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SUBICULAR LESION INDUCED IMPAIRMENT IN OPERANT BEHAVIOUR AND ALTERED DENDRITIC MORPHOLOGY CF CA1, CA3 HIPPOCAMPAL NEURONS

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Abstract : The effects of bilateral electrolytic subicular lesions were examined on the operant behaviour for food reward on a continuous reinforcement schedule as well as the dendritic morphology of CA1 and CA3 hippocampal areas. The subjects were female Wistar rats 20 days of age and were divided into four groups. 1. Age matched control 2. Sham operated 3. Operant behaviour for food reward and 4. Subicular lesion. Animals were starved twentyfour hours prior to operant behaviour training sessions. Two trial sessions with continuous reinforcement (CRF) of 10 min duration/day were done during training sessions following which the rats were allowed CRF for ten minutes per day for ten days. On the eleventh day, the operant behaviour and sham operated animals were taken up for bilateral subicular lesion and sham surgery respectively. After seventy two hours of surgical recovery, operant behavioural testing was done as before for a further period of ten days. Later all the groups of animals were sacrificed and the hippocampi were processed for rapid Golgi staining technique. Our results suggest that subicular lesions do produce a significant reduction in operant learning behaviour for food reward. Further the Golgi studies revealed a reduction in dendritic branching points and intersections of apical and basal CA1, CA3 neurons in lesioned animals.

Key words : subicular lesion, hippocampal neurons, dendritic morphology, behaviour

INTRODUCTION

Subiculum is the major output structure of the hippocampus and hence its role in learning and memory needs to be elucidated. Subicular complex comprises of four components, pre-subiculum, para-subiculum, pro-subiculum, and subiculum (1). The subiculum is located adjacent to the hippocampal CA1 sub-field, and receives a massive excitatory projection from these hippocampal cells (2). It has diverse efferent system and its major projection areas include the nucleus accumbens, involved in instrumental learning (3), prefrontal cortex, thought to be involved in working memory (4), anterior thalamic nuclei and posterior cingulate cortex, which are said to play a role in discrimination avoidance learning (5). It is connected to perirhinal cortex which is involved in visual and tactual memory (6).

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Subicular lesions have shown to produce memory impairment in spatial memory tasks in animal models (7, 8). As there was no study available on operant food reward behaviour task as well as dendritic arborization of CA1 and CA3 pyramidal neurons after subicular lesions, the present study was aimed at evaluating these effects.

METHODS

Subjects

Wistar female rats of 20 days old were taken up for the study. There were randomly assigned to any of the four groups having 6 animals in each group. 1. Control (C) – age matched but not exposed to behavioural training. 2. Sham control group (S) – trained on continuous reinforcement schedule then taken up for surgery but not lesioned. 3. Trained control group (T) – trained on continuous reinforcement schedule. 4. Lesioned group (L) – trained on continuous reinforcement schedule and then lesioned. Throughout the experiment the animals were maintained on a restricted diet (except for controls) to maintain 80% of their initial weight.

Behavioural studies

The animals were provided operant behavioural taining for food reward in the operant conditioning chamber (Campden Inst. Ltd, USA). The chamber is provided with two levers which on being pressed activates the pellet dispenser. The pellet dispenser has a reservoir capacity of approximately 2000 pellets and can deliver a pellet with a latency of 0.12 sec. The force required to press the lever is 10 grams. The three groups of animals namely – sham, trained and lesioned were taken up for operant training. All the animals were starved 24 hours prior and then individually introduced into the chamber for a period of 10 min twice a day. During the trial session, a pellet was kept on each of the levers in order to motivate the animals towards the lever. On approaching the lever, a pellet was delivered manually by the experimenter. Trial sessions were continued till the animal correlated between pressing of the lever and obtaining the food reward. Once learning had occured test sessions of 10 minutes duration per day were given for 10 days. The pellets used for the study were prepared using 18% casein wheat diet (18 CW) (9). Each pellet weighed about 40 mgs.

Surgery

Rats were taken up for surgery on completion of behavioural studies. Ketamine 10 (150 mg/kg body weight) was used for anaesthesia and bilateral electrolytic lesion of subiculum was done using stereotactic instrument (INCO, Ambala). The predetermined coordinates were adopted from Pelligrino and Cushman atlas (10) [A.P. = 2.7 mm, ML = 4.5 mm, DV = 8 mm]. The sham group underwent the same procedure as the lesioned group but no current was passed through the electrodes.

Post operative behavioural studies

Seventy two hours post operatively all the groups of animals were tested again for their behaviour using operant chamber as done before for a further period of ten days.

Histological procedures

On completion of behavioural studies, the three groups of rats and also the age

matched control group were sacrificed and the brains were removed immediately. Then the hipocampi were dissected and processed for rapid golgi staining technique (11). The CA1 and CA3 hippocampal fields were studied for their dendritic alterations. The camera lucida drawings for CA1 and CA3 neurons were done at a magnification of 40 x. The branching points and intersection points of CA1 and CA3 neurons were determined by Scholls method (12).

The behavioural data was analysed by one way and two way ANOVA and the morphological data was analysed by one way ANOVA.

RESULTS

Pedal Press Rate (PRR) : The animals with similar pedal press rates were included and put into 3 groups : (1) sham, (2) trained (3) lesioned. The PPR of total ten days before surgery showed no difference among the groups. However, the PPR after surgery showed that the lesioned rats had a significantly lower PPR as compared to the sham and trained rats ($P<0.01$). By the 8th day of behavioral study the lesioned rats improved their performance significantly and the differences were found to be insignificant among the groups (Fig.1 a, b).

Intersection and Branching Points

The intersection and branching points of both CA1 and CA3 neurons was quantified using camera lucida drawings. In case of CA1 apical dendrites the branching point and intersection points of the lesioned group showed a significant decrease upto 200 microns distance from soma. However, in case of basal dendrites the branching points

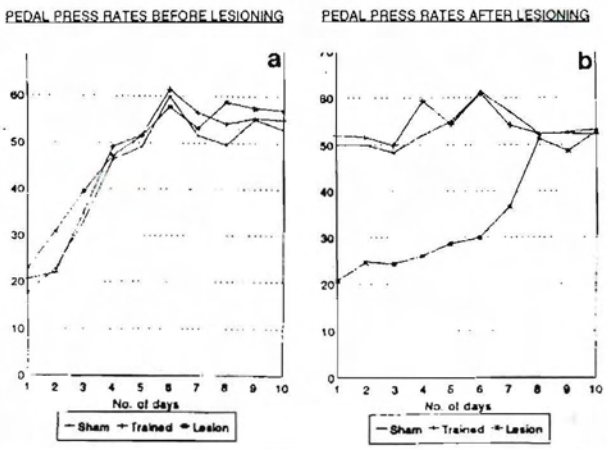

Fig. 1 : Evaluation of pedal press rates, before lesioning (a) and after lesioning (b) for a total of ten days (sham ⊖, trained +, lesioned *).

showed a significant reduction upto a distance of 200 microns (Fig.2) and the intersections upto 160 microns. The dendritic arborization of the CA3 neurons of the lesioned rats was reduced in a similar fashion as the CA1 and CA3 (Fig. 3). The golgi photographs of CA3 neurons are depicted in (Fig. 4).

DISCUSSION

The present study indicates that bilateral electrolytic lesions of subiculum causes an impairment in operant learning. The lesioned animals had decreased pedal press rate without loss of the retention of operant behaviour. Results from previous studies reported a severe and long lasting deficit in spatial learning in rats with neurotoxic lesions of subiculum (7), and post subiculum (8). In the Morris water maze task (13), it has been shown that ibotenic acid subicular lesioned rats showed similar changes to that of hippocampal lesioned rats in being impaired on some components of spatial memory task.

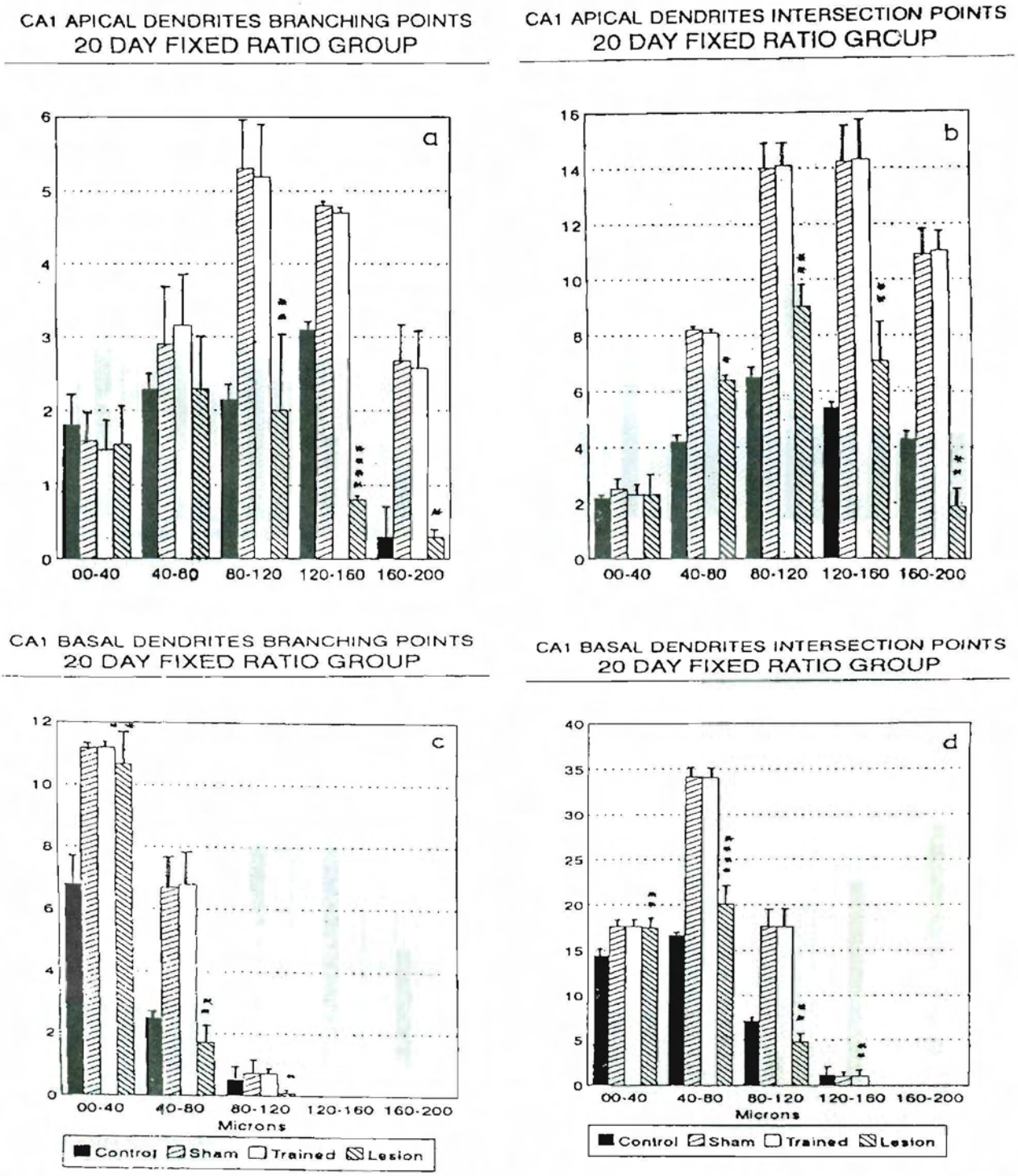

Fig. 2 : Number of branching points, intersection points of apical (a, b) and branching and intersection points of basal (c, d) dendrites of CA1 neurons. (* = 0.1; ** = 0.01; *** = 0.001; **** = 0.0001; ***** = 0.00001).

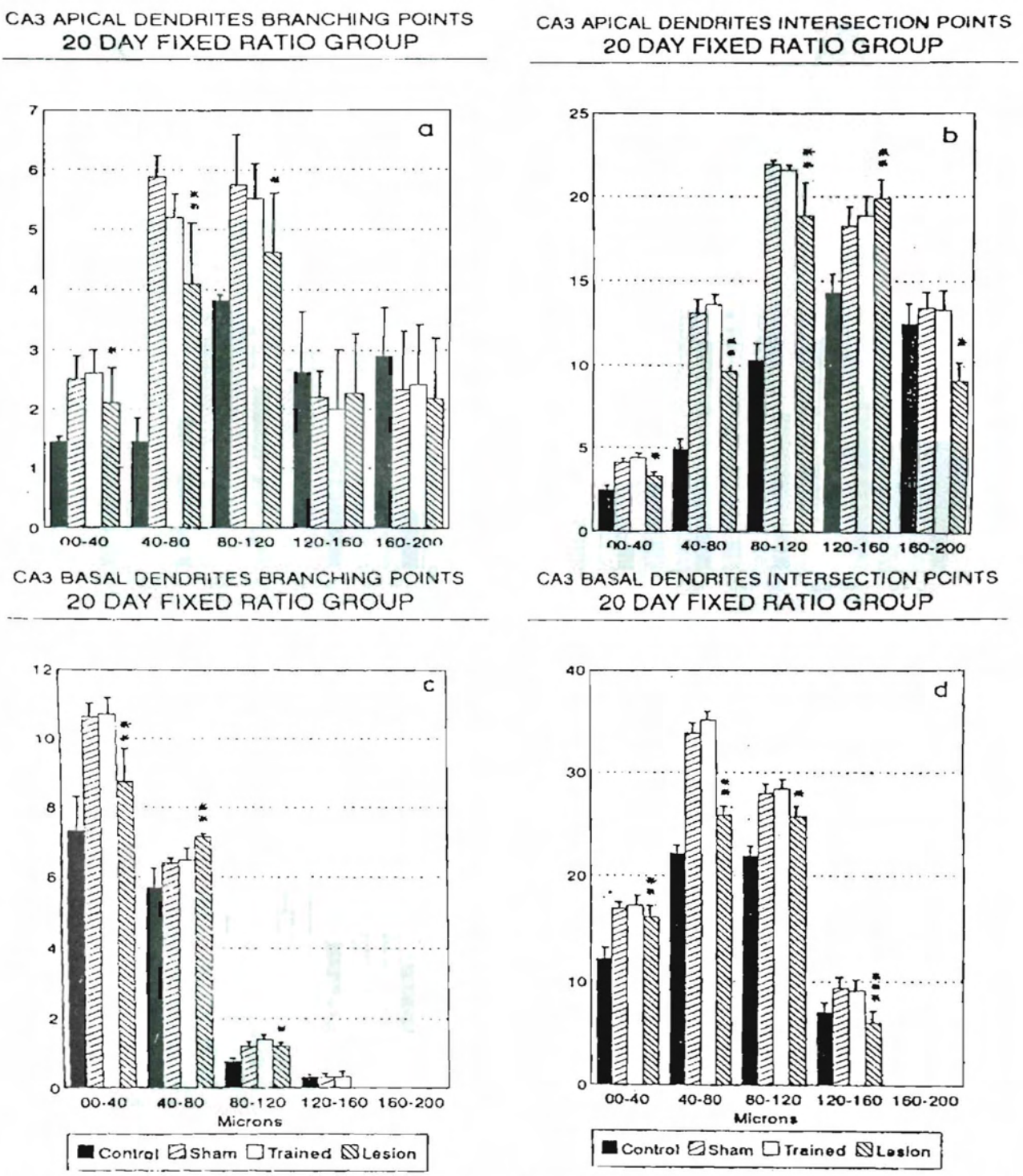

Fig. 3 : Number of branching points, intersection points of apical (a, b) and branching and intersection points of basal (c, d) dendrites of CA3 neurons. (* = 0.1; ** = – 0.01; *** = – 0.001; **** = 0.0001; ***** = 0.00001).

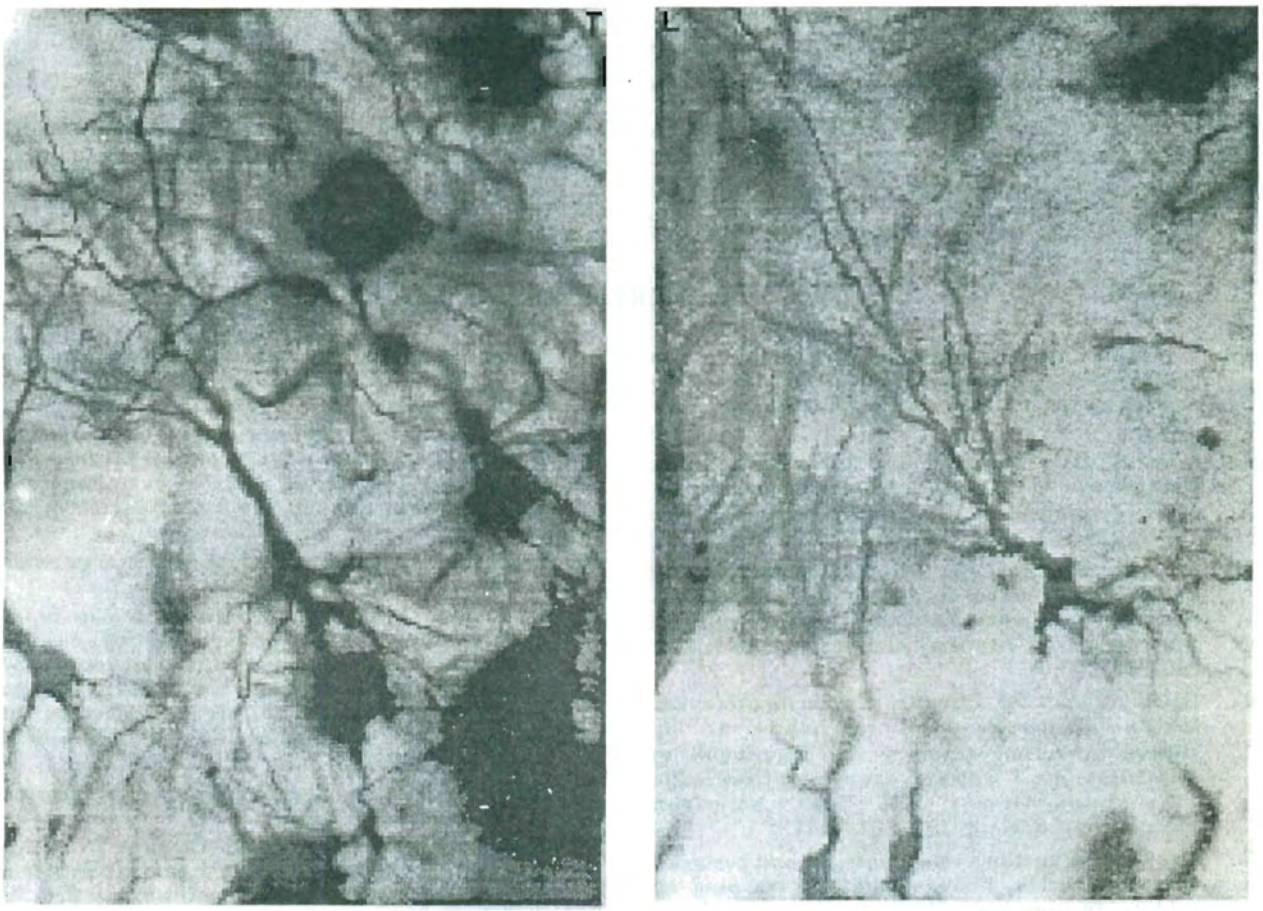

Fig. 4 : Golgi photographs of neurons (T–trained, L–lesioned).

In our studies retention of operant behaviour is not affected though the PPR are lower in lesioned animals. This is due to the fact that long term memory is stored in other areas of brain as the hippocampus processes the short term memory and transfers it into long term memory. The subiculum is major output structure of the hippocampus having diverse connection with other cortical and sub-cortical strucutures (14), and is ideally suited for modifying or transfer of information from hippocampus. Disruption of hippocampal connections by way of subicular lesions has produced behavioural impairment (15). Our morphological data reveals a reduction in dendritic arborization of both apical and basal branches of CA1 and CA3 neurons in lesioned group. This change was more prominent in CA1 area. The extent of loss of CA1 due to neurotoxins or ischemic lesions can be correlated significantly with performance on delayed spatial discrimination (16). Monkeys with bilateral lesions of CA1 field have severe impairment on retention of conditional task learnt before surgery and on new acquisition of several types of this tasks (17). As the

subiculum is a major input source of CA1 neurons which are topographically organized (2), the lesions of subiculum thus found to reduce the CA1 neuronal target. The CA1 neurons in turn are the post synaptic targets for the CA1 neruons. Their altered morphology could be one of the reasons of an altered CA3 neuronal profile.

Thus subicular lesions impairs operant performance and also alters the dendritic morphology of both CA1 and CA3 hippocampal neurons.

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