Research article

The Differential Effects of high dose Magnetic Seizure Therapy (MST) and ElectroConvulsive Shock (ECS) on the number of Neuron and Glial cells in the hippocampus of a rhesus monkey model.

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Abstract

Background: Anatomical evidence of brain damage from electroconvulsive therapy (ECT) is deficient; but most of the previous studies were done in rodents and there are no modern stereological studies in primates documenting its safety. Magnetic seizure therapy (MST) is under development as a less invasive form of convulsive therapy, and there are only two prior reports on its anatomical effects. The first report showed no histological lesions in the brains of higher mammals subjected to electroconvulsive shock (ECS) or MST, under conditions that model closely those used in humans. The second report reported no significant effects of ECS and moderate dose MST on the number of neuron and glial cells in the hippocampus and frontal cortex. We sought to test these findings by determining whether these interventions at higher dosage affected the number of neurons or glia in the hippocampus. **Subjects and methods**: Eighteen animals received ٦ weeks of ECS, MST, or anesthesia alone, ٤ days per week. After perfusion fixation, numbers of neurons and glia in the hippocampus were determined by unbiased stereological methods. **Results:** We found no effect of either interventions compared to anesthesia a lone on volumes or total number or numerical density of neurons or glia in all hippocampal areas. **Conclusions:** Induction of seizures in animal model of human ECT and MST therapy does not cause a change in the number of neurons or glia in potentially vulnerable regions of brain. This study provides further evidence that ECT and high dose MST, when appropriately applied, do not cause structural damage to the brain.

Key words: Stereology; Non-human primate brain; neuropathology; hippocampus; ECT; rTMS.

Introduction

Despite the discovery of new antidepressant medications and psychotherapies, severe major depressive disorder (MDD) remains an important public health concern, responsible for significant morbidity and mortality,[١]. For these patients with treatment resistant MDD, bipolar disorder, or schizophrenia, the main therapeutic option is electroconvulsive therapy (ECT)[٢]. The absolute number of patients who receive ECT is large, annually estimated at ١ million worldwide [٣]. Treatment with ECT produces rapid response and remission rates and is safe for patients across the adult life span[٤]. However, ECT also results in adverse neurocognitive effects[٥]. However, the forms of ECT with the most well established efficacy/side effect profile still result in a substantial side effect burden[٦,٧] ECT use is also delimited by concerns about stigma and fears of brain damage, although structural damage has not been confirmed[٨-١٠]. The principle of ECT is the induction of a brief generalized grand mal seizure. It is well known that seizures of long duration, for example status epilepticus, are associated with cell death in certain brain regions. It is therefore a reasonable assumption that some cell death also might occur after seizures of short duration, i.e. ECT. Results from research on electroconvulsive stimulation (ECS), is

however inconclusive, with reports of both neuronal cell death and neuroprotective effects[١١]. Aside from this current study and its earlier version[٩], animal models to determine whether cells are lost because of ECT have been limited to rodent models that don't include modern safeguards like modern clinical human ECT. It is worth to mention that, research has found that the antidepressant properties of ECT and its effects on cognition are uncorrelated and considerable progress has been made in altering the ECT technique to maintain efficacy while reducing cognitive side effects[١٢]. The efficacy and side effects of ECT are determined by the site of seizure initiation and patterns of seizure spread, but these factors cannot be adequately controlled with current ECT technique[١٣]. A form of convulsive therapy that preserve the therapeutic efficacy of ECT, but with a better side effect profile, should improve the quality of life for patients in need for convulsive therapy and should increase access to effective treatment. Magnetic seizure therapy (MST) is under development as a means of achieving that goal[١٤]. The hypothesis of using magnetic pulses for the induction of therapeutic seizures was proposed by Sackeim and appeared in the mid-١٩٩٠s,[١٥], but it was not until ٢٠٠١ that the first case report was published that documented the feasibility and safety of MST . MST is a mixture of TMS and ECT. TMS produces an approximate ١٥٪ remission rate with minimal adverse effects[١٦] whereas ECT is the most effective antidepressants available with an approximate ٨٧٪ remission rate, but with moderate adverse effects[١٧,١٨]. Thus, the development of MST aims to combine the optimal antidepressant effects of ECT with the minimal adverse effect profile of TMS[١٩]. Induction of seizure by magnetic seizure stimulation (MST), which produces a more localized current than electrical stimulation, is still under development as a less invasive form of convulsive therapy intended to improve the risk/ benefit ratio by sparing memory. Studies to date demonstrate the feasibility of MST in a monkey model,[٢٠]. Open studies of MST in humans provide preliminary evidence for antidepressant effects[٢١]. Initial reports suggest that MST improve mood in refractory major depression[٢٢,٢٣] and demonstrate a superior side effect profile to ECT with acute[١٤]. However only one report has addressed the anatomical effects of moderate dose MST and ECS[٩]. We now report a stereological assessment of the effect of high dose MST and ECS in the hippocampus using an expanded sample and mimicking clinical ECT and MST. The rhesus monkey has been chosen because it is the species of choice for many experimental models of aging, brain diseases, and new treatment strategies, since it shares with humans many aspects of neuroanatomy and cognitive function[٢٤,٢٥].

Subjects and methods

This study was conducted at the New York State Psychiatric Institute (NYPSI) and approved by the Institutional Animal Care and Use Committee (IACUC) and Columbia University, New York, USA. Subjects were eighteen pathogen-free male rhesus macaca mulatta monkeys obtained from NIH breeding colony. They were all male and divided into six cohorts of three, matched for age, weight and sex. Each cohort was group-housed. Within each cohort, subjects were randomly assigned to ECS, MST, or sham interventions. All staff not involved in the delivery of the interventions was masked to group assignment. The study was designed to allow observation of either acute or delayed pathology. Interventions were administered ٤ days/week for a total of ٦ weeks (٢٤ sessions). The number and frequency of treatments were selected to be more intense than that typically administered in human ECT, to maximize the chance of detecting any neuropathological consequences of the interventions. A ٥- week recovery period was interposed before the last intervention week to permit maturation of any neuropathological effects. Sacrifice was done three days after the last intervention. We avoided the use of pre-intervention sedation (ketamine) to remove the monkey from the home cage prior to general anesthesia. Ketamine represent a confound to both cognitive and anatomical measures. Ketamine has been reported, at high doses, to attenuate Mossy Fiber Sprouting (MFS) with ECS in rodents[٢٦].

Subjects were trained to extend a leg voluntarily to permit IV access and an initial induction dose of methohexital was administered. Like human ECT, interventions were administered under general anesthesia with methohexital (٠.٥ mg/kg IV), muscle relaxation with succinylcholine (٣.٥mg/kg IV) and continuous ventilatory support (١٠٠٪ O٢ positive pressure). Sham involved anaesthesia and monitoring only, with no stimulation or seizure induction. Bilateral ECS was administered with the MECTA Spectrum ٥٠٠٠Q ECT device at ٢.٥X titrated seizure threshold and at a frequency of ٥٠ Hz. ECS electrodes were placed bilaterally on the right and left temples, mimicking electrode placement for Bilateral ECT in humans. Seizure threshold titration for ECS was performed by increasing duration of stimulation by١٦٠ms with each stimulation (with current held at ٨٠٠mA and pulse width at ٠.٥ms) until seizure was induced. Subsequent daily dosage of ECS was set at ٢.٥X titrated seizure threshold and at a frequency of ٥٠ Hz. MST was administered via a ١٠٠ Hz Magstim MST device with a pediatric-sized round coil on the vertex. MST seizure threshold was defined by the number of pulses required to elicit a seizure. Seizure threshold titration was performed by starting with a ٥٠Hz, ١s stimulation (٥٠ pulses) and delivering stimulations approximately every ٢٠ seconds, increasing the duration by ١ second with each stimulation until a seizure was induced. Subsequent daily dosage of MST was set at ١٠٠ Hz and either ٦X seizure threshold, or the maximum output capacity of the device (١٠٠٠ pulses at ١٠٠ Hz). Seizure thresholds for ECS and for MST were determined using the ascending method of limits titration procedure[٢٧]. An ECS frequency of ٥٠ Hz is comprised of ٥٠ pulse pairs (upward and downward going) per second, resulting in ١٠٠ total pulses per second. A MST frequency of ١٠٠ Hz is comprised of ١٠٠ pulses per second, thus the conditions were matched in total number of pulses per second. Stereological assessments focused on hippocampus which is the primary site of seizure-induced damage in epilepsy models, and because of the relevance of this area to cognitive side effects. Three days following the last intervention, subjects transported to the perfusion laboratory where they were anesthetized to the surgical depth, heparinized, and transcardially perfused. The calvarium was opened with a Stryker saw. Brains removed and post fixed in ٤٪ phosphate-buffered formaldehyde for ٤ h. General neuropathological examination for any microscopic or macroscopic evidence of acute or chronic damage was done for the left hemisphere by an expert neuropathologist (Andrew J Dwork, MD).

In the hippocampus, we examined the subregions CA١, CA٢, CA٣ and CA٤ with special emphasis on CA١ and CA٣. The hippocampus was selected since these hippocampal areas have been repeatedly found to be particularly vulnerable to damage associated with frequent or prolonged seizures in humans, nonhuman primates, and other animal[٢٨]. The definition of the borders of hippocampal areas was based on criteria from studies on humans[٢٩] and rhesus monkeys[٣٠].

In this study, we used the optical fractionator design and the Cavalieri principle to examine for neuron and glial numbers and volume. The optical fractionator design provides a direct and simple method to estimate the total cell number and is in principle unaffected by tissue shrinkage[٣١,٣٢]. The basic principle is to count every cell in a systematic and uniform random sample (SURS) that constitutes a known fraction of the region of interest. All sections were ٤٠ microns thick, except that every ١٩th and ٢٠th (wells ١٩, ٢٠ and ٣٩, ٤٠) was ٨٠ microns thick to allow for sufficient guard zones around an optical dissecter. For each case, a random choice of well ٢٠ or ٤٠ was made, and all the sections in that well stained with cresyl violet. The regions of interest were demarcated on each slide based on the conventional cytoarchitectonic divisions of the hippocampal formation, and the interleaved Timm stained sections for delineation of CA٣ from CA٢. Staining was done with a modified Vogt Cresyl Violet, which provided the best staining of the relevant cells. Microscopic examination was done using a high-resolution microscope connected to a computer via a

video camera. A counting frame was applied to the tissue using CAST software.

Counting principles: For all stereological methods, it is critical that all particles in each sample are counted only once and with the same probability, which is provided by the optical dissector [٣٣]. With the optical dissector, it is possible to dissect optically the chosen sample using the focal plane, which is moved through the thick section in the z-axis. After having established a constant density within the dissector height, all sampled particles that come into focus inside the counting frame are counted and added to is the sum of all cells counted in all dissectors in a region, provided they do not touch the exclusion lines. An upper guard area of ٥ mm was used to avoid loss of sampled particles due to tissue preparation

Differentiation of neurons and glial cells: While it is usually easy to distinguish large- and middle-sized neurons from glial cells, the distinction between small neurons and large glial cells can be challenging. The following criteria were used as characteristic for neurons: a centrally located nucleolus, a distinctive nucleus, visible cytoplasm, presence of dendritic processes, and larger cell body size. Glial cells were identified by the following criteria: heterochromatin clumps, sparse cytoplasm, and smaller cell body size[٣٤].

Results

The coefficient of error [٣٥] for each individual measurement was < ٠.١٠. There were no significant effects of treatment on the coefficient of error of any measurement. Cell counts, volumes, and densities are shown in Fig. (١-٨). One-way ANOVA revealed no significant effects of treatment on volume, neuronal density, glial density, total number of neurons, or total number of glia in any of the hippocampal areas.

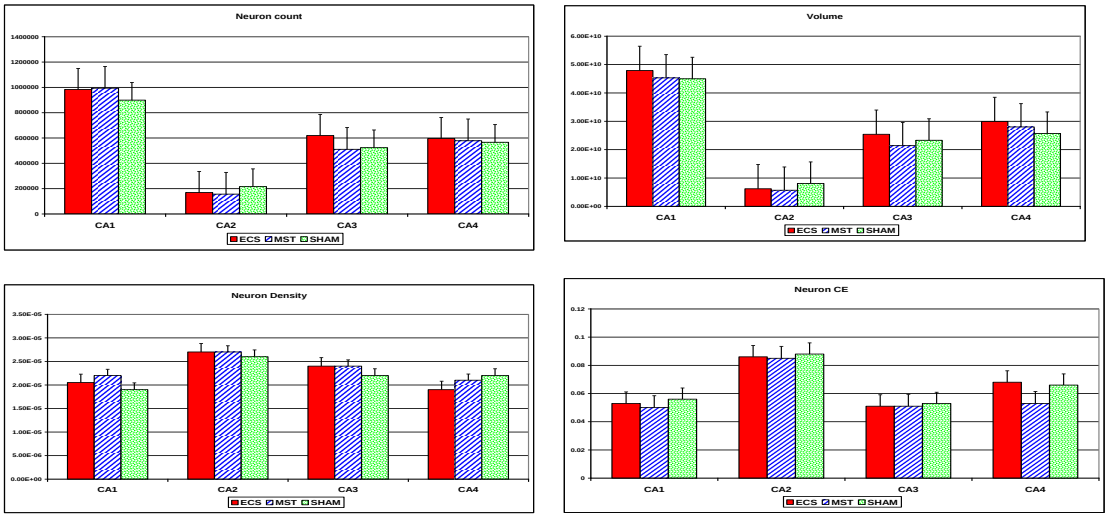

Figure (١ to ٤): Means and standard deviations of the total estimated neuronal number, volume, density and CE in all hippocampal areas (CA١, CA٢, CA٣ and CA٤) following different interventions (ECS, MST or sham). As shown, there is no significant differential effect of these interventions.

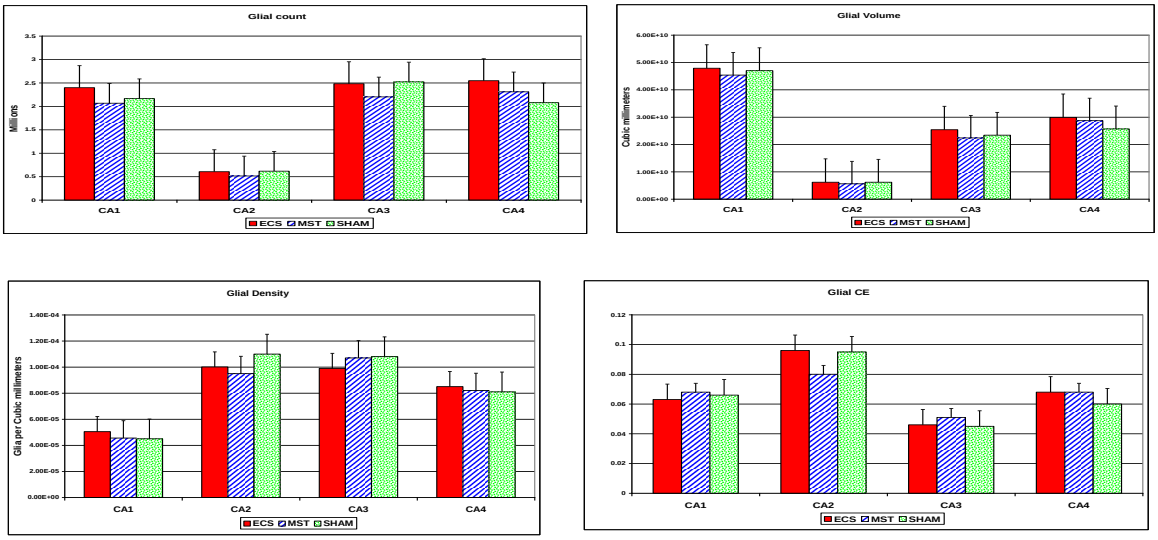

Figure (٥-٨): Means and standard deviations of the estimated total number, volume, density and CE of glial cells in all hippocampal areas (CA١, CA٢, CA٣ and CA٤) following different interventions (ECS, MST or sham). As shown, there is no significant differential effect of these interventions.

Discussion

In our study, we didn't find any significant effect of ECS, high dose MST or sham on volume, neuronal density, glial density, total number of neurons, or total number of glia in any of hippocampal areas. Our findings are consistent with the results of several other studies which showed no evidence of destructive lesions from clinical modern ECT as currently applied in human , nor in animal models mimicking these conditions[٩,٣٦]. While there have been no quantitative autopsy studies of ECT in humans, there are several reports of individuals who had received large numbers of treatments and showed no qualitative abnormalities on neuropathological examination[٣٦,٣٧]. However, up to ٩٠٪ of cases of temporal lobe epilepsy are accompanied by hippocampal sclerosis[٣٨]. In a stereological study done in rats, decreases of ١٧–٣٢٪ in neuronal density throughout CA١ and CA٣ was found after ٣٠ kindled seizures, and subregions of CA١ and CA٣ showed ١٧–١٨٪ decrements after only three kindled seizures[٣٩]. And in humans, the pyramidal neurons of CA١ are among the most vulnerable to generalized excitotoxic, hypoxic, ischemic and hypoglycemic insults[٤٠]. Thus, it would be reasonable to assume that ECS or MST, while not producing hippocampal sclerosis, might cause some loss of neurons in CA١ or CA٣, and that the absence of such loss would be a strong evidence for the absence of excitotoxicity. Dwork et al., (٢٠٠٩), in a similar study, tested for the differential effects of ECS, MST and sham on the number of neuron and glial cells in hippocampus and prefrontal areas. Dr. Dwork reported no significant effects of those interventions on these areas. However, the dosage used in Dwork study was ٢.٥ seizure threshold for both ECS and MST. The current study is first stereological study to examine the effects of high dose MST (٦ seizure threshold) in animal model and is the second stereological assessment of ECS or MST in higher mammals, and the second in any animal model of ECT or MST with muscle paralysis and supported oxygenation. Supporting our findings for the safety of ECT is the quantitative studies of unmodified ECS in rats which found no effects on neuronal number in neocortex[٤١], hippocampus[٤٢]. In the only stereological study to include hippocampal granular cell neurons, these were greater in number, and the granular cell layer and hilus were greater in volume, in rats receiving daily unmodified ECS for ١٠ days, compared with rats receiving sham treatments. However, CA١ and CA٢–٣ were unaffected[٤٣].

However, in contrast to studies that modeled ECT, neuronal loss does occur in models of epilepsy. For example, Cardoso et al.,[٤٤] used stereological methods to examine the entorhinal cortex and the hippocampal dentate gyrus and hilus in ٣-month-old rats treated at age ٢ months with daily ECS for ٥ days and a sixth ECS ٢ hours after the fifth. This protocol, specifically designed to induce the last seizure while glutamate reuptake capacity was impaired (i.e., to facilitate seizure-induced excitotoxicity), resulted in lower numbers of neurons but normal volume in the hippocampal hilus, and in lower numbers of neurons and smaller volume in layer ٣ of the entorhinal cortex.

Our findings for the MST intervention were also negative. This is consistent with the findings provided by Dwork et al.,[٩,١٠]. To our knowledge, other than these two studies and our study, no other study has looked for histological damage or loss of cells following cases of seizures induced by MST. Studies of repetitive transcranial magnetic stimulation (rTMS), with or without unintended seizures, are mostly negative. Magnetic resonance imaging studies of rTMS have revealed no abnormalities of brain structure or diffusion studies[٤٥].

The only histological study in humans qualitatively examined temporal lobectomy specimens from two epileptic subjects who underwent rTMS with approximately ٢٠٠٠ stimulations over the preceding ٢–٤ weeks before surgery[٤٦]. In one, rTMS induced an unintended partial motor seizure. Neither temporal lobectomy specimen showed pathological changes, except for a vascular malformation that obviously preceded rTMS.

In lower mammals, most studies are negative, but two reported subtle, qualitative abnormalities. There is a report of cortical microvacuolar changes, visible by light microscopy, following rTMS in unanesthetized rats by[٤٧]. Another study by[٤٨] found no abnormalities by light microscopy of the brains of rats treated with ECS or rTMS, but reported "edematous changes" visible by electron microscopy that were milder after rTMS than after ECS. Sgro et al.,[٤٩] found no qualitative abnormalities after rTMS of anesthetized rats. Also, Post et al. (١٩٩٩), in a qualitative study, found neither pathological changes nor increased immunoreactivity for glial fibrillary acidic protein (GFAP) in anesthetized or un-anesthetized rats after rTMS, and they specifically ruled out microvacuolation. On the same direction, Ravnborg et al.,[٥٠] found no change in the permeability of cerebral blood vessels after rTMS in anesthetized or unanesthetized rats. Qualitative studies by Counter et al.,[٥١] and Nishikiori et al.,[٥٢] found no abnormalities of the brain after extensive rTMS in unanesthetized rabbits. In summary, there is very little evidence that rTMS produces structural changes, whether seizures are induced or not. These very subtle positive findings found only on experiment in lower mammals and examined only by light microscopy and the procedures was done without anesthesia. The number of the glia cells examined in our study was a total number and no effort was done to subclassify them. So, we do not know if there was perhaps a decrease in one type and an increase in another one. However, any microglial or astrocytic reaction will be associated with neuronal loss which was not observed in our case. Another thing, general and microscopic examination of the left hemisphere didn't show any signs of pathological lesions.

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