Hippocampal Neurons Are Damaged by Caffeine-Augmented Electroshock Seizures

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Caffeine-augmented electroconvulsive therapy has been introduced into medical practice without experimental confirmation that such seizure modification does not result in neuronal injury. In this report rats pretreated with caffeine prior to a series of nine electrically induced convulsions showed neuronal injury confined to hippocampal sectors and striatum. Electrically induced convulsions without caffeine pretreatment did not result in injury. The potential deleterious effects of caffeine augmentation of human electroconvulsive therapy require rigorous clinical assessment.

Key Words: Caffeine, electrically induced seizures, neuronal damage, electroconvulsions, hippocampus

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Introduction

Electroconvulsive therapy (ECT) is an established and accepted treatment for major mood disorders in North American psychiatry. After an extensive review of the practice of ECT, the American Psychiatric Association published a Task Force Report that endorsed the continued use of this sometimes controversial treatment (American Psychiatric Association 1990). At the forefront of the controversy surrounding the use of ECT are the known memory-related side effects and claims that the treatment causes "brain damage" (Breggin 1979; Squire 1986).

Numerous experimental animal studies have been conducted to determine whether electrically induced convulsions (electroconvulsive stimulations or ECSs) produce neuropathological damage. Hartelius demonstrated in a cat ECS study that a small number of cortical neurons (<0.1% of those examined) were subject to possibly irreversible damage (shadow cells and neuronophagia) following 11–16 unmodified ECSs (Hartelius 1952). Colon and Notermans demonstrated in a rat ECS study that a small but statistically significant decrease in cortical neuron nuclear volume (<3%) occurred following 12 unmodified ECSs (Colon and Notermans 1975). Both of these studies, however, were done without oxygenation or muscle paralysis, modifications that are known to protect against pathologic changes due to prolonged seizures (Meldrum et al 1975). With the exception of these two reports, controlled ECS studies have failed to demonstrate differences between control and experimental animals with regard to irreversible changes (Weiner 1984). An especially noteworthy study by Dam et al found that rats treated with three ECSs per day to a total of 140 seizures did not suffer hippocampal neuron loss (Dam et al 1980). A recent comprehensive review of animal ECS and human ECT literature concluded that there is no credible evidence that

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ECS in animals or ECT in humans causes structural brain damage (Devanand et al 1994).

In recent years, the practice of augmenting ECT seizures with methylxanthines (caffeine, theophylline) has been developed (Calev et al 1993; Coffey et al 1987; Hinkle et al 1987; Shapira et al 1987; Swartz and Lewis 1991). The pharmacologic effects of methylxanthines include (but are not limited to) adenosine receptor blockade (Snyder et al 1981). Since a substantial body of evidence suggests that adenosine is a neuroprotective substance (Dragunow and Faull 1988), the use of adenosine antagonists in ECT is a potential cause for concern. This study was undertaken to examine the histopathological effects of caffeine-augmented electrically induced seizures.

Materials and Methods

Male Sprague–Dawley rats weighing 250–350 grams were randomly assigned to one of four experimental groups, each receiving a total of nine genuine ECSs or sham ECSs. For each of the nine treatments, the rats were preoxygenated with 100% O_2 for 5 min, and pretreated with atropine (0.05 mg/kg). Anesthesia was induced using isoflurane before endotracheal intubation. The animals were then ventilated with oxygen and 1–2% isoflurane. Expired CO_2 was continuously monitored using a Beckman CO_2 analyzer, and the ventilatory rate was adjusted to maintain end tidal CO_2 at 4.5–5.5%. During each series of experiments an arterial blood gas sample was obtained from 1 rat to confirm adequate ventilation and oxygenation. For each rat, venous blood was obtained for determination of blood glucose level. Body temperature was continuously monitored and maintained at 37.5°C. When stable, the rats received an intraperitoneal dose of sodium thiopenthal (30 mg/kg) and succinylcholine (0.45 mg/kg). Isoflurane was discontinued 3 min after the intraperitoneal injections, and endotracheal ventilation was maintained until recovery from the anesthetic.

The nine treatments were conducted three times per

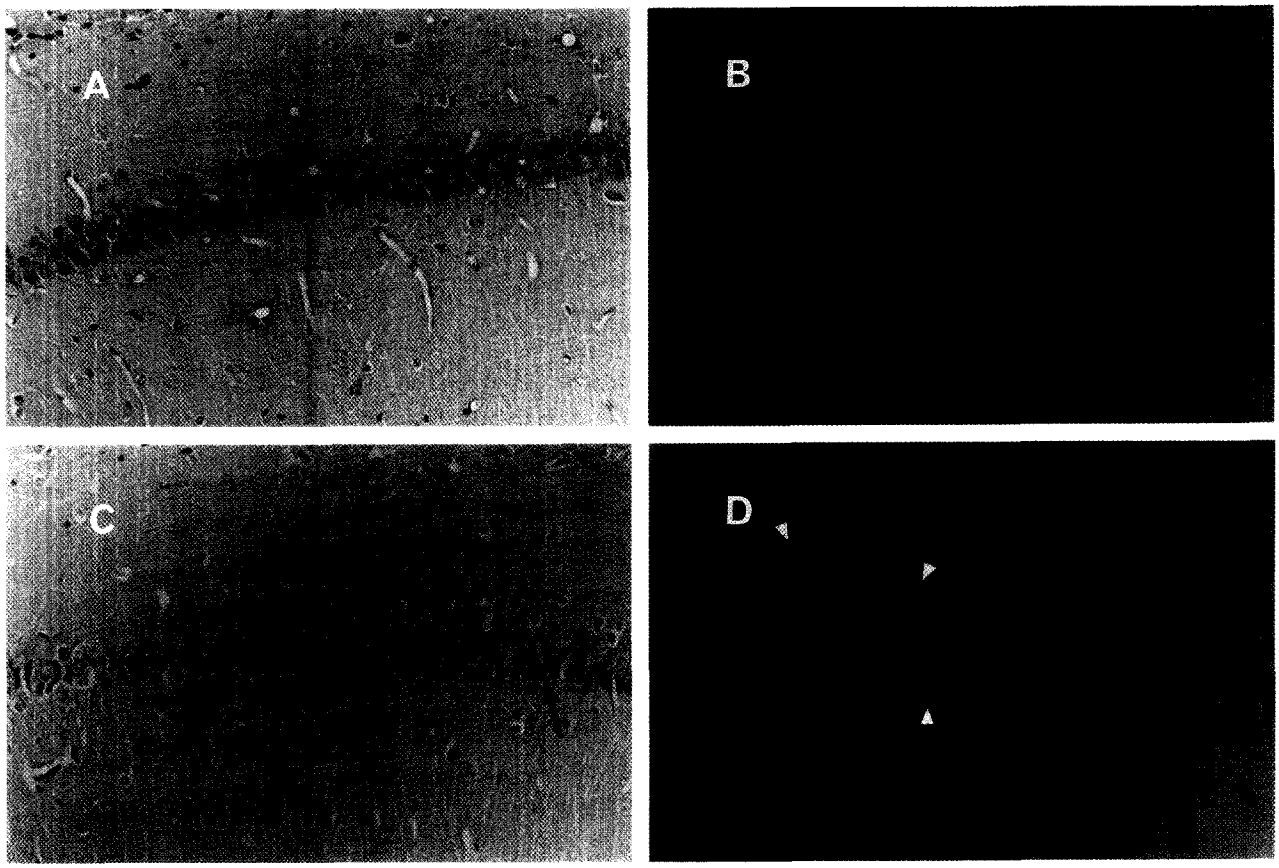

Figure 1. Effect of caffeine-augmented electroshock seizures on hippocampal CA1 sector morphology. **(A), (B)** Control: anesthetic only. **(C), (D)** Caffeine-augmented electroconvulsive stimulation. Hematoxylin/eosin [**(A), (C)**] and glial fibrillary acid protein [**(B), (D)**] stained sections show neuronal injury characterized by retraction of the cell body, eosinophilia of the cytoplasm, deformation of the nucleus [black arrows, **(C)**], and associated glial cell activation/mobilization [white arrow heads, **(D)**] in the caffeine-augmented electroconvulsive-stimulated rat. Original magnification ×200.

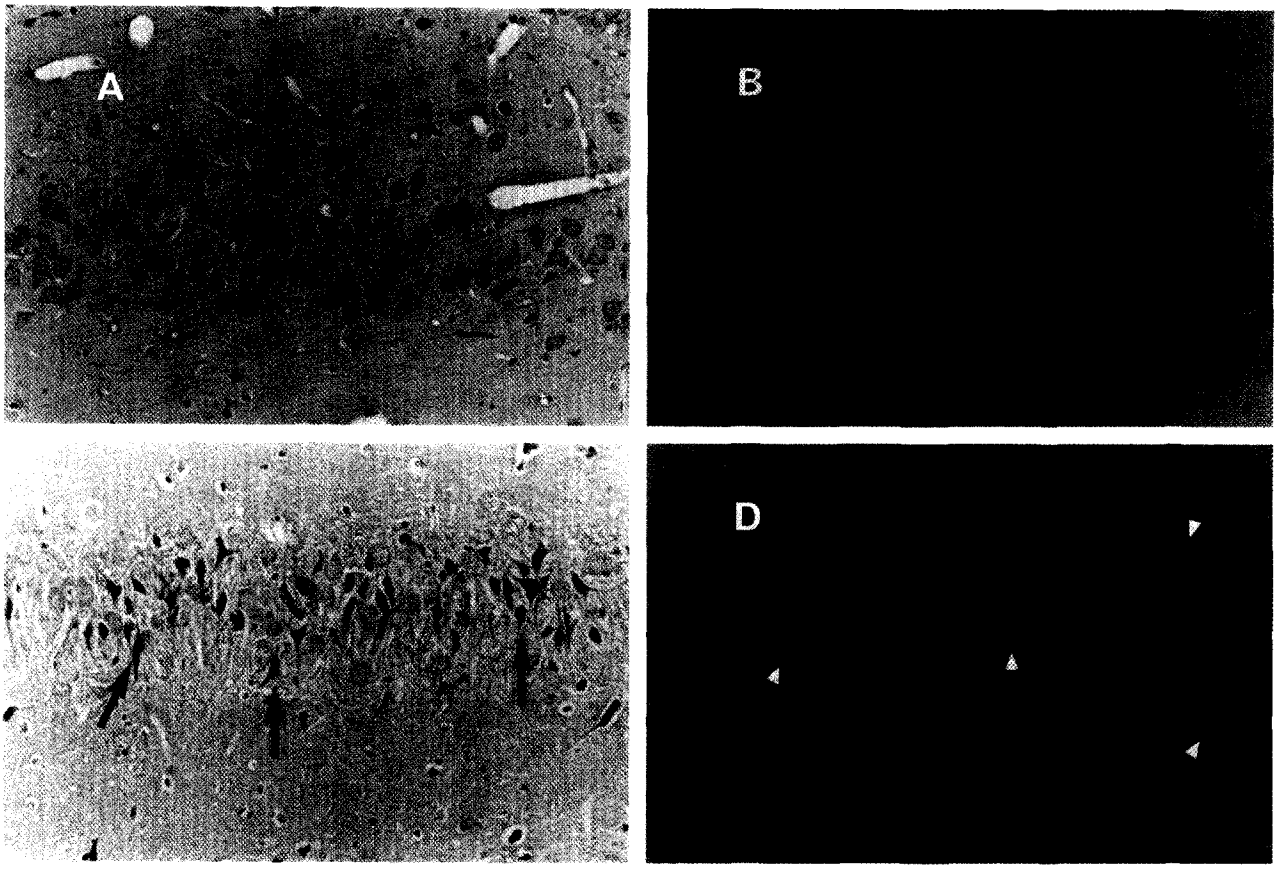

Figure 2. Effect of caffeine-augmented electroshock seizures on hippocampal CA4 sector morphology. **(A), (B)** Control: anesthetic only. **(C), (D)** Caffeine-augmented electroconvulsive stimulation. Hematoxylin/eosin [**(A), (C)**] and glial fibrillary acid protein [**(B), (D)**] stained sections show neuronal injury [black arrows, **(C)**] and associated glial cell activation/mobilization [white arrow heads, **(D)**] in the caffeine-augmented electroconvulsive-stimulated rat. Compared to the CA1 sector of the same rat (Figure 1), gliosis was more evident in the CA4 sector. Original magnification ×200.

week for 3 weeks as follows. One group of animals (n = 11) was treated with the anesthetic protocol only. The second group (n = 11) received the anesthetic plus an intraperitoneal dose of caffeine (15 mg/kg) at the time of preoxygenation. The third group (n = 12) received the anesthetic plus an ECS treatment 5 min after the administration of succinylcholine. ECS was administered using ear clip electrodes and a brief pulse alternating current stimulus. Stimulus parameters were: frequency 60 Hz; pulse width 1.0 msec; duration 1.25 sec; current 90 mA. The fourth group (n = 12) received both caffeine and an ECS treatment as described. In every instance, ECS resulted in tonic flexion/extension and clonic motor seizure activity, which was partially modified by muscle paralysis. Seizure activity was recorded using needle scalp electrodes.

Following the ninth treatment, each animal was sacrificed by perfusion–fixation with 10% buffered formaldehyde. The brain was fixed with the same solution for a further 2 weeks prior to sectioning. Paraffin sections (8 μm thick) were cut and stained with hematoxylin and eosin. All sections were examined to determine the distribution and extent of neuronal damage. Neuronal injury was quantitated using a standard section of the hippocampus by direct visual counting of acidophilic and/or pyknotic neurons. The examiner was blind to the identity of the sections examined. The presence of reactive gliosis was substantiated with glial fibrillary acid protein (GFAP) stains.

Results

Body weight, blood glucose, temperature, PaO_2, $PaCO_2$, and duration of anesthetic are presented in Table 1, showing no significant differences between groups. The seizure duration in the anesthesia–ECS group was 9.8 ± 2.1 sec, compared with 14.0 ± 2.2 sec in the anesthesia–caffeine–ECS group (t test, $p < .001$).

Experimental groups that did not receive ECS and the ECS group not pretreated with caffeine showed occasional

Table 1. Physiological Variables (Mean ± SEM) in the Experimental Groups

	Experimental groups			
	Group 1 (n = 11) (anesthetic only)	Group 2 (n = 12) (anesthetic & ECS)	Group 3 (n = 11) (anesthetic & caffeine)	Group 4 (n = 12) (anesthetic & caffeine & ECS)
Weight (g)				
Day 1	313 ± 7	318 ± 4	348 ± 13	342 ± 12
Day 9	342 ± 5	340 ± 6	357 ± 10	350 ± 8
Blood glucose (mmol/L)	4.6 ± 0.2	4.7 ± 0.2	5.3 ± 0.2	5.2 ± 0.1
Temperature (°C)	37.72 ± 0.04	37.80 ± 0.05	37.40 ± 0.05	37.70 ± 0.05
PaO_2 (mmHg)	215 ± 11	228 ± 7	223 ± 10	231 ± 9
$PaCO_2$ (mmHg)	38.8 ± 0.3	39.1 ± 0.3	37.3 ± 0.6	38.2 ± 0.4
Duration of anesthetic (min)	11.6 ± 0.6	11.7 ± 0.5	11.5 ± 0.6	11.6 ± 0.4

There are no significant differences between groups.
ECS = electroconvulsive stimulation.

injured neurons in the hippocampal sectors (Table 2). Injury was more severe and extensive in the caffeine-pretreated ECS group. Severe neuronal injury in the hippocampus was characterized by retraction of the cell body, eosinophilia of the cytoplasm, disappearance of Nissl bodies, deformation of the nucleus, and associated glial cell activation/mobilization (Figures 1 and 2). Within the striatum, neuronal injury was mild and confined to 4 of 11 animals in the caffeine–ECS group. In this region, injury was restricted to small and medium-sized neurons. In all groups, mild Purkinje cell injury was observed confined to the vermis with no significant intergroup differences. Neocortical injury was not evident in any of the experimental groups.

Discussion

This study demonstrates that caffeine-augmented ECS causes neuronal damage in hippocampal sectors and striatum, sparing the neocortex. The histopathological findings are consistent with irreversible neuronal damage (Brown and Brierley 1972; Bubis et al 1976; Kirino and Sano 1984; Nadler et al 1978; Olney 1969). As documented in previous reports (Devanand et al 1994), there was no evidence of neuronal damage with ECS alone. Neuronal injury occurred in the caffeine–ECS group despite the use of seizure modification (oxygenation, barbiturate anesthesia, muscle relaxation), controlled physiologic conditions (blood glucose, body temperature, ventilation), moderately suprathreshold electrical stimulus intensity, and a clinically relevant treatment schedule.

Caution is advisable when attempting to extrapolate from an animal model to the human condition. The results of the present study are strengthened, however, by the use of methods that reproduce as closely as possible the conditions of human ECT and extensive physiologic control and monitoring. It should be acknowledged that the dose of caffeine used in the present study (15 mg/kg) was higher than the usual dose of caffeine used to augment human ECT seizures; however, clinical reports have indicated the use of as much as 750 mg of caffeine (Coffey et al 1987) or 2000 mg of caffeine–sodium benzoate (968 mg caffeine) (Shapira et al 1987) to augment ECT seizures. Also, there is evidence that different species (or even different strains) of mammals differ in their sensitivity to the central nervous system toxicity of caffeine (Seale et al 1984). This may limit the generalizability of the present findings. The data presented herein show a statistically nonsignificant trend toward more injured neurons in the CA1 hippocampus in the anesthesia–caffeine group, which could conceivably indicate some intrinsic toxicity of the caffeine dose on its own.

It has been demonstrated previously that acute caffeine pretreatment accelerates ischemic neuronal injury (Sutherland et al 1991). The ability of caffeine to promote neuronal injury in both repeated ECSs and ischemia is consistent with the evidence that implicates adenosine as a neuroprotective substance (Dragunow and Faull 1988) and the known ability of methylxanthines to block adenosine receptors (Snyder et al 1981). Both the hippocampus and striatum have been shown to have a high concentration of adenosine A1 receptors that are up-regulated following chronic caffeine treatment (Daval et al 1989; Onodera and Kogure 1985; Sutherland et al 1991).

Hippocampal and striatal neurons are known to be selectively vulnerable to damage during repeated epileptic seizures (Dam 1980), hypoglycemia (Auer et al 1984), status epilepticus (Lothman and Collins 1981; Nevander et al 1985), and forebrain ischemia (Smith et al 1984; Sutherland et al 1991; Suzuki et al 1983). Neuronal hyperexcitability has been shown to be an important factor contributing to neuronal injury in these pathological states (Dunwiddie 1985; Ingvar et al 1988; Nevander et al 1985; Suzuki et al 1983). The neuroprotective properties of

Table 2. Quantitated Neuronal Injury in Experimental Groups

Region	Experimental groups			
	Group 1 (n = 11) (Anesthetic only)	Group 2 (n = 12) (anesthetic & ECS)	Group 3 (n = 11) (anesthetic & caffeine)	Group 4 (n = 12) (anesthetic & caffeine & ECS)
CA1	7 ± 3	7 ± 4	18 ± 5	26 ± 8[a]
CA2/3	2 ± 1	0.6 ± 0.3	3.1 ± 0.7	25 ± 9[b]
CA4	0.09 ± 0.09	0.6 ± 0.4	1.1 ± 0.5	10 ± 4[c]
Striatum	0	0	0	0.3 ± 0.1[d]

For hippocampal sectors, data represent percentage of injured neurons (± SEM) in a standard section. In the striatum, neuronal injury was graded as follows: <10% = 1, 10–50% = 2, 50–100% = 3. Differences between groups were assessed by analysis of variance followed by Scheffe's test for multiple comparisons.
ECS = electroconvulsive stimulation.
[a] $p < .05$ group 4 different from groups 1 and 2.
[b] $p < .02$ group 4 different from all other groups.
[c] $p < .01$ group 4 different from all other groups.
[d] $p < .05$ group 4 different from all other groups.

adenosine may be related to inhibition of neuronal excitability both directly (Dunwiddie 1985) and by inhibiting release of excitatory amino acid neurotransmitters (Corradetti et al 1984). Thus the mechanism by which caffeine promotes neuronal injury may involve dysregulation of adenosine-modulated neuronal excitability. Caffeine is also known to enhance the release of intracellular calcium stores, which is another mechanism by which neuronal damage may be produced (Nehlig et al 1992). Caffeine–ECS-treated rats had significantly longer seizures than animals in the ECS alone group. This modest prolongation of seizure duration by itself is unlikely to account for the present findings; prolonged periods of status epilepticus are required to produce hippocampal neuronal injury (Ingvar et al 1988).

The practice of methylxanthine enhancement of ECT seizures developed without preclinical studies to document its safety. The results of the present study suggest the possibility that clinical use of caffeine-enhanced ECT may result in hippocampal and striatal neuronal damage. This is particularly noteworthy considering the generally accepted role of the hippocampus as a major neuroanatomical region subserving memory function (Olton et al 1979). Investigators to date have not reported increased memory side effects of ECT when caffeine augmentation was used (Calev et al 1993; Coffey et al 1990; Shapira et al 1987). In fact, one open trial found modestly decreased cognitive effects of caffeine-augmented ECT (Calev et al 1993). Further detailed studies of the effects of caffeine-enhanced clinical ECT including longer-term follow-up are needed.

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