

PLASTICITY OF HIPPOCAMPAL AND MOTOR CORTICAL PYRAMIDAL NEURONS INDUCED BY SELF-STIMULATION EXPERIENCE

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The self-stimulation (SS) induced neuronal plasticity was observed in CA3 hippocampal and layer V motor cortical pyramidal neurons. SS experience was allowed daily for a total of 1 hour for 10 days through four bipolar electrodes implanted bilaterally in lateral hypothalamus (LH) and substantia nigra-ventral tegmental area (SN-VTA) in adult male Wistar rats. Examination of pyramidal neurons stained by rapid Golgi technique was made in a total of 1600 neurons out of 80 rats consisting of 4 groups. The dendritic intersections were quantified upto 200 and 120 μm radial distances in apical and basal dendrites respectively. The CA3 hippocampal and layer V motor cortical pyramidal neurons of SS group revealed significant increase ($P < 0.001$, two-way ANOVA) in dendritic intersections in both apical and basal dendrites, compared to normal control (NC), sham control (SH) and experimenter-administered (EA) group of animals. These results demonstrate that SS experience promotes increase in dendritic length in hippocampal and motor cortical pyramidal neurons.

Key words : self-stimulation reward hippocampus motor cortex
pyramidal neurons dendritic intersections neuronal plasticity

INTRODUCTION

The property of neuronal plasticity has been well established. Several studies have revealed structural and functional alterations in neurons of the brain under different conditions of nurture such as learning and experience (1,2,3,4,5). Previous studies from our laboratory have demonstrated the neuronal plasticity in limbic and neocortical neurons following the operant learning condition in preweaning age (6), nutritional restriction during brain growth spurt (7, 8) and electrical self-stimulation reward (9).

It has been reported that repetitive stimulations of neuronal pathways could induce changes in the excitability of the dendrites (10). Such a phenomenon is also observed in long-term potentiation (LTP) (11,12,13) or long-term depression (LTD) (14), as well as in other types of modulation of synaptic functions

(3,15). Activity induced gene expression in neurons has been recognised in recent years (15,16). SS experience which involves frequent engagements of rewarding circuits may result in significant changes in dendritic arborization of pyramidal neurons of limbic and neocortical areas, as it involves both learning and motor activity.

Accordingly the present work was aimed at assessing self-stimulation (SS) experience induced alterations in dendritic intersections in CA3 pyramidal neurons of hippocampus and layer V pyramidal neurons of motor cortex.

METHODS

Adult male Wistar rats (n=80, 130-140 days old) weighing in the range of 270-300 g were the subjects for the present study. They were divided into four groups,

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each containing 20 rats; i) electrical self-stimulation experienced (SS) group, ii) experimenter-administered electrical stimulation (EA) group, iii) sham control (SH) group and, iv) normal control (NC) group. In each animal of the SS, EA and SH group, bipolar stainless steel electrodes (30 s.g.w) were implanted bilaterally in the lateral hypothalamus (LH) and substantia nigra - ventral tegmental area (SN-VTA) stereotaxically. The coordinates were chosen according to Paxinos and Watson (17). After three days of surgical recovery SS group of animals were trained in a Skinner box for pedal pressing to obtain self-stimulation (9). Each pedal press delivered a stimulus train of square waves of 65 Hz for 0.25 s. The current intensity was adjusted for each electrode in the rat to elicit a maximum possible pedal press rate. This was in the range of 25-75 μ A for different electrodes and animals. Animals of SS group were allowed 15 min of daily self-stimulation for each site, over a period of 10 days. Only those rats which could self-stimulate above 1000 per 15 minutes in all 4 sites, were retained in SS group. The animals of EA group were not allowed to undergo self-stimulation experience, however the electrical stimulations were administered by experimenter through each electrode daily for 10 days. The stimulus parameters were similar to those of SS group. During these imposed stimulations the rats were very active, moving about and exploring in the Skinner box. The EA group consisted of 5 sub groups of four animals each. The experimenter administered electrical stimulations at 1000, 1200, 1500, 1800 and 2000 per site for 15 minutes duration in different sub groups. This was carried out because in SS group of animals pedal press responses ranged from 1000 to 2200 per 15 minutes per site. The EA group is studied to provide the electrical stimulation effects of LH and SN-VTA. The animals of the SH group had the electrodes implanted but were neither allowed self-stimulation experience nor subjected to experimenter-administered electrical stimulations. The NC group of animals had no electrodes implanted.

After 10 days, all groups of animals were sacrificed and the hippocampus and motor cortex of both hemispheres were processed for rapid Golgi method (6, 7). The CA3 pyramidal neurons of hippocampus and layer V pyramidal neurons of motor cortex were examined in 120 μ m thick sections. The slides were coded to overcome observer bias. Well impregnated

neurons having all dendrites intact without truncations were chosen. Neurons were sampled from both hemispheres of the brain since both sides had the electrodes implanted. Camera lucida drawings of neurons were made at 600 X magnification using a binocular Olympus microscope. At the same magnification, concentric rings were drawn on a tracing paper at 40 μ m equivalent intervals with the aid of stage micrometer. The number of dendritic intersections were counted in successive radial segmental areas of 40 μ m distances, taking into consideration the center of the soma as a reference point. Dendritic intersection is the point where the dendrite cuts the centric ring. In both types of neurons, apical and basal dendrites were studied upto 200 and 120 μ m distances respectively. For each rat, 10 neurons were drawn separately from both regions namely, hippocampus and motor cortex. Since 80 rats were involved in this study, a grand total of 1600 neurons (800 hippocampal and 800 motor cortical neurons) were analysed. Histology with Nissl staining was used to verify placement of electrodes in specific regions (LH, SN-VTA) of the brain.

Statistical Analysis

Statistical analysis of data from each segmental area was carried out by two-way analysis of variance (18) (ANOVA, treatments x subjects, data of neurons averaged within each subject). In segments where the F-test showed significant effect of treatments, intergroup differences were assessed by least significant difference (LSD) analysis.

RESULTS

Alterations of dendritic intersections in CA3 pyramidal neurons of hippocampus :

Apical dendrites: The dendritic intersections in apical dendrites increased progressively upto a radial distance of 160 μ m from the soma and further increase was not observed beyond this point in NC, SH and EA group of animals. However, further increase in dendritic intersections was observed in SS group of animals upto 200 μ m distance. The values of NC, SH and EA control groups were not significantly different amongst them in any of the radial lines. Only the values of SS group of animals were significantly higher ($P < 0.001$) than those of the other 3 groups at the distances of 80, 120, 160 and 200 μ m (Table I, Fig. 1).

TABLE I : Dendritic intersections of hippocampal CA3 pyramidal neurons.

Group	Apical dendrites					Basal dendrites		
	40 μ	80 μ	120 μ	160 μ	200 μ	40 μ	80 μ	120 μ
NC	0.68 \pm 0.42	3.24 \pm 1.26	7.80 \pm 1.84	10.61 \pm 1.33	10.45 \pm 1.53	8.36 \pm 1.50	13.98 \pm 1.27	14.15 \pm 1.49
SH	0.92 \pm 0.48	3.57 \pm 1.18	7.46 \pm 1.72	9.62 \pm 2.40	9.00 \pm 2.59	8.47 \pm 2.07	13.10 \pm 1.89	12.82 \pm 2.68
EA	1.12 \pm 0.63	3.33 \pm 1.67	6.95 \pm 1.93	10.16 \pm 2.21	10.50 \pm 2.49	9.33 \pm 1.38	15.19 \pm 2.22	15.52 \pm 2.55
SS (%NC)	1.18 \pm 0.70 (173.52)	6.08 \pm 1.68 (187.65)	12.12 \pm 1.65 (155.38)	16.84 \pm 2.84 (158.71)	17.44 \pm 3.13 (166.88)	9.24 \pm 1.58 (110.52)	17.56 \pm 3.16 (125.60)	20.10 \pm 3.49 (142.04)
ANOVA								
F (d.f. 3,57)	2.88	17.79	32.35	48.34	48.53	1.82	17.81	30.58
P (2)	NS	0.001	0.001	0.001	0.001	NS	0.001	0.001

Dendritic intersections in successive 40 μ m radial lines along apical and basal dendrites of hippocampal CA3 pyramidal neurons of normal control (NC), sham control (SH), experimenter - administered (EA) and self-stimulation (SS) groups of rats. Each value represents the mean \pm SD of data, obtained from 200 neurons per group. Significance comparison : NC, SH and EA groups versus SS group of animals. NS-Not significant.

CA₃ PYRAMIDAL NEURONS OF HIPPOCAMPUS

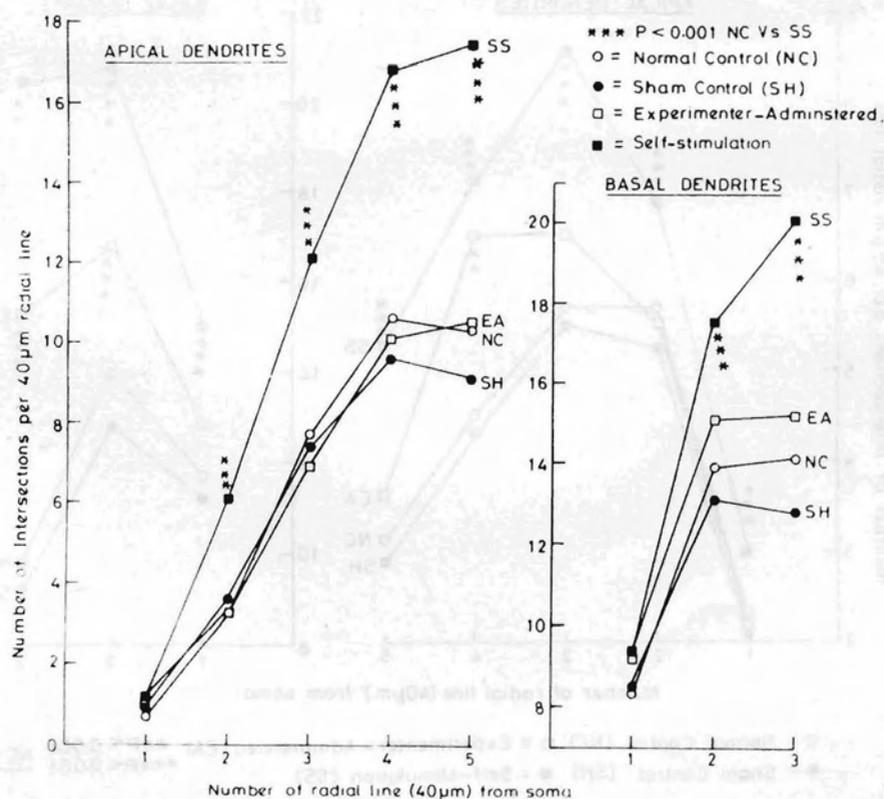


Fig. 1 : Dendritic intersections of CA3 pyramidal neurons in hippocampus of the four groups of rats represented in 40 μ m successive radial lines. Each point in the graph is the mean data obtained from 200 neurons in a group of 20 rats.

Basal dendrites: The number of dendritic intersections increased in basal dendrites upto 80 μm radial distance from the soma in NC, SH and EA groups. No further increase in number was observed between 80 and 120 μm distances. On the other hand the number continued to increase even upto 120 μm distance from soma in SS group. The number of intersections observed in the dendrites of SS group of animals at both 80 and 120 μm radial lines were significantly higher ($P < 0.001$) than those in other three groups (Table I, Fig. 1).

Alteration in dendritic intersections in layer V pyramidal neurons of motor cortex :

Apical dendrites : The number of intersections in apical dendrites reach its peak at 120 μm radial line in all four groups. Although the EA group showed a significant increase in the intersections at 120, 160 and 200 μm distances compared to NC and SH groups,

the number of intersections observed in SS group of animals was even higher ($P < 0.001$) than that of EA group at all the radial lines. The percentile values of EA and SS groups over NC group at 120, 160 and 200 μm radial distances are 15 and 49.73, 23.77 and 57.77, 14.46 and 68.23 respectively (Table II, Fig. 2).

Basal dendrites : The basal dendritic intersections increased upto 80 μm in all groups of animals. The basal dendrites also showed an increase in the number of intersections in EA group which was significantly higher ($P < 0.05$ at 40 μm and $P < 0.001$ at 80 and 120 μm) than NC and SH groups. As seen in apical dendrites, the number of intersections in basal dendrites of SS group of animals in all radial lines was significantly higher ($P < 0.001$) than that observed in EA animals. At 120 μm radial line EA and SS group values were higher by 55.01% and 108.20% respectively over NC group (Table II, Fig.2).

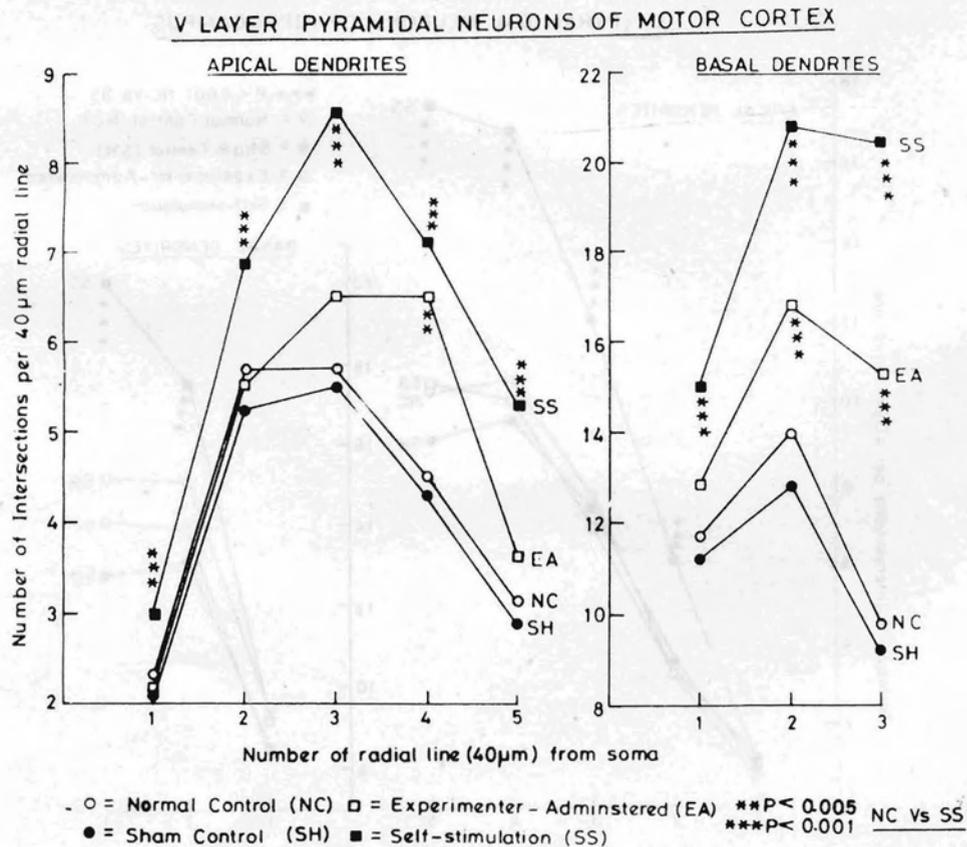


Fig. 2: Dendritic intersections of layer V pyramidal neurons in motor cortex of the four groups of rats represented in 40 μm successive radial lines. Each point on the graph is the mean data obtained from 200 neurons in a group of 20 rats. Comparison were made between NC versus EA and SS groups.

TABLE II : Dendritic intersections of layer V pyramidal neurons of motor cortex.

Group	Apical dendrites					Basal dendrites		
	40 μ	80 μ	120 μ	160 μ	200 μ	40 μ	80 μ	120 μ
NC	2.36 \pm 0.71	5.77 \pm 1.07	5.73 \pm 0.90	4.50 \pm 0.72	3.18 \pm 0.40	11.74 \pm 1.63	14.05 \pm 2.15	9.87 \pm 2.59
SH	2.16 \pm 0.56	5.24 \pm 0.97	5.56 \pm 0.92	4.32 \pm 0.76	2.89 \pm 0.72	11.02 \pm 1.47	12.83 \pm 2.13	9.28 \pm 2.00
EA	2.23 \pm 0.61	5.55 \pm 1.58	6.59 \pm 1.79	5.57 \pm 1.42	3.64 \pm 0.91	12.78 \pm 1.72*	16.87 \pm 2.59**	15.30 \pm 3.52**
SS (%NC)	3.02 \pm 0.52 (127.96)	6.89 \pm 1.18 (119.41)	8.58 \pm 2.34 (149.73)	7.10 \pm 1.86 (157.77)	5.35 \pm 1.70 (168.23)	15.06 \pm 1.57 (128.27)	20.81 \pm 3.70 (148.11)	20.55 \pm 4.61 (208.20)
ANOVA								
F (d.f. 3,57)	7.65	6.63	16.11	21.91	22.59	29.93	43.15	61.13
P (2)	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001

Dendritic intersections in successive 40 μ m radial lines along apical and basal dendrites of layer V pyramidal neurons of motor cortex of normal control (NC), sham control (SH), experimenter - administered (EA) and self-stimulation (SS) groups of rats. Each value represents the mean \pm SD of data, obtained from 200 neurons per group. Significance comparison : NC, SH and EA groups versus SH group; NC and SH groups versus EA group (*P<0.05, **P<0.001).

DISCUSSION

The dendritic plasticity is known to be influenced by experiential or nutritional or environmental conditions. Mahajan and Desiraju (9) reported alterations in dendritic branching and spine densities of CA3 pyramidal neurons in rats subjected to operant conditioning experience for 8 days during the brain growth spurt period. Moderate undernourishment in rats resulted in the reduction of dendritic spines of layer V pyramidal neurons of motor and visual cortices (7) and synaptic changes in developing cingulate cortex and hippocampus in undernourished rats (8). Environmental influence on dendritic elaborations has been reported by several investigators (4, 19-23). Dendrites of granule cells in dentate gyrus showed an increase in length and branching in rats which were reared in complex environment in contrast to those reared in isolation (20).

Recent studies from our laboratory by Shankaranarayana Rao et al. (Unpublished observations) showed that experience of self-stimulation (SS) of LH and SN-VTA for 10 days resulted in a significant increase of dendritic branching in CA3 pyramidal neurons of hippocampus and layer V pyramidal neurons of motor cortex. Another study by Bindu and Deisraju

(9) reported an increase in dendritic branching in two self-stimulated regions namely LH and SN-VTA and also in pyramidal neurons of hippocampus. The present study showed a significant increase of dendritic intersections in CA3 pyramidal neurons of hippocampus and layer V pyramidal neurons of motor cortex in rats undergone SS experience. Significant increase of intersections was observed in both apical and basal dendrites of the pyramidal neurons studied. These observations suggest that changes in neuronal morphology and synaptic organisation can also be brought about by a rewarding experience.

Increase in the number of intersections of dendrites at all radial lines suggest that SS experience promotes considerable elaborations (growth) of dendritic processes. The possibility that irritation due to electrode implantation alone causing such dendritic change has been ruled out since the SH group of animals which had electrodes implanted but not subjected to SS experience nor EA stimulations showed no dendritic change in the pyramidal neurons of hippocampus and motor cortex. The results also suggest that dendritic change is related to SS behavioural experience and not to electrical stimulation effects of LH and SN-VTA. However, an increase in dendritic intersections in few segments of motor cortical neurons was also observed

in EA group. This may be due to an increased exploratory locomotor activity observed in these rats during experimenter administered stimulations.

We have demonstrated that SS experience produces changes in neuronal morphology in hippocampus (area concerned with learning and retention) and motor cortex (area concerned with planning and execution of motor activity), the structures of brain concerned with successful performance of self-stimulation. It is possible that changes in the dendritic arborization brought about by SS experience is through gene expression. The work of Bailey et al (15) has

shown that learning experience in aplysia is associated with the down regulation of the gene responsible for synthesis of cell adhesion molecule leading to defasciculation of the neurons and formation of new connections. Apart from structural changes, this learning experience may also bring about changes in synaptic efficacy leading to LTP.

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