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LTP-like synaptic efficacy changes following electroconvulsive stimulation

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SYNAPTIC plasticity is thought to represent a mechanism for memory formation. Memory disturbances commonly follow electroconvulsive therapy (ECT) for depression. Accordingly, we examined the development and duration of the effects of electroconvulsive stimulation (ECS) on hippocampal synaptic plasticity in rats. Daily recording of field potentials during 10 spaced ECS revealed an increase in the dentate gyrus evoked response, reaching a maximum after the fifth seizure. In a second experiment, an identical ECS series substantially reduced the degree to which electrically induced synaptic changes (long-term potentiation—LTP) could be elicited under anaesthesia for up to 40 days. These findings suggest that ECS induces LTP-like long-lasting synaptic changes which may underlie the neuropsychological sequelae of ECT treatment in humans.

Key words: Electroconvulsive stimulation; Long-term potentiation; Synaptic plasticity; Hippocampus; Depressive illness; Memory; Rat

Introduction

Electroconvulsive therapy (ECT) is used safely and effectively in the treatment of severe depressive illness, yet continues to provoke public controversy. This is because patients treated with ECT commonly experience a marked, but transient, disorder of memory function and that the neural basis of the therapeutic action ECT remains largely unknown. Detailed examination of the neurobiological effects of ECT may lead to the refinement of treatment and a better understanding of the disease that ECT treats so effectively.

In recent years, many investigations into the neurobiology of memory have focused on an electrophysiological phenomenon known as long-term potentiation (LTP). LTP is an experimentally induced form of synaptic plasticity which can be readily demonstrated in the hippocampus and constitutes a long-lasting increase in excitatory synaptic strength.[1] It has been proposed as a plausible candidate mechanism for the neural substrate of learning. Several experiments have strengthened the proposed link between LTP and learning and memory: first, *N*-methyl-D-aspartate (NMDA) receptor antagonists which block induction of LTP in the hippocampus *in vivo* also block the acquisition of a spatial learning task in rats in a dose-dependent manner;[2] second, mutant mice which lack a gene controlling LTP (alpha-CaMKII) also fail in spatial learning tasks;[3] third, there is evidence that driving synaptic plasticity in the hippocampus of the rat to an experimental maximum by repeatedly inducing LTP ('saturation' of LTP) occludes formation of new spatial memories.[4,5]

We have previously reported that repeated, spaced electroconvulsive stimulation (ECS) in the rat has a profound effect on hippocampal LTP,[6] reducing the degree to which LTP can be induced in the dentate gyrus of anaesthetized rats compared with control subjects. Analysis of absolute excitatory postsynaptic potential (EPSP) and population spike size prior to LTP induction suggested that LTP had already been partially induced following repeated seizure activity, thereby reducing the degree to which further potentiation could be elicited experimentally. The impact of ECS on hippocampal LTP may account for the memory impairment it causes in humans and other species. Consistent with this proposal, ECS treated rats show a marked spatial learning deficit in the open field water-maze over 3 days following the last of a series of 10 spaced electroconvulsive stimulations.[7] Similar findings have since been reported elsewhere.[8]

With regard to the clinical situation, the following characteristics of ECT treatment have been noted: a single seizure is usually ineffective in the treatment of depression;[9] the effects on memory and mood appear to accumulate with increasing numbers of seizures; successful treatment usually requires between four and eight treatments;[10] and the anterograde memory disturbance lasts between 1 and 12 weeks.[11] In the present study, we wished to determine whether electrophysiological findings in rats would parallel these clinical observations. Accordingly, we examined the development of the effect of ECS on EPSP size by recording evoked potentials daily in the dentate gyrus of the hippocampus throughout a series of ECS applications, and attempted to measure the duration of the effect of repeated ECS on both EPSP size and synaptic plasticity by examining separate groups of anaesthetized animals at various intervals following repeated ECS.

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Materials and Methods

Male hooded Lister rats (200–350 g) were used. ECS was induced under light halothane anaesthesia via ear-clip electrodes using a purpose built stimulator set to deliver 200 V (sine wave), 40–60 mA, for 2 s. Sham treatment involved anaesthesia and equal handling but no current was passed through the electrodes. Treatments were always given at 48-h intervals.

Development of ECS effect: Rats were prepared for chronic recording as previously described.[12] Stimulating electrodes were positioned in the perforant path (7.5 mm posterior and 4.0 mm lateral to Bregma) and recording electrodes in the hilus of the dentate gyrus (3.8 mm posterior and 2.2 mm lateral to Bregma). Rats were allowed at least 2 weeks to recover from surgery. The electrode assembly was then connected to a long flexible cable allowing the rats free movement within the recording chamber. Following a 20 min habituation period evoked potentials were generated for stimulus intensity intensities between 100 and 1000 μA (input/output curve) using a stimulus isolator (Digitimer Ltd, UK) delivering monophasic square-wave pulses of 100 μs duration at 0.067 Hz. The waveform was amplified (Grass Instruments, Quincy, MA), filtered (1 Hz–10 kHz), then sampled by computer (Acorn RISC-OS, UK) at 10 000 Hz using an analogue-to-digital converter board (Wild Vision, UK). A stimulus intensity was selected to give an 80% maximal EPSP initial rising slope and 20 evoked potentials were recorded for 5 days to provide baseline means. Subjects were then assigned in a quasi-random manner to either the ECS group or the sham group. Recordings (mean of 20 waveforms) were made 24 and 48 h after each of 10 treatments. EPSP slope and population spike values were expressed as a percentage increase over the baseline mean.

Duration of the ECS effect: Field potentials were recorded under urethane anaesthesia (1.5 g kg^{-1}) in separate groups of rats at intervals of 1, 5, 10 or 40 days after the last in a series of ECS or 5 days after a series of sham treatments. The response was monitored at low frequency for 60 min and LTP was expressed at the percentage increase in EPSP slope (mean of the last 10 samples divided by the mean of the 10 samples recorded just before tetanus). Core body temperature was maintained at 36.5°C throughout recording by a homeothermic blanket system (Harvard Apparatus Ltd, UK).

Results

Development of ECS effect on field potentials in chronically implanted animals: Throughout the experiments, ECS application reliably elicited a full tonic–clonic seizure in all animals. While the mean EPSP slope gradient value remained stable throughout the recording period in the sham group, a significant and progressive increase over time was observed in the ECS group (analysis of variance, Group $F(1,9) = 9.34$, $p < 0.05$; Time $F(19,171) = 3.42$ $p < 0.0001$; Group × Time interaction $F(19,171) = 2.74$, $p < 0.0005$) reaching a value of 34.4% (± 9.4% s.e.m.) above original baseline 24 h after the last seizure (see Fig. 1A). A subsequent analysis of simple main effects confirmed that EPSP slope values in the sham group did not differ with respect to time ($F < 1$) while the ECS group showed a significant increase ($p < 0.0001$) over time. The first significant effects were seen 24 h, but not 48 h, after the second and third seizure ($p < 0.05$) and lasting increases (beyond 48 h) did not occur until after the fourth seizure. The corresponding increase in population spike height (see Fig. 1B) during ECS treatment was also significant (ANOVA, Group $F(1,8) = 11.92$, $p < 0.01$; Time $F(19,152) = 2.32$, $p < 0.0001$; Group × Time interaction $F(19,152) = 3.1$, $p < 0.0001$) with values recorded from the ECS group reaching 70.5% (± 15.3% s.e.m.) above baseline. In the ECS group, the mean EPSP slope and population spike height values were typically higher 24 h post-seizure than at 48 h post-seizure particularly over the first five treatments. Thereafter, EPSP slope and population spike amplitude appeared to have reached a plateau with smaller and less consistent increments obtained following the last five seizures. Spontaneous seizure activity or hippocampal afterdischarge were not observed during recording.

Duration of ECS effect on EPSP slope size and LTP induction: EPSP slope size and percentage of LTP generated at various delays (1, 5, 10 and 40 days) after a series of 10 spaced ECS are displayed in Figure 2. The amount of LTP induced was least in those animals examined 1 day after the last ECS (15.3 ± 2.5%) and greatest at the longest delay (32.7 ± 3.6%) with progressive recovery seen at intermediate delays (see Fig. 2A). Differences between the groups were significant (ANOVA, $F(4,41) = 11.65$, $p < 0.0001$). *Post hoc* testing indicated that an ECS course significantly altered the amount of LTP generated by tetanus compared to sham treatment, in groups examined 1, 5 and 10 days, but not 40 days, following the final treatment (Newman–Keuls $p < 0.01$). As observed in the previous experiment, absolute EPSP slope values (see Fig. 2B) differed significantly pre-tetanus ($F(4,41) = 7.38$, $p < 0.0001$), with all ECS groups (i.e. at 1, 5, 10 and 40 days post ECS) significantly greater than the sham group (Newman–Keuls $p < 0.05$).

Discussion

In chronically implanted animals, the increase in EPSP slope developed gradually over the course of the first five seizures, and thereafter appeared to have reached a maximum. The fact that one seizure did not induce a significant change, and that increases seen 24 h after early seizures decayed back towards baseline after

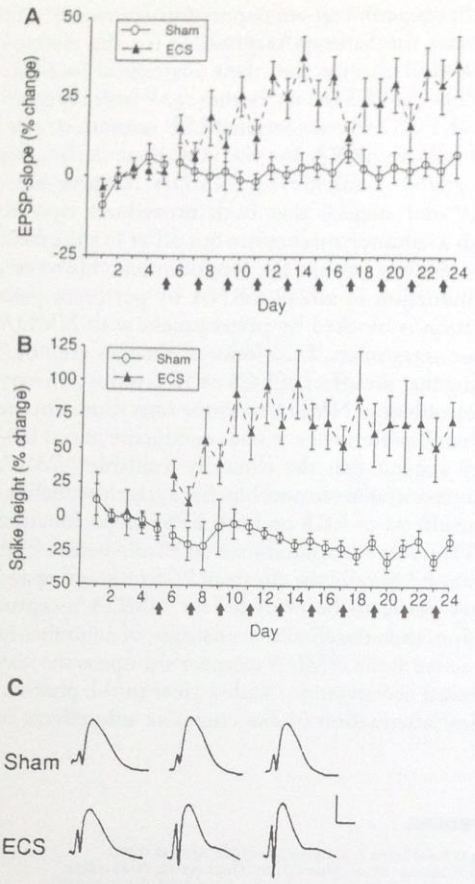

FIG. 1. (**A**) Changes in EPSP slope function expressed as a percentage of the first 5 baseline days during a course of 10 spaced ECS (filled triangles: $n = 6$) or 10 sham (open circles: $n = 5$) treatments. The arrows indicate application of either ECS or sham ECS following the relevant recording session. (**B**) Changes in population spike height in the same subjects excluding one sham subject who did not exhibit a population spike throughout the experiment. (**C**) Examples of representative waveforms from the two groups during the baseline recording phase—on the left; midway through the course of treatment—centre; and at the end of the course—far right. Calibration bar 5 mV, 5 ms.

48 h indicates that a lasting increase in EPSP slope size requires repeated seizure induction. Repeated, spaced ECS markedly elevated EPSP slope size in the dentate gyrus of the hippocampus and concomitantly reduced the degree to which LTP could be induced under anaesthesia for at least 10 days after the last seizure. The effect of repeated ECS on EPSP slope gradient was still detectable in animals studied 40 days after the last of a series of 10 ECS.

The measurement of EPSPs in freely moving animals may be confounded by activity related brain temperature artefacts,[13] but this is unlikely to be the case in this instance for two reasons. First, the concomitant rise in population spike amplitude observed throughout the study does not conform to the pattern observed for temperature related changes. While the EPSP slope increases with brain temperature, the population spike magnitude declines.[13] In contrast, following induction of LTP both EPSP slope and population spike measures increase,[5] as observed here. Second, and more compellingly, the same magnitude of EPSP slope size increase is observed following repeated ECS in anaesthetized animals (Fig. 2b), in which activity related brain temperature artefacts obviously cannot occur.

The finding of increased EPSP slope and population spike amplitude of long duration, associated with a reduction in the amount of LTP that can be obtained, is consistent with the notion that repeated ECS drives hippocampal plasticity toward its limit. This may account for some of the cognitive deficits described following repeated ECS in a variety of species. Earlier reports suggesting that driving synaptic plasticity in the hippocampus of the rat to an experimental maximum by local stimulation can occlude the formation of new spatial memories,[4,5] have proved controversial.[14] It has been suggested that 'conventional' saturation procedures may simply fail to generate sufficiently extensive effects throughout the hippocampus to consistently produce a robust spatial learning deficit.[8] Electroconvulsive stimulation, in contrast, may cause more widespread (though perhaps less complete) disruption of synaptic plasticity and therefore a greater and more reliable learning deficit. Watermaze task acquisition deficits observed following repeated ECS[7,8] may however be due to extra-hippocampal disruption, or to the effects of ECS on aspects of cerebral function other than synaptic plasticity.

While these possibilities may limit the use of ECS as an investigative tool in studying the role of hippocampal synaptic plasticity in learning and memory, important clinical implications remain. The gradual development of the ECS induced change on dentate field potentials mirrors the clinical situation in terms of the cumulative amnesic deficit and gradual recovery from depression observed. Strikingly, the number of ECS required to elevate maximally the EPSP (5) is similar to the average number of treatments usually required in ECT treatment to relieve depression (4–8).[10] Furthermore, the duration of the effect on LTP induction described here is of a similar order of magnitude to the duration of amnesic deficit described after ECT in humans (1–12 weeks)[11] and in animal studies (21 days).[15] The limited and short-lived effect on EPSP slope seen after the first seizure, when compared with the substantial and long lasting effects of 10 spaced ECS, parallels the clinical action of ECT still further and meets the important stricture that effects of ECT observed in clinical studies are only likely to be explained by biological changes which develop after more than one seizure.[9]

In drawing these parallels, we are aware that they refer to both the cognitive and therapeutic (affective) actions of ECT when used clinically as a treatment for depressive illness. Although most studies of LTP have

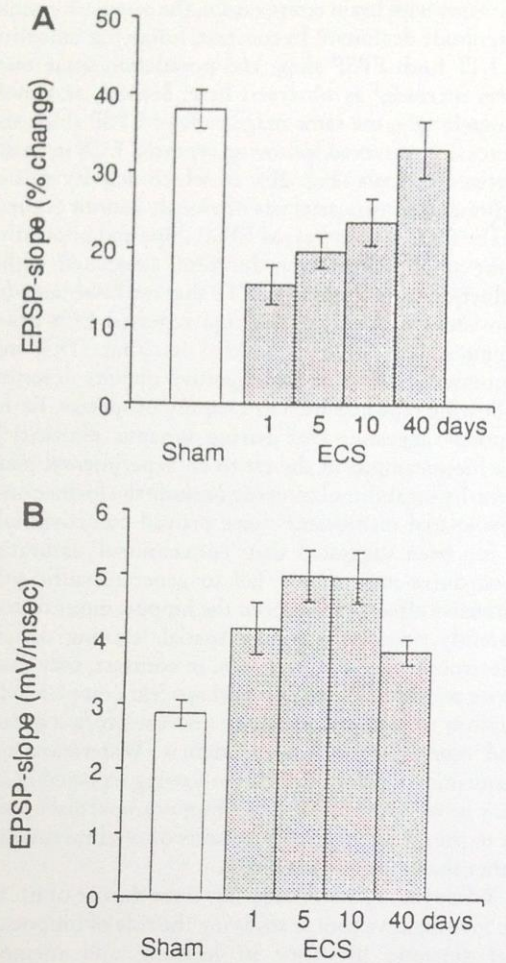

FIG. 2. (**A**) The magnitude of LTP as expressed by percentage change in EPSP slope, relative to baseline, 60 min after tetanic stimulation. (**B**) The pre-tetanus values for EPSP initial slope in all groups. In each case the white bar represents the control group receiving anaesthesia and equivalent handling only ($n = 10$) and shaded bars represent the groups treated with 10 ECS (one every 48 h) and then left for 1 ($n = 9$), 5 ($n = 10$), 10 ($n = 9$) or 40 ($n = 8$) days before electrophysiological testing.

focused on issues relating to learning and memory, a number of recent reports have implicated alterations in LTP in affective states. Studies conducted by Foy *et al*[16] and Shors *et al*[17] indicate that exposing animals to inescapable stress (the behavioural correlates of which have been used as a model of depressive illness in rats) reduces the degree to which LTP can be elicited in area CA1 of hippocampal slices. In addition, drugs active at the NMDA receptor complex have antidepressant properties in animal models of depressive illness.[18]

Further experiments are required to determine both the precise mechanisms responsible for the electrophysiological changes and their anatomical localization following ECS in rats. Barnes *et al*[8] have reported that both ECS and conventional LTP saturation techniques induce mRNA for the immediate early gene (IEG) zif268, a marker for NMDA receptor activation,[19] and suggest that both procedures operate through a common mechanism but differ in the extent of their activity within the hippocampus. However, while induction of zif268 mRNA by perforant path stimulation is blocked by pretreatment with NMDA receptor antagonists, ECS-induced changes are not,[20] implying that the effect of ECS on IEG induction may be independent of NMDA receptor activation. On the other hand, preliminary studies conducted in our laboratory suggest that the clinically available NMDA receptor associated channel blocker ketamine ameliorates the effects of ECS on both EPSP slope function and LTP in rats when administered 10 min before ECS application.[21] Should the effects of ECS on learning and memory prove to be mediated by NMDA receptor activation, then the clinical availability of a number of drugs active at the NMDA receptor site opens the way for clinical investigation, with a view to the pharmacological attenuation of the cognitive side effects of ECT.

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