 17 days and we observed that SS and not spontaneous recovery, if any, contributed for the reversal of stress-induced dendritic atrophy (Figs. 2, 3, 4). Moreover, clinically it may not always be possible to provide a stress-free period or permit spontaneous recovery to occur without therapeutic intervention. Thus, it is imperative that strategies to counter the effects of stress be developed. In this context, our study demonstrates that SS following stress produces recovery of stress-induced deficits and indicates that activation of specific neurotransmitter systems could be beneficial in the amelioration of stress-induced deficits.

Restoration of stress-induced decrease in the levels of neurotransmitters in the hippocampus by self-stimulation

In the present study AChE activity was decreased in stressed rats, while SS group of rats had enhanced AChE activity than controls, confirming our previous reports [26]. However, when stressed rats were subjected to SS experience, the AChE activity was restored to the control level, indicating that SS experience restores stress-induced decrease in the hippocampal AChE activity.

The hippocampus has a robust cholinergic innervation from the basal forebrain, medial septum and the diagonal band of Broca [47]. These cholinergic inputs may be important in regulating the excitability of the hippocampal neurons [48]. Earlier studies have suggested the involvement

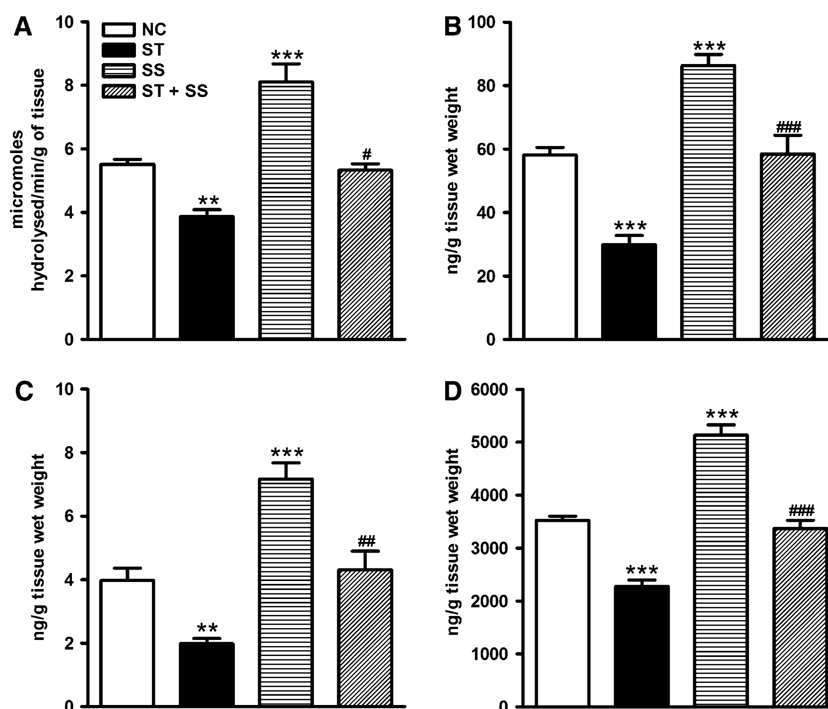

Fig. 5 Restoration of stress-induced decrease in AChE activity (**A**) and levels of norepinephrine (**B**), dopamine (**C**) and 5-hydroxytryptamine (**D**) in the hippocampus by SS. Groups are as described in Fig. 4. Note a decrease in AChE activity, NA, DA, and 5-HT levels in stressed animals, an increase in SS group and restoration in the ST + SS group. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. ST, One-way ANOVA followed by Tukey's post hoc test. Values expressed as mean ± SEM

of cholinergic system in learning and memory [49]. Further, ACh release was increased in the hippocampus during acquisition of the operant behavior or exposure to novel environment [50, 51]. Our recent study has demonstrated the role of the cholinergic drug, oxotremorine in the reversal of stress-induced deficits in a partially baited radial arm maze task [2].

Apart from its involvement in learning and memory, septo-hippocampal cholinergic pathway is very sensitive to stress. A reduction in the brain ACh levels following stress was observed [52]. Subsequent studies have shown that chronic stress decreases the activity of the septo-hippocampal cholinergic system. For example, GC administration for 2 months caused degeneration of septo-hippocampal cholinergic neurons prior to the loss of hippocampal neurons [53]. Thus, our data along with other reports suggest that chronic stress disrupts the cholinergic transmission, which could affect the spatial cognition as observed in some of the neurological disorders like Alzheimer's disease and dementia.

The hippocampus apart from being innervated by cholinergic inputs also receives direct dopaminergic projections from VTA [54]. Dopamine receptor agonists have been shown to increase ACh release in the cortex and further stimulation of D1 receptors is known to activate cortically projecting cholinergic neurons [55]. Moreover, the dopamine neurons from substantia nigra pars compacta when stimulated are known to release AChE in a neurotransmitter-like fashion along with dopamine [56]. AChE is also known to induce long-term potentiation (LTP) in pyramidal cells of the hippocampus, by a mechanism dependent on metabotropic glutamate receptors [55, 57].

Acetylcholinesterase, apart from its catalytic (cholinergic) function, is also known to exhibit non-cholinergic trophic effects. It is shown to influence neuronal cell differentiation, adhesion, neuritogenesis and synaptogenesis [56]. It was suggested that enhanced AChE activity following chronic l-deprenyl administration could be responsible for increased dendritic arbors in CA3 pyramidal neurons [58] and prefrontal cortical neurons [59] in primates.

The restoration of AChE activity in the hippocampus of stressed rats by self-stimulation from SN-VTA can be mainly explained in terms of dopaminergic and cholinergic activation. This is substantiated by our previous studies, where it was shown that self-stimulation experience from SN-VTA, increases the levels of noradrenaline, dopamine, glutamate, and AChE activity in the hippocampus [26]. Thus, above studies indicate that direct stimulation of SN-VTA by ICSS may be enhancing DA release and in turn facilitating AChE release.

In addition to AChE, the levels of NA, DA, and 5-HT in the hippocampus were also brought to normal values in ST + SS group of rats. Stress-induced decrease in hippocampal amine levels is in agreement with our previous reports [3, 18]. The role of neurotransmitters in the regulation of the synaptic density also needs to be considered. The depletion of noradrenaline, serotonin, and acetylcholine is found to decrease the synaptic numbers in rats [60, 61]. Previous studies from our laboratory have shown a

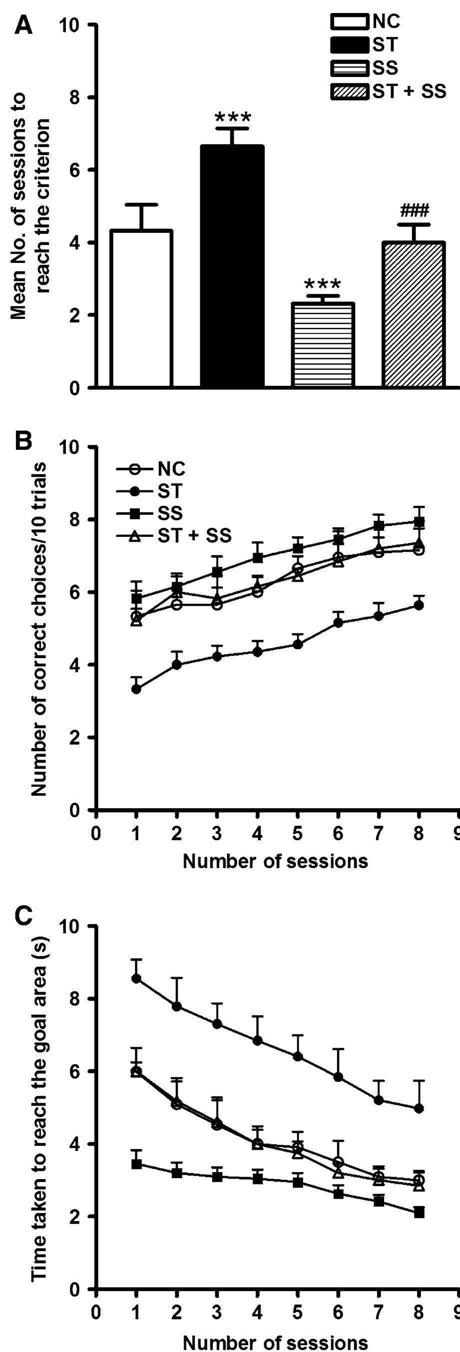

Fig. 6 Effect of SS rewarding experience on the effects of stress on the mean number of sessions to reach the criterion (**A**), number of correct choices per session (**B**), and the time taken to reach the goal area (**C**) in the rewarded alternation T-maze task. Stressed (*ST*) group of rats took more number of sessions ($p < 0.001$) to reach the criterion compared to the other groups (**A**). The number of correct choices was significantly decreased in ST group compared to control and was reversed in the ST + SS group (**B**). The mean latency to reach the goal area was higher in ST group of rats in all the sessions and was reversed in the ST + SS group (**C**). $^{***}p < 0.001$ vs. NC; $^{\#\#\#}p < 0.001$ vs. ST, One-way ANOVA followed by Newman Keul's post hoc test. Values expressed as mean ± SEM

significant increase in the levels of noradrenaline, dopamine, glutamate and AChE activity in the hippocampus following SS experience [26]. This was related to increased number of synapses in the hippocampus [24]. Thus, altered concentrations of these neurotransmitters may play a role in regulating the synaptic activity and thus influencing the neural activity in the hippocampus, which may have an effect on the behavior. We have recently demonstrated the restoration of dopamine levels to normal in stressed rats by bromocriptine treatment [3]. All these studies suggest the involvement of multiple neurotransmitter systems in the reversal of stress-induced morphological and behavioral deficits.

Amelioration of stress-induced spatial learning deficits by self-stimulation

The significant findings of the present study are that the SS rewarding experience from SN-VTA reversed the chronic stress induced behavioral impairments in T-maze rewarded alternation task. Chronic stress is known to impair the performance of rats in various learning tasks; T-maze, radial arm maze, Y-maze, and Morris water maze [1–3, 12]. In contrast, SS rewarding experience facilitates the acquisition of operant, spatial [27] and shuttle-box [62] tasks and also ameliorates fornix lesion induced spatial learning deficits [28]. Stress induced behavioral impairments might be due to regressive structural changes in CA3 hippocampal pyramidal neurons [12, 13]. It is also shown that lesions of the CA3 region of the hippocampus impair spatial memory [63]. In addition, single as well as chronic corticosterone injections inhibit LTP in the hippocampus [64]. Furthermore, continuous blockade of brain GC receptors is shown to facilitate spatial learning and memory in rats [12].

Mesohippocampal dopaminergic system may also be involved in the modulation of spatial learning and memory processes in rats [65]. Dopamine agonists improved the cognitive performance significantly in memory-impaired aged rats in the Morris water maze task by modulating the hippocampal ACh release [66]. Since there is a direct dopaminergic innervation to the hippocampus from ventral tegmental area, dopamine can alter the ACh release locally. It has also been reported that during acquisition of the operant behavior or exposure to novel environment, the ACh release was increased in the hippocampus [50]. The facilitation of spatial learning seen in ST + SS group of rats might have occurred due to dopamine induced enhancement in ACh release, since our previous studies have shown that the levels of dopamine and AChE activity were increased in the hippocampus following self-stimulation experience [26].

Thus, changes in multiple neurotransmitters and neuronal morphology could underlie the reversal of stress-induced deficits by ICSS.

Functional and clinical implications

It is well documented that enriched environment and behavioral training can lead to improved learning and memory, accompanied by morphological changes in the hippocampal neurons [67]. It has been hypothesized that such experience-dependent cognitive improvement results from these structural modifications. SS, a rewarding behavioral experience, which brings about changes in the hippocampal neurons, may ameliorate the spatial learning impairments in the stressed rats by accelerating the recovery process. Our previous study has demonstrated that stress-induced hippocampal dendritic atrophy could be reversed after 45 days of rehabilitation [13], whereas, the behavioral recovery observed in the present study could be achieved within 10 days following SS experience. Stress-induced dendritic atrophy, biochemical and behavioral deficits may manifest as different types of neuropsychiatric disorders and any means to reverse the stress-induced deficits is of clinical importance. Although directly, ICSS may not be applied to the clinical setting, understanding the neurochemical pathways involved in the reversal of stress-induced deficits would lead to the development of pharmacological targets. In support of this notion, we recently reported that a cholinergic agonist (oxotremorine) and a dopaminergic agonist (bromocriptine) reverse the stress-induced deficits [2, 3]. Understanding the mechanism of reversal of stress-induced deficits by ICSS would lead to identification of many such targets for pharmacotherapy of stress-induced disorders. Further, transcranial magnetic stimulation (TMS) or vagus nerve stimulation is emerging as an important therapeutic tool to treat depression and other stress disorders [68]. In relevance to this, our study that electrical stimulation of specific brain regions produce favorable effects and demonstrate that stimulation of specific brain regions could be a treatment option for stress-induced disorders and opens an avenue to treat such diseases.

In conclusion, the current findings that stress induced deficits are reversed by SS experience, which may be due to an enhanced neuronal plasticity induced by SS at morphological, biochemical and behavioral levels point to the brain's inherent property of plasticity and its therapeutic potential.

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References

1. Sunanda, Shankaranarayana Rao BS, Raju TR (2000) Chronic restraint stress impairs acquisition and retention of spatial memory task in rats. Curr Sci 79:1581–1584
2. Srikumar BN, Raju TR, Shankaranarayana Rao BS (2006) The involvement of cholinergic and noradrenergic systems in behavioral recovery following oxotremorine treatment to chronically stressed rats. Neuroscience 143:679–688
3. Srikumar BN, Raju TR, Shankaranarayana Rao BS (2007) Contrasting effects of bromocriptine on learning of a partially baited radial arm maze task in the presence and absence of restraint stress. Psychopharmacology (Berl) 193:363–374
4. Conrad CD, Galea LA, Kuroda Y et al (1996) Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. Behav Neurosci 110:1321–1334
5. McLay RN, Freeman SM, Zadina JE (1998) Chronic corticosterone impairs memory performance in the Barnes maze. Physiol Behav 63:933–937
6. Bodnoff SR, Humphreys AG, Lehman JC et al (1995) Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. J Neurosci 15:61–69
7. Herbert J, Goodyer IM, Grossman AB et al (2006) Do corticosteroids damage the brain? J Neuroendocrinol 18:393–411
8. de Quervain DJ, Roozendaal B, McGaugh JL (1998) Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature 394:787–790
9. Krugers HJ, Goltstein PM, van der LS et al (2006) Blockade of glucocorticoid receptors rapidly restores hippocampal CA1 synaptic plasticity after exposure to chronic stress. Eur J Neurosci 23:3051–3055
10. Luine VN, Spencer RL, McEwen BS (1993) Effects of chronic corticosterone ingestion on spatial memory performance and hippocampal serotonergic function. Brain Res 616:65–70
11. Magarinos AM, McEwen BS (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. Neuroscience 69:89–98
12. McEwen BS (1999) Stress and hippocampal plasticity. Annu Rev Neurosci 22:105–122
13. Shankaranarayana Rao BS, Madhavi R, Sunanda et al (2001) Complete reversal of dendritic atrophy in CA3 neurons of the hippocampus by rehabilitation in restraint stressed rats. Curr Sci 80:653–659
14. Sunanda, Meti BL, Raju TR (1997) Entorhinal cortex lesioning protects hippocampal CA3 neurons from stress-induced damage. Brain Res 770:302–306
15. Vyas A, Mitra R, Shankaranarayana Rao BS et al (2002) Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neurosci 22:6810–6818
16. Sunanda, Rao MS, Raju TR (1995) Effect of chronic restraint stress on dendritic spines and excrescences of hippocampal CA3 pyramidal neurons—a quantitative study. Brain Res 694:312–317
17. Pawlak R, Shankaranarayana Rao BS, Melchor JP et al (2005) Tissue plasminogen activator and plasminogen mediate stress-induced decline of neuronal and cognitive functions in the mouse hippocampus. Proc Natl Acad Sci USA 102:18201–18206
18. Sunanda, Shankaranarayana Rao BS, Raju TR (2000) Restraint stress-induced alterations in the levels of biogenic amines, amino acids, and AChE activity in the hippocampus. Neurochem Res 25:1547–1552
19. Olds J (1962) Hypothalamic substrates of reward. Physiol Rev 42:554–604

20. Shankaranarayana Rao BS, Desiraju T, Raju TR (1993) Neuronal plasticity induced by self-stimulation rewarding experience in rats—a study on alteration in dendritic branching in pyramidal neurons of hippocampus and motor cortex. Brain Res 627:216–224
21. Shankaranarayana Rao BS, Desiraju T, Meti BL et al (1994) Plasticity of hippocampal and motor cortical pyramidal neurons induced by self-stimulation experience. Indian J Physiol Pharmacol 38:23–28
22. Shankaranarayana Rao BS, Raju TR, Meti BL (1998) Long-lasting structural changes in CA3 hippocampal and layer V motor cortical pyramidal neurons associated with self-stimulation rewarding experience: a quantitative Golgi study. Brain Res Bull 47:95–101
23. Shankaranarayana Rao BS, Raju TR, Meti BL (1998) Alterations in the density of excrescences in CA3 neurons of hippocampus in rats subjected to self-stimulation experience. Brain Res 804:320–324
24. Shankaranarayana Rao BS, Raju TR, Meti BL (1999) Increased numerical density of synapses in CA3 region of hippocampus and molecular layer of motor cortex after self-stimulation rewarding experience. Neuroscience 91:799–803
25. Shankaranarayana Rao BS, Raju TR, Meti BL (1999) Self-stimulation rewarding experience induced alterations in dendritic spine density in CA3 hippocampal and layer V motor cortical pyramidal neurons. Neuroscience 89:1067–1077
26. Shankaranarayana Rao BS, Raju TR, Meti BL (1998) Self-stimulation of lateral hypothalamus and ventral tegmentum increases the levels of noradrenaline, dopamine, glutamate, and AChE activity, but not 5-hydroxytryptamine and GABA levels in hippocampus and motor cortex. Neurochem Res 23:1053–1059
27. Yoganarasimha D, Shankaranarayana Rao BS, Raju TR et al (1998) Facilitation of acquisition and performance of operant and spatial learning tasks in self-stimulation experienced rats. Behav Neurosci 112:725–729
28. Yoganarasimha D, Meti BL (1999) Amelioration of fornix lesion induced learning deficits by self-stimulation rewarding experience. Brain Res 845:246–251
29. Shankaranarayana Rao BS, Raju TR (2001) Intracranial self-stimulation: an animal model to study drug addiction, depression and neuronal plasticity—a review. Proc Indian Natl Sci Acad (Biol Sci) B67:155–188
30. Paxinos G, Watson C (1986) The Rat Brain in Stereotaxic Coordinates. Academic Press, Sydney
31. Ramkumar K, Raju TR, Shankaranarayana Rao BS (2004) Intracranial self-stimulation. In: Raju TR, Kutty BM, Sathyaprabha TN et al (eds) Brain and Behavior. NIMHANS, Bangalore, pp 121–126
32. Shankaranarayana Rao BS, Raju TR (2004) The Golgi techniques for staining neurons. In: Raju TR, Kutty BM, Sathyaprabha TN et al (eds) Brain and Behavior. NIMHANS, Bangalore, pp 108–111
33. Gundappa G, Desiraju T (1988) Deviations in brain development of F2 generation on caloric undernutrition and scope of their prevention by rehabilitation: alterations in dendritic spine production and pruning of pyramidal neurons of lower laminae of motor cortex and visual cortex. Brain Res 456:205–223
34. Fitch JM, Juraska JM, Washington LW (1989) The dendritic morphology of pyramidal neurons in the rat hippocampal CA3 area. I. Cell types. Brain Res 479:105–114
35. Ellman GL, Courtney KD, Andres V Jr et al (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95
36. Srikumar BN, Ramkumar K, Raju TR et al (2004) Assay of acetylcholinesterase activity in the brain. In: Raju TR, Kutty BM, Sathyaprabha TN et al (eds) Brain and behavior. NIMHANS, Bangalore, pp 142–144
37. Deepti N, Ramkumar K, Srikumar BN et al (2004) Estimation of neurotransmitters in the brain by chromatographic methods. In: Raju TR, Kutty BM, Sathyaprabha TN et al (eds) Brain and Behavior. NIMHANS, Bangalore, pp 134–141
38. Srikumar BN, Bindu B, Priya V et al (2004) Methods of assessment of learning and memory in rodents. In: Raju TR, Kutty BM, Sathyaprabha TN et al (eds) Brain and Behavior. NIMHANS, Bangalore, pp 145–151
39. Bures J, Buresova O, Huston JP (1983) Techniques and basic experiments for the study of brain and behaviour. Elsevier Science Publishers B.V., Amsterdam
40. Deacon RM, Rawlins JN (2006) T-maze alternation in the rodent. Nat Protoc 1:7–12
41. Watanabe Y, Gould E, McEwen BS (1992) Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res 588:341–345
42. Sapolsky RM, Krey LC, McEwen BS (1985) Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. J Neurosci 5:1222–1227
43. Woolley CS, Gould E, McEwen BS (1990) Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res 531:225–231
44. Uno H, Tarara R, Else JG et al (1989) Hippocampal damage associated with prolonged and fatal stress in primates. J Neurosci 9:1705–1711
45. Magarinos AM, Verdugo JM, McEwen BS (1997) Chronic stress alters synaptic terminal structure in hippocampus. Proc Natl Acad Sci USA 94:14002–14008
46. Conrad CD, LeDoux JE, Magarinos AM et al (1999) Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. Behav Neurosci 113:902–913
47. Amaral DG, Witter MP (1995) Hippocampal Formation. In: Paxinos G (ed) The Rat Nervous System. Academic Press, New York, NY, pp 443–493
48. Ibata Y, Desiraju T, Pappas GD (1971) Light and electron microscopic study of the projection of the medial septal nucleus to the hippocampus of the cat. Exp Neurol 33:103–122
49. Petrillo M, Ritter CA, Powers AS (1994) A role for acetylcholine in spatial memory in turtles. Physiol Behav 56:135–141
50. Orsetti M, Casamenti F, Pepeu G (1996) Enhanced acetylcholine release in the hippocampus and cortex during acquisition of an operant behavior. Brain Res 724:89–96
51. Rasmusson D, Szerb JC (1975) Cortical acetylcholine release during operant behaviour in rabbits. Life Sci 16:683–690
52. Anisman H (1975) Time-dependent variations in aversively motivated behaviors: nonassociative effects of cholinergic and catecholaminergic activity. Psychol Rev 82:359–385
53. Tizabi Y, Gilad VH, Gilad GM (1989) Effects of chronic stressors or corticosterone treatment on the septohippocampal cholinergic system of the rat. Neurosci Lett 105:177–182
54. Verney C, Baulac M, Berger B et al (1985) Morphological evidence for a dopaminergic terminal field in the hippocampal formation of young and adult rat. Neuroscience 14:1039–1052
55. Day J, Fibiger HC (1993) Dopaminergic regulation of cortical acetylcholine release: effects of dopamine receptor agonists. Neuroscience 54:643–648
56. Mattson MP (1988) Neurotransmitters in the regulation of neuronal cytoarchitecture. Brain Res 472:179–212
57. Appleyard ME (1995) Acetylcholinesterase induces long-term potentiation in CA1 pyramidal cells by a mechanism dependent on metabotropic glutamate receptors. Neurosci Lett 190:25–28
58. Lakshmana MK, Shankaranarayana Rao BS, Dhingra NK et al (1998) Chronic (-) deprenyl administration increases dendritic arborization in CA3 neurons of hippocampus and AChE activity in specific regions of the primate brain. Brain Res 796:38–44

59. Shankaranarayana Rao BS, Lakshmana MK, Meti BL et al (1999) Chronic (-) deprenyl administration alters dendritic morphology of layer III pyramidal neurons in the prefrontal cortex of adult Bonnett monkeys. Brain Res 821:218–223
60. Matsukawa M, Ogawa M, Nakadate K et al (1997) Serotonin and acetylcholine are crucial to maintain hippocampal synapses and memory acquisition in rats. Neurosci Lett 230:13–16
61. Mazer C, Muneyyirci J, Taheny K et al (1997) Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: a possible model of neurodevelopmental disorders with cognitive deficits. Brain Res 760:68–73
62. Segura-Torres P, Portell-Cortes I, Morgado-Bernal I (1991) Improvement of shuttle-box avoidance with post-training intracranial self-stimulation, in rats: a parametric study. Behav Brain Res 42:161–167
63. Handelmann GE, Olton DS (1981) Spatial memory following damage to hippocampal CA3 pyramidal cells with kainic acid: impairment and recovery with preoperative training. Brain Res 217:41–58
64. Pavlides C, Watanabe Y, McEwen BS (1993) Effects of glucocorticoids on hippocampal long-term potentiation. Hippocampus 3:183–192
65. Gasbarri A, Sulli A, Innocenzi R et al (1996) Spatial memory impairment induced by lesion of the mesohippocampal dopaminergic system in the rat. Neuroscience 74:1037–1044
66. Hersi AI, Rowe W, Gaudreau P et al (1995) Dopamine D1 receptor ligands modulate cognitive performance and hippocampal acetylcholine release in memory-impaired aged rats. Neuroscience 69:1067–1074
67. Rosenzweig MR, Bennett EL (1996) Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res 78:57–65
68. George MS, Nahas Z, Borckardt JJ et al (2007) Brain stimulation for the treatment of psychiatric disorders. Curr Opin Psychiatry 20:250–254