

ELECTROCONVULSIVE SHOCK (ECS) INDUCED DENDRITIC LENGTH MODIFICATION AS AN INDICATOR OF NEUROPLASTICITY

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ABSTRACT

Antidepressant treatments like Electroconvulsive therapy act by inducing neuroplastic changes in the brain. It protects against and even reverses some but not all of this stress-induced neurohistological changes. The aim of the study was to quantify the effect of ECS on the length of pyramidal neurons in CA1 region of hippocampus. Thirty Wistar rats were randomly chosen, divided into experimental group (n=15) which received ECS and the other group was control (n=15). Brains were processed with rapid Golgi technique. Length of pyramidal cells was measured in both groups by using neuroLucida software. The mean of experimental group was 1368 ± 81.09 and control was 739 ± 32.12 ; with p value < 0.001 . Neurohistological effects of ECS, a manifestation of neuroplasticity may be an important contributor for proper re-wiring so necessary for relearning of healthier cognitions, emotional responses and behavioural expressions in psychological disorders.

Key Words: *Neuroplasticity, Electroconvulsive Seizure, Depression*

INTRODUCTION

Depression is conventionally viewed as a state of chemical imbalance, and antidepressants are suggested to act through increasing monoaminergic neurotransmission. Pathological stress and depression are associated with changes such as loss of dendritic spines, shrinkage of the dendritic tree and loss of synapses in the hippocampus and prefrontal cortex. There is also a decrease in glia (Andrade, 2010). Antidepressant treatment protects against and even reverses some but not all of these stress-induced neurohistological changes (Pittenger, 2008). These treatments include drugs, electroconvulsive therapy (ECT), repetitive transcranial magnetic stimulation (rTMS), vagus nerve stimulation (VNS), transcranial direct current stimulation (tDCS), light therapy, sleep deprivation therapy and others glia (Andrade, 2010). The hippocampus is a key structure involved in learning and memory, especially explicit (consciously acquired) memory. Perhaps as a result of hippocampal impairment, stressed animals and depressed humans show impaired learning and memory (Sapolsky, 2000; Diamond, 2004).

In animal models, stress-induced histological changes in the hippocampus include the following (D'Sa, 2002; Sheline, 2004; Gorwood 2009 and Jay, 2009)

- Loss of dendritic spines
- Decrease in the number and length of dendrites
- Loss of synapses
- Loss of glia
- Impairment of neurogenesis
- Possibly apoptosis (under extreme conditions)

In animal models ECT is known as electroconvulsive shock (ECS). It has been shown to result in neuroplasticity in the hippocampus. Neuroplasticity is a well characterized phenomenon in the developing adult brain. It refers to the capacity of a single neuron to modify morphology, synaptic connections and activity. Neuronal connections and capacity for plastic events are known to be compromised in several pathological disorders, such as major depression (Kessler, 2007).

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The aim of the study was to determine neuroplasticity in rat hippocampus using length of apical dendrite as criteria. To the best of our knowledge, there are no studies reported on the effect of ECS in normal rat.

MATERIALS AND METHODS

Male Wistar rats (180- 250g) obtained from Central Animal Research Facilities (CARF), St. John's medical college, Bangalore were used as subjects. Subjects were housed 3 per cage in polypropylene cages (22.5 cm×35.5 cm× 15 cm) with temperature($25\pm 2^{\circ}\text{C}$), humidity (50 – 55%) and light (12 hours light- dark cycle) in controlled environment with food and water ad libidum.

Inclusion Criteria

Adult male healthy rats of 2-3 months old

Exclusion Criteria

Rats which are injured during ECS and rats affected with infections.

ECS procedure

30 Subjects were randomly sorted and allotted into ECS and control groups

The ECS group received an ECS treatment consecutive 6 days at 10:00AM by using electrode gel coated stainless steel ear clip electrodes

The electrical stimulus was delivered through Naviquire machine, Bangalore, India, constant current brief pulse ECT device

Parameters: the stimulus parameters were computer controlled

Amplitude 250mA, pulse width 1.5ms, pulse frequency 50Hz, stimulus duration 0.54 sec (10mC)

Each stimulus resulted in a tonic – clonic seizure lasting 8 – 12 sec

Sham treated control animals received identical handling but no electrical stimulation

Rats were sacrificed after one month and hippocampus processed with rapid Golgi solution. Rapid Golgi being a gold standard technique for staining neurons, this method was chosen (Smitha, 2012).

Morphometric Analysis

The hippocampal sections were observed using Leitz microscope. The neurons were viewed randomly and the neurons, which fulfilled the following criteria, were selected for the study.

Dark and consistent silver impregnation throughout the extent of all the dendrites

The presence of untruncated dendrites

Relative isolation from the neighboring impregnated neuron

Camera Lucida tracings were obtained from randomly selected CA1 pyramidal neurons at a magnification of 625X using Leitz microscope. The quantification and analysis of dendritic branching points and dendritic intersections were carried out using Sholl's analysis.

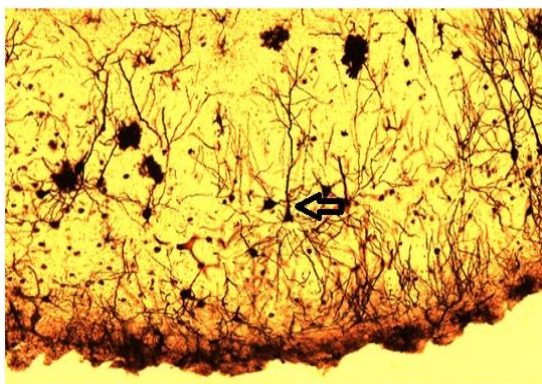


Figure 1: Pyramidal neurons in CA1 region under 10X

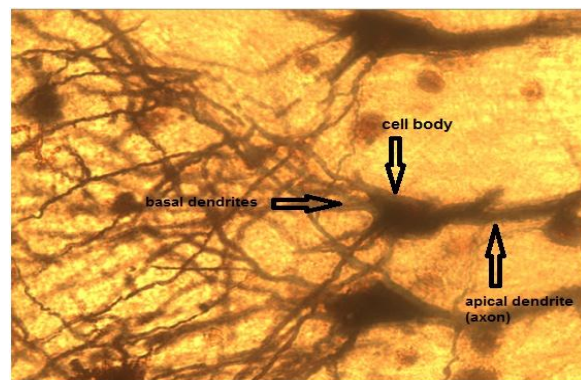


Figure 2: Pyramidal neuron in CA1 region under 40X

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Sholl's Analysis

The neurons were traced with the help of neurolucida software by keeping the reference point in the center of the cell body and trace the apical dendrite of the pyramidal neuron under 40X (Fig. 1, 2 and 3).

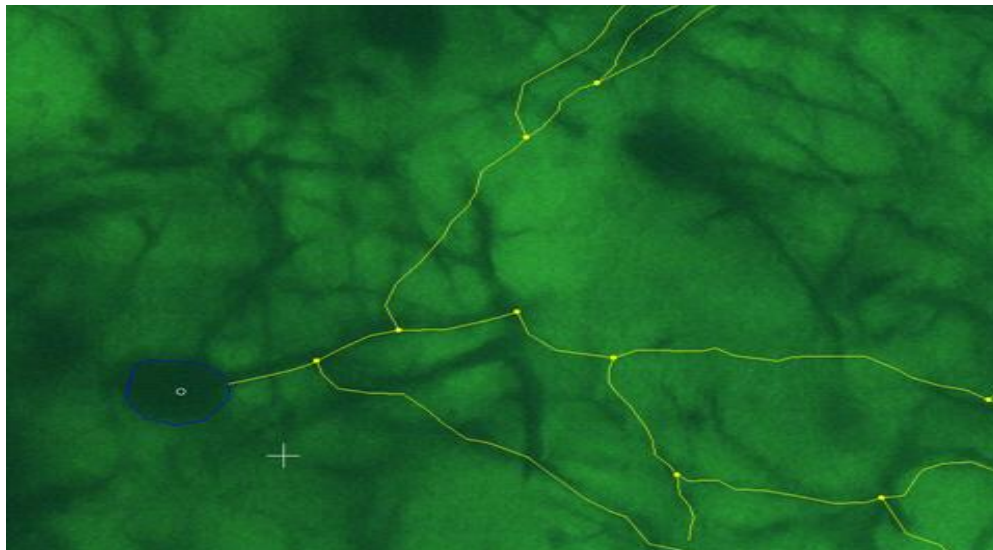


Figure -3: Traced apical dendrite with the neurolucida software

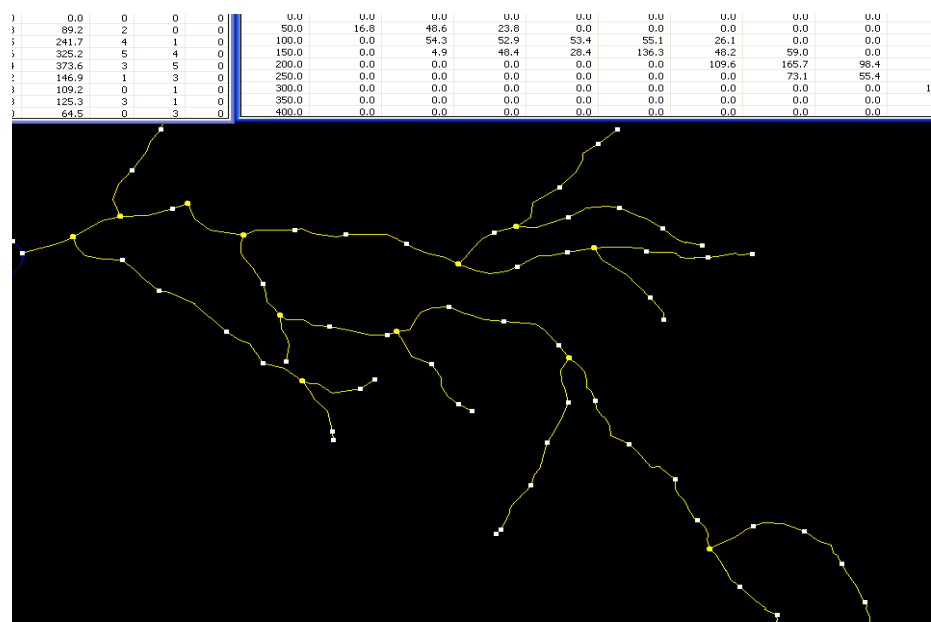


Figure 4: calculation of length with neurolucida explorer

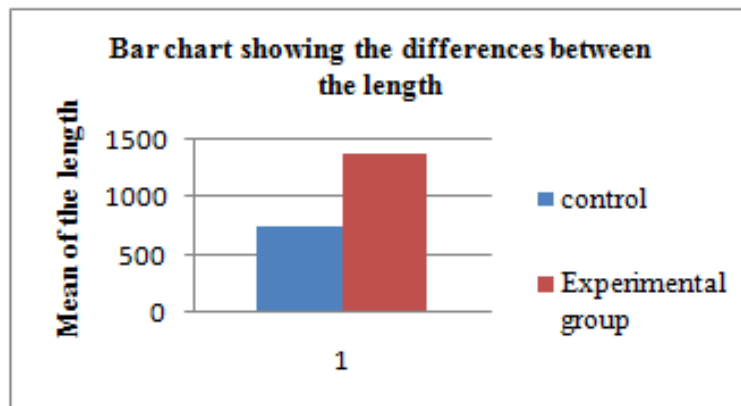
The traced neurons were explored with the Neurolucida explorer software (Fig. 4). The software gives the details about the dendritic intersections, length and branching points from the soma by calculating values in each successive concentric segment. The dendritic branching and intersections were studied up to a length of 250 μ m distance from the center of the soma.

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Statistical analyses were performed using statistical software (SPSS for windows, version 16). Independent sample t test was used to evaluate significant changes in the length of the axon and Levene's test for homogeneity.

RESULTS

The results are graphed in bar chart. The mean of the experimental group was 1368 ± 573.43 in experimental group; and 739.19 ± 32.12 in control group. In the study the mean difference in the length of the two groups was 628.858, 95% confidence of the difference - lower and upper boundaries were 455.763 & 801.952 with a P value of ≤ 0.001 and 99% power. There was a significant difference in length of the ECS treated group and control group.



DISCUSSION

Chronic antidepressants like ECS treatment increases plastic events including change in the length; arborization and branching of neurons in distinct brain areas, such as hippocampus. Antidepressants not only change the morphology of neuron but also increase the number of new neurons (Andrade, 2010; D'Sa, 2002). Neuronal connections and capacity for plastic events are compromised in several pathological disorders, such as major depression. Glutamate binds to the postsynaptic NMDA receptor, which gates the calcium ion channel. Entry of calcium into the postsynaptic neuron initiates a series of changes; these involve neurotransmitter and neuromodulator molecules such as arachidonic acid, prostanooids, endogenous cannabinoids, platelet activating factor and others (Andrade, 2010).

Antidepressant treatments including ECS have been shown to increase synaptogenesis, accelerate dendritic arborizations of newly generated neurons, and spine maturation of dentate gyrus cells in hippocampus. However, a direct demonstration of the effect of ECS on dendritic complexity of mature granule cells is still lacking. After ECS, WT mice showed increased dendrite length and dendritic intersections at $60\mu\text{m}$ and greater distances from the soma, suggesting a robust response to the treatment (Sudhirkumar, 2012).

Meta analysis suggests that depression may be associated with a disruption of mechanisms that govern cell survival and neural plasticity in the brain. Antidepressants could mediate their effects by increasing neurogenesis and modulating the signalling pathways involved in plasticity and survival (Andrade, 2008; Carrol, 2002).

The present study was designed to examine the effect of ECS on neuroplasticity in the CA1 region of adult male rat. There are many studies in literature about dentate gyrus mossy fibers sprouting after ECS, but less in cornu ammonis region. It was found that six ECS seizures increased the length of the axon of

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pyramidal cells. There is a significant increase in length of apical dendrites of pyramidal neurons (p value <0.01) compared to the control group.

Clinical Relevance

A major function of hippocampus is memory. In denial, an ego defence mechanism which occurs in psychological trauma memory is affected. Restoration of memory is an important indicator of recovery in such major life events. This work on neuroplasticity involving ECS treatment may play an important mechanism in proper re-wiring.

Conclusion

From the present study we have shown that ECS administration at appropriate dose could be an important contributor for proper re-wiring and relearning of healthier cognitions.

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