

Research Report

Caspase-dependent programmed cell death pathways are not activated in generalized seizure-induced neuronal death

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ABSTRACT

Activation of the caspase-dependent cell death pathways has been shown in focal seizures, but whether this occurs in prolonged generalized seizures is not known. We investigated whether the initiator caspase in the extrinsic pathway, caspase-8, or the intrinsic pathway, caspase-9, is activated during the first 24 h following lithium–pilocarpine-induced status epilepticus, when neuronal death is maximal and widespread. The thymuses of rats given methamphetamine were used as positive controls for caspase-3-activated cellular apoptosis. Following methamphetamine treatment, caspase-9 but not caspase-8 was activated in thymocytes. However, 6 or 24 h following status epilepticus, none of 26 brain regions studied showed either caspase-8 or -9 activation by immunohistochemistry, western blotting and enzyme activity assays. Our results provide evidence against the activation of the extrinsic and intrinsic caspase pathways in generalized seizures, which produce morphologically necrotic neurons with internucleosomal DNA cleavage (DNA laddering), a programmed process. In contrast, there is increasing evidence that caspase-independent programmed mechanisms play a prominent role in seizure-induced neuronal death.

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1. Introduction

Based upon a developmental classification of cell death (Clarke, 1990), three major morphological subtypes are currently recognized: apoptotic (type I), autophagic (type II) and necrotic (type IIIb). In recent years attention has been focused on the programmed mechanisms contributing to apoptotic cell death—more specifically, the intrinsic (mitochondrial) and extrinsic (Fas death receptor-mediated) caspase-dependent pathways (Cohen, 1997; Earnshaw et al., 1999; Philchenkov, 2004; Reed, 2000; Riedl and Shi, 2004; Stefanis, 2005; Zimmerman et al., 2001). The notion that pathologically

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Abbreviations: Ac-DEVD-AFC, acetyl-aspartyl-glutamyl-valyl-aspartyl-7-amino-4-trifluoromethylcoumarin; Z-IETD-AFC, carbobenzoxy-isoleucyl-glutamyl-threonyl-aspartyl-7-amino-4-trifluoromethylcoumarin; Ac-LEHD-AFC, acetyl-leucyl-glutamyl-histidyl-aspartyl-7-amino-4-trifluoromethlcoumarin; CAD/ICAD, caspase-activated DNase and inhibitor of caspase-activated DNase; DFF40/45, 40 kDa and 45 kDa DNA fragmentation factors; KA, kainic acid; LM, light microscopy; LPC, lithium–pilocarpine; METH, methamphetamine; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline+0.2% Tween-20; PC, pilocarpine; PCD, programmed cell death; SE, status epilepticus; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling

induced neuronal death is apoptotic was based initially upon application of two techniques thought at the time to be specific for apoptotic cell death, terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL), labeling doublestranded DNA fragments (Gavrielli et al., 1992), and agarose gel electrophoresis, showing 180 base pair, internucleosomal DNA cleavage or DNA "laddering" (Wyllie, 1980). However, both TUNEL positivity and DNA laddering may be found in necrotic, as well as apoptotic, cells (Fujikawa et al., 1999, 2000, 2002). This, plus a lack of attention to the morphological similarities and differences between apoptotic and necrotic cells, and controversy regarding the activation of caspase-dependent pathways in excitotoxic neuronal death, has created confusion in the literature (Fujikawa, 2000, 2002; Roy and Sapolsky, 1999; Sloviter, 2002).

The conventional view that necrotic cell death is a passive process in which cells swell, then lyse, has been called into question by accumulating evidence that necrotic cell death may involve caspase-independent, programmed mechanisms (Denecker et al., 2001; Kitanaka and Kuchino, 1999; Leist and Jäättelä, 2001; Proskuryakov et al., 2003). Seizure-induced neuronal death is morphologically necrotic (Fujikawa et al., 1999, 2000, 2002), but involves programmed processes such as DNA laddering (Filipkowski et al., 1994; Fujikawa et al., 1999, 2000, 2002; Kondratyev and Gale, 2000; Kondratyev et al., 2002; Pollard et al., 1994).

There is conflicting information as to whether the central effector caspase, caspase-3, contributes to neuronal death from prolonged seizures, or status epilepticus (SE) (Ananth et al., 2001; Fujikawa et al., 2002; Henshall et al., 2000; Kondratyev and Gale, 2000; Narkilahti et al., 2003; Puig and Ferrer, 2002; Weise et al., 2005). However, even if caspase-3 does not contribute to SE-induced neuronal death, this does not rule out activation of either caspase-9 or caspase-8, upstream cysteine proteases in the intrinsic mitochondrial and Fas death receptor extrinsic caspase-dependent pathways respectively. For example, it was recently shown in an adult model of hypoxia-ischemia that both caspase-8 and -9 are activated without activation of caspase-3 (Adhami et al., 2006). Both caspase-8 and -9 are activated in a model of focal seizures (Henshall et al., 2001a,b; Li et al., 2006), but unlike caspase-3, there are no reports of whether either is activated or not in generalized seizure-induced neuronal death. We show for the first time that neither caspase-8 nor -9 is activated during the first 24 h following generalized seizures, when neuronal necrosis is maximal and widespread. This suggests that caspase-independent mechanisms are involved in producing generalized seizure-induced neuronal necrosis with DNA laddering. In fact, there is increasing evidence that caspase-independent mechanisms play a prominent role in seizure-induced neuronal death (see Discussion).

2. Results

2.1. Extent of neuronal damage 6 and 24 h following SE

Six hours following SE, 15 of 26 brain regions examined showed a significant number of acidophilic neurons by H&E

stain compared to controls (Table 1), ranging from 10 to 25% (e.g., the ventral subiculum, rhinal and entorhinal cortex, neocortex and septal nuclei) to more than 50% (e.g., the ventral hippocampal dentate hilus). We have shown previously that these acidophilic neurons are necrotic by ultrastructural examination in lithium–pilocarpine-induced SE (LPCSE) (Fuji-

Brain region6 h recers24 h recoveryControlSEControlSEDorsal hippocampus0.5 ±00.0 ±01.0 ±0.5**CA10.0 ±00.2 ±0.20.0 ±00.5 ±0CA20.0 ±00.2 ±0.20.0 ±00.5 ±0CA30.0 ±00.5 ±00.0 ±00.5 ±0Dorsal dentate gyrus0.1 ±0.6**0.0 ±00.7 ±0.2*Hilus0.0 ±01.7 ±0.6**0.0 ±00.7 ±0.2*Hilus0.0 ±01.5 ±00.2 ±0.2**0.1 ±0.2**CA10.0 ±00.5 ±00.0 ±02.3 ±0.2***.1**CA20.0 ±00.5 ±00.2 ±0.2**0.1 ±0.2**CA10.0 ±00.5 ±00.2 ±0.2***.1**CA30.0 ±00.3 ±0.40.402.7 ±0.2***.1**CA30.0 ±00.5 ±00.5 ±0.00.5 ±0.0Dentate granule cels0.401.3 ±0.2***0.400.5 ±0.0Hilus0.401.3 ±0.2***0.401.5 ±0.5****Amygdala0.401.3 ±0.2***0.401.5 ±0.5****Piriform cortex0.401.0 ±0.0*0.401.5 ±0.5****Nucleus0.401.2 ±0.2***0.401.5 ±0.5****Hilan0.401.2 ±0.2***0.401.5 ±0.5****Indertification0.401.2 ±0.2***0.401.5 ±0.2****Indertification0.401.2 ±0.2***0.401.5 ±0.2****Indertification0.401.2 ±0.2***0.401.5 ±0.2****	Table 1 – Damage scores by H&E stain					
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Subiculum 0 ± 0 $1\pm 0.2^{**}$ 0 ± 0 $0.7\pm 0.2^*$ CA1 0 ± 0 0.5 ± 0 0 ± 0 $2.3\pm 0.2^{***,1+1}$ CA2 0 ± 0 0.3 ± 0.4 0 ± 0 $2.7\pm 0.0^{***,1+1+1}$ CA3 0 ± 0 0.3 ± 0 0 ± 0 $1.7\pm 0.2^{***,1+1+1}$ Ventral dentate gyrus $Dentate granule cells$ 0 ± 0 0.7 ± 0 0 ± 0 Hilus 0 ± 0 0.7 ± 0 0 ± 0 0.5 ± 0.0 Hilus 0 ± 0 $2.8\pm 0^{***}$ 0 ± 0 $2.8\pm 0.2^{***}$ Amygdala 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $1.0\pm 0.2^{**}$ Piriform cortex 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $2.3\pm 0.0^{***,1+}$ Dorsal endopiriform 0 ± 0 $2.2\pm 0.2^{**}$ 0 ± 0 $1.5\pm 0.5^{***+}$ nucleus $U= 0$ $1.0\pm 0.6^{**}$ 0 ± 0 $1.5\pm 0.5^{***+}$ ND nucleus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1+}$ LP nucleus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1+}$ LD nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $0.8\pm 0.0^{**}$ Centromedian 0 ± 0 $0.22\pm 0.2^{***}$ 0 ± 0 $0.8\pm 0.0^{**}$ Frontoparietotemporal 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex $S=$ $S=$ $S=$ $S=$ Septal nuclei 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex $S=$ $S=$ $S=$ $S=$ Substantia nigra $S=$ $S=$ $S=$ $S=$ <	Hilus	0 ± 0	$1.7 \pm 0.6^{***}$	0 ± 0	1.3±0.9***	
CA1 0 ± 0 0.5 ± 0 0 ± 0 $2.3\pm 0.2^{***,1++}$ CA2 0 ± 0 0.3 ± 0 0 ± 0 $2.7\pm 0.0^{***,1++}$ CA3 0 ± 0 0.3 ± 0 0 ± 0 $1.7\pm 0.2^{***,1++}$ Ventral dentate gyrus $Dentate granule cells$ 0 ± 0 0.7 ± 0 0 ± 0 0.5 ± 0.0 Hilus 0 ± 0 $2.8\pm 0^{***}$ 0 ± 0 $2.8\pm 0.2^{***}$ Amygdala 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $2.8\pm 0.2^{***}$ Amygdala 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $1.0\pm 0.2^{**}$ Piriform cortex 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $1.5\pm 0.5^{***+}$ Dorsal endopiriform 0 ± 0 $2.2\pm 0.2^{**}$ 0 ± 0 $1.5\pm 0.5^{***+}$ nucleus $Hinal cortex$ 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.2\pm 0.5^{***+}$ MD nucleus 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ LP nucleus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1^+}$ LD nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1^+}$ LD nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $0.8\pm 0.0^{**}$ Centromedian 0 ± 0 0.5 ± 0.0 0.2 ± 0.2 0.2 ± 0.2 nucleus 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex $Septal nuclei$ 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 Septal nuclei 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex $Septal nuclei$ 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 </td <td>Ventral hippocampus</td> <td></td> <td></td> <td></td> <td></td>	Ventral hippocampus					
CA2 0 ± 0 0.3 ± 0.4 0 ± 0 $2.7\pm0.0^{***,1+1}$ CA3 0 ± 0 0.3 ± 0 0 ± 0 $1.7\pm0.2^{***,1+1}$ Ventral dentate gyrus U 0 ± 0 0.5 ± 0.0 Hilus 0 ± 0 $2.8\pm0^{***}$ 0 ± 0 $2.8\pm0.2^{***}$ Amygdala 0 ± 0 $1.3\pm0.2^{***}$ 0 ± 0 $2.8\pm0.2^{***}$ Amygdala 0 ± 0 $1.3\pm0.2^{***}$ 0 ± 0 $2.3\pm0.0^{***,1+1}$ Dorsal endopiriform 0 ± 0 $2.2\pm0.2^{**}$ 0 ± 0 $1.0\pm0.2^{**}$ Piriform cortex 0 ± 0 $1.2\pm0.2^{***}$ 0 ± 0 $1.5\pm0.5^{***+1}$ Dorsal endopiriform 0 ± 0 $2.2\pm0.2^{**}$ 0 ± 0 $1.5\pm0.5^{***+1}$ Dorsal endopiriform 0 ± 0 $1.0\pm0.6^{**}$ 0 ± 0 $1.2\pm0.4^{***}$ Nucleus 0 ± 0 $1.0\pm0.0^{**}$ 0 ± 0 $1.2\pm0.4^{***}$ MD nucleus 0 ± 0 $1.2\pm0.2^{***}$ 0 ± 0 $1.3\pm0.5^{***}$ LP nucleus 0 ± 0 0.2 ± 0.2 0.2 ± 0.2 0.2 ± 0.2 nucleus 0 ± 0 0.2 ± 0.2 0.2 ± 0.2 0.2 ± 0.2	Subiculum	0 ± 0	1±0.2**	0 ± 0		
CA3 0 ± 0 0.3 ± 0 0 ± 0 $1.7\pm 0.2^{***,111}$ Ventral dentate gyrusDentate granule cells 0 ± 0 0.7 ± 0 0 ± 0 0.5 ± 0.0 Hilus 0 ± 0 $2.8\pm 0^{***}$ 0 ± 0 $2.8\pm 0.2^{***}$ Amygdala 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $2.8\pm 0.2^{***}$ Amygdala 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $1.0\pm 0.2^{**}$ Piriform cortex 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $2.3\pm 0.0^{***,111}$ Dorsal endopiriform 0 ± 0 $2.2\pm 0.2^{**}$ 0 ± 0 $1.5\pm 0.5^{***1}$ nucleus 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.5\pm 0.5^{***1}$ Rhinal cortex 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ Thalamus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.3\pm 0.5^{***}$ LP nucleus 0 ± 0 $1.2\pm 0.4^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1}$ LD nucleus 0 ± 0 $0.2\pm 0.4^{***}$ 0 ± 0 $0.8\pm 0.2^{**}$ Centromedian 0 ± 0 0.0 ± 0 0.2 ± 0.2 0.2 ± 0.2 nucleus 0 ± 0 $2.3\pm 0.2^{***}$ 0 ± 0 0.5 ± 0.0 cortexSeptal nuclei 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortexSeptal nuclei 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 substantia nigra p 0 ± 0 0.3 ± 0.2 0 ± 0 $0.7\pm 0.2^{**}$ Francomparte 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortexSeptal nuclei 0 ± 0 0	CA1	0 ± 0	0.5 ± 0	0±0		
Ventral dentate gyrusDentate granule cells 0 ± 0 0.7 ± 0 0 ± 0 0.5 ± 0.0 Hilus 0 ± 0 $2.8\pm 0^{***}$ 0 ± 0 $2.8\pm 0.2^{***}$ Amygdala 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $1.0\pm 0.2^{**}$ Piriform cortex 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $2.3\pm 0.0^{***,1†}$ Dorsal endopiriform 0 ± 0 $2.2\pm 0.2^{**}$ 0 ± 0 $1.5\pm 0.5^{***†}$ nucleus 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.5\pm 0.5^{***†}$ Rhinal cortex 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ Thalamus 0 ± 0 $1.0\pm 0.0^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ MD nucleus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.3\pm 0.5^{***}$ LP nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1}$ LD nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $0.8\pm 0.2^{**}$ reuniens nucleus 0 ± 0 0.0 ± 0.0 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 $0.2\pm 0.2^{***}$ 0 ± 0 0.5 ± 0.0 cortex $Septal nuclei$ 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 Septal nuclei 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex $Septal nuclei$ 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 Substantia nigra $Pars$ 0 ± 0 0.0 ± 0.0 0 ± 0 $0.2\pm 0.2^{**}$	CA2	0 ± 0	0.3 ± 0.4	0 ± 0		
Dentate granule cells 0 ± 0 0.7 ± 0 0 ± 0 0.5 ± 0.0 Hilus 0 ± 0 $2.8\pm 0^{***}$ 0 ± 0 $2.8\pm 0.2^{***}$ Amygdala 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $1.0\pm 0.2^{**}$ Piriform cortex 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $2.3\pm 0.0^{***,1\dagger}$ Dorsal endopiriform 0 ± 0 $2.2\pm 0.2^{**}$ 0 ± 0 $1.5\pm 0.5^{***\dagger}$ nucleus 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.5\pm 0.5^{***\dagger}$ Rhinal cortex 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 0.5 ± 0.0 Entorhinal cortex 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ Thalamus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.3\pm 0.5^{***}$ LP nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1}$ LD nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $0.8\pm 0.2^{**}$ Centromedian 0 ± 0 0.0 ± 0.0 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 $0.2\pm 0.2^{***}$ 0 ± 0 0.5 ± 0.0 cortex $Septal nuclei$ 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 Septal nuclei 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex $Septal nuclei$ 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 Substantia nigra $Pars$ compacta 0 ± 0 0.0 ± 0.0 0 ± 0	CA3	0 ± 0	0.3 ± 0	0±0	1.7±0.2***,†††	
Hilus 0 ± 0 $2.8\pm 0^{***}$ 0 ± 0 $2.8\pm 0.2^{***}$ Amygdala 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $1.0\pm 0.2^{**}$ Piriform cortex 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $2.3\pm 0.0^{***,1\dagger}$ Dorsal endopiriform 0 ± 0 $2.2\pm 0.2^{**}$ 0 ± 0 $1.5\pm 0.5^{***\dagger}$ nucleus ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.5\pm 0.5^{***\dagger}$ Rhinal cortex 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 0.5 ± 0.0 Entorhinal cortex 0 ± 0 $1.0\pm 0.0^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ Thalamus ± 0 $1.0\pm 0.0^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ MD nucleus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.3\pm 0.5^{***}$ LP nucleus 0 ± 0 $2.2\pm 0.4^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1}$ LD nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $0.8\pm 0.2^{**}$ Centromedian 0 ± 0 0.0 ± 0.0 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 0.2 ± 0.2 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 $0.2\pm 0.2^{***}$ 0 ± 0 $0.2\pm 0.2^{***}$ Frontoparietotemporal 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 5 ± 0.0 0.5 ± 0.0 0.5 ± 0.0 0.5 ± 0.0 cortex $5\pm 0.2^{***}$ 0 ± 0 0.5 ± 0.0 Septal nuclei 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex $5\pm 0.2^{***}$ 0 ± 0 0.5 ± 0.0 0.5 ± 0.0 Substantia nigra $2\pm 0.2^{**}$	Ventral dentate gyrus					
Amygdala 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $1.0\pm 0.2^{**}$ Piriform cortex 0 ± 0 $1.3\pm 0.2^{***}$ 0 ± 0 $2.3\pm 0.0^{***,1^{\dagger}}$ Dorsal endopiriform 0 ± 0 $2.2\pm 0.2^{**}$ 0 ± 0 $1.5\pm 0.5^{***^{\dagger}}$ nucleus $1.0\pm 0.6^{**}$ 0 ± 0 $1.5\pm 0.5^{***^{\dagger}}$ Rhinal cortex 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 0.5 ± 0.0 Entorhinal cortex 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ Thalamus ± 0 $1.0\pm 0.0^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ MD nucleus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.3\pm 0.5^{***}$ LP nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1}$ LD nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $0.8\pm 0.2^{**}$ Centromedian 0 ± 0 0.0 ± 0.0 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 0.2 ± 0.2 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 $0.2\pm 0.2^{***}$ 0 ± 0 $0.2\pm 0.2^{***}$ Frontoparietotemporal 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex <td< td=""><td>Dentate granule cells</td><td>0 ± 0</td><td>0.7 ± 0</td><td>0±0</td><td>0.5 ± 0.0</td></td<>	Dentate granule cells	0 ± 0	0.7 ± 0	0±0	0.5 ± 0.0	
Piriform cortex 0 ± 0 $1.3 \pm 0.2^{***}$ 0 ± 0 $2.3 \pm 0.0^{***,\dagger\dagger}$ Dorsal endopiriform 0 ± 0 $2.2 \pm 0.2^{**}$ 0 ± 0 $1.5 \pm 0.5^{***\dagger}$ nucleus Rhinal cortex 0 ± 0 $1.0 \pm 0.6^{**}$ 0 ± 0 0.5 ± 0.0 Entorhinal cortex 0 ± 0 $1.0 \pm 0.6^{**}$ 0 ± 0 $1.2 \pm 0.4^{***}$ Thalamus mD nucleus 0 ± 0 $1.2 \pm 0.2^{***}$ 0 ± 0 $1.3 \pm 0.5^{***}$ LP nucleus 0 ± 0 $1.2 \pm 0.2^{***}$ 0 ± 0 $1.5 \pm 0.2^{***,1}$ LD nucleus 0 ± 0 $1.2 \pm 0.6^{***}$ 0 ± 0 $1.5 \pm 0.2^{***,1}$ LD nucleus 0 ± 0 $1.2 \pm 0.6^{***}$ 0 ± 0 $0.8 \pm 0.2^{**}$ Centromedian 0 ± 0 0.0 ± 0.0 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 $0.2 \pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 contromedian 0 ± 0 $0.8 \pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex Septal nuclei 0 ± 0 $0.8 \pm 0.2^{**}$ 0 ± 0	Hilus	0 ± 0	$2.8 \pm 0^{***}$	0 ± 0	2.8±0.2***	
$\begin{array}{c c c c c c c } Dorsal endopiriform & 0\pm0 & 2.2\pm0.2^{**} & 0\pm0 & 1.5\pm0.5^{***\dagger} \\ nucleus & & & & & & & & & & \\ \end{tabular}$ Rhinal cortex & 0\pm0 & 1.0\pm0.6^{**} & 0\pm0 & 0.5\pm0.0 \\ Entorhinal cortex & 0\pm0 & 1.0\pm0.0^{**} & 0\pm0 & 1.2\pm0.4^{***} \\ \end{tabular} Thalamus & & & & & & & \\ \end{tabular} MD nucleus & 0\pm0 & 1.2\pm0.2^{***} & 0\pm0 & 1.3\pm0.5^{***} \\ LP nucleus & 0\pm0 & 2.2\pm0.4^{***} & 0\pm0 & 1.5\pm0.2^{***,\dagger} \\ LD nucleus & 0\pm0 & 1.2\pm0.6^{***} & 0\pm0 & 0.8\pm0.0^{**} \\ \end{tabular} Centromedian & 0\pm0 & 0.0\pm0.0 & 0\pm0 & 0.2\pm0.2 \\ nucleus & & & & & & \\ \end{tabular} Reuniens nucleus & 0\pm0 & 2.3\pm0.2^{***} & 0\pm0 & 0.5\pm0.0 \\ \end{tabular} Cortex & & & & & & \\ \end{tabular} Septal nuclei & 0\pm0 & 0.8\pm0.2^{**} & 0\pm0 & 1.0\pm0.2^{**} \\ \end{tabular} Gaudate-putamen & 0\pm0 & 0.3\pm0.2 & 0\pm0 & 0.7\pm0.2^{**} \\ \end{tabular} Pars compacta & 0\pm0 & 0.0\pm0.0 & 0\pm0 & 0\pm0 \\ \end{tabular}	50	0 ± 0	$1.3 \pm 0.2^{***}$	0±0		
nucleusRhinal cortex 0 ± 0 $1.0\pm 0.6^{**}$ 0 ± 0 0.5 ± 0.0 Entorhinal cortex 0 ± 0 $1.0\pm 0.0^{**}$ 0 ± 0 $1.2\pm 0.4^{***}$ Thalamus MD nucleus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.3\pm 0.5^{***}$ LP nucleus 0 ± 0 $2.2\pm 0.4^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1}$ LD nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $0.8\pm 0.0^{**}$ Centromedian 0 ± 0 0.0 ± 0.0 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 $0.2\pm 0.2^{***}$ 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 $0.8\pm 0.2^{***}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 Substantia nigra 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 $0.7\pm 0.2^{**}$ Pars compacta 0 ± 0 0.0 ± 0.0 0 ± 0 0 ± 0	Piriform cortex	0 ± 0	$1.3 \pm 0.2^{***}$	0 ± 0		
$ \begin{array}{c} {\rm Entorhinal \ cortex} \\ {\rm Thalamus} \\ \\ {\rm MD \ nucleus} \\ {\rm MD \ nucleus} \\ {\rm MD \ nucleus} \\ {\rm 0\pm0} \\ 1.2\pm0.2^{***} \\ 0\pm0 \\ 1.2\pm0.2^{***} \\ 0\pm0 \\ 1.2\pm0.4^{***} \\ 0\pm0 \\ 1.2\pm0.4^{***} \\ 0\pm0 \\ 1.2\pm0.6^{***} \\ 0\pm0 \\ 1.2\pm0.6^{***} \\ 0\pm0 \\ 0.8\pm0.2^{***},^{\uparrow} \\ 1.5\pm0.2^{***,\uparrow} \\ 0\pm0 \\ 0.8\pm0.2^{**} \\ 0\pm0 \\ 0.2\pm0.2 \\ 0\pm0 \\ 0\pm$	1	0±0	2.2±0.2**	0±0	1.5±0.5*** [†]	
Thalamus MD nucleus 0 ± 0 $1.2\pm 0.2^{***}$ 0 ± 0 $1.3\pm 0.5^{***}$ LP nucleus 0 ± 0 $2.2\pm 0.4^{***}$ 0 ± 0 $1.5\pm 0.2^{***,1}$ LD nucleus 0 ± 0 $1.2\pm 0.6^{***}$ 0 ± 0 $0.8\pm 0.0^{**}$ Centromedian 0 ± 0 0.0 ± 0.0 0 ± 0 0.2 ± 0.2 nucleus 0 ± 0 0.2 ± 0.2 0.2 ± 0.2 nucleus 0 ± 0 0.2 ± 0.2 0.2 ± 0.2 Reuniens nucleus 0 ± 0 $2.3\pm 0.2^{***}$ 0 ± 0 $2.3\pm 0.2^{***}$ Frontoparietotemporal 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex $Septal nuclei$ 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 Gaudate-putamen 0 ± 0 0.3 ± 0.2 0 ± 0 $0.7\pm 0.2^{**}$ Substantia nigra $Fars compacta$ 0 ± 0 0.0 ± 0.0 0 ± 0 0 ± 0	Rhinal cortex	0 ± 0	$1.0 \pm 0.6^{**}$	0±0	0.5 ± 0.0	
$\begin{array}{cccc} LP \ nucleus & 0\pm0 & 2.2\pm0.4^{***} & 0\pm0 & 1.5\pm0.2^{***,\dagger} \\ LD \ nucleus & 0\pm0 & 1.2\pm0.6^{***} & 0\pm0 & 0.8\pm0.0^{**} \\ Centromedian & 0\pm0 & 0.0\pm0. & 0\pm0 & 0.2\pm0.2 \\ nucleus & & & & \\ Reuniens \ nucleus & 0\pm0 & 2.3\pm0.2^{***} & 0\pm0 & 2.3\pm0.2^{***} \\ Frontoparietotemporal & 0\pm0 & 0.8\pm0.2^{**} & 0\pm0 & 0.5\pm0.0 \\ cortex & & & & \\ Septal \ nuclei & 0\pm0 & 0.8\pm0.2^{**} & 0\pm0 & 1.0\pm0.2^{**} \\ Caudate-putamen & 0\pm0 & 0.3\pm0.2 & 0\pm0 & 0.7\pm0.2^{*} \\ Substantia \ nigra & & \\ Pars \ compacta & 0\pm0 & 0.0\pm0. & 0\pm0 & 0\pm0 \end{array}$		0±0	1.0±0.0**	0±0	$1.2 \pm 0.4^{***}$	
$\begin{array}{cccc} LP \ nucleus & 0\pm0 & 2.2\pm0.4^{***} & 0\pm0 & 1.5\pm0.2^{***,\dagger} \\ LD \ nucleus & 0\pm0 & 1.2\pm0.6^{***} & 0\pm0 & 0.8\pm0.0^{**} \\ Centromedian & 0\pm0 & 0.0\pm0. & 0\pm0 & 0.2\pm0.2 \\ nucleus & & & & \\ Reuniens \ nucleus & 0\pm0 & 2.3\pm0.2^{***} & 0\pm0 & 2.3\pm0.2^{***} \\ Frontoparietotemporal & 0\pm0 & 0.8\pm0.2^{**} & 0\pm0 & 0.5\pm0.0 \\ cortex & & & & \\ Septal \ nuclei & 0\pm0 & 0.8\pm0.2^{**} & 0\pm0 & 1.0\pm0.2^{**} \\ Caudate-putamen & 0\pm0 & 0.3\pm0.2 & 0\pm0 & 0.7\pm0.2^{*} \\ Substantia \ nigra & & \\ Pars \ compacta & 0\pm0 & 0.0\pm0. & 0\pm0 & 0\pm0 \end{array}$	MD nucleus	0±0	1.2±0.2***	0±0	1.3±0.5***	
$\begin{array}{cccc} \text{LD nucleus} & 0\pm0 & 1.2\pm0.6^{***} & 0\pm0 & 0.8\pm0.0^{**} \\ \text{Centromedian} & 0\pm0 & 0.0\pm0. & 0\pm0 & 0.2\pm0.2 \\ \text{nucleus} & & & & & \\ \text{Reuniens nucleus} & 0\pm0 & 2.3\pm0.2^{***} & 0\pm0 & 2.3\pm0.2^{***} \\ \text{Frontoparietotemporal} & 0\pm0 & 0.8\pm0.2^{**} & 0\pm0 & 0.5\pm0.0 \\ \text{cortex} & & & & \\ \text{Septal nuclei} & 0\pm0 & 0.8\pm0.2^{**} & 0\pm0 & 1.0\pm0.2^{**} \\ \text{Caudate-putamen} & 0\pm0 & 0.3\pm0.2 & 0\pm0 & 0.7\pm0.2^{*} \\ \text{Substantia nigra} & & & \\ \text{Pars compacta} & 0\pm0 & 0.0\pm0. & 0\pm0 & 0\pm0 \end{array}$	LP nucleus	0±0	2.2±0.4***	0±0		
nucleus 0 ± 0 $2.3\pm 0.2^{***}$ 0 ± 0 $2.3\pm 0.2^{***}$ Frontoparietotemporal 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 0.5 ± 0.0 cortex 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 $1.0\pm 0.2^{**}$ Septal nuclei 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 $1.0\pm 0.2^{**}$ Caudate-putamen 0 ± 0 0.3 ± 0.2 0 ± 0 $0.7\pm 0.2^{**}$ Substantia nigra $Pars$ compacta 0 ± 0 0.0 ± 0.0 0 ± 0 0 ± 0	LD nucleus	0±0	1.2±0.6***	0±0		
$\begin{array}{ccc} Frontoparietotemporal & 0\pm0 & 0.8\pm0.2^{**} & 0\pm0 & 0.5\pm0.0 \\ cortex & & & & \\ Septal nuclei & 0\pm0 & 0.8\pm0.2^{**} & 0\pm0 & 1.0\pm0.2^{**} \\ Caudate-putamen & 0\pm0 & 0.3\pm0.2 & 0\pm0 & 0.7\pm0.2^{*} \\ Substantia nigra & & & \\ Pars compacta & 0\pm0 & 0.0\pm0.0 & 0\pm0 & 0\pm0 \end{array}$		0±0	0.0 ± 0.0	0±0	0.2 ± 0.2	
cortexSeptal nuclei 0 ± 0 $0.8\pm 0.2^{**}$ 0 ± 0 $1.0\pm 0.2^{**}$ Caudate-putamen 0 ± 0 0.3 ± 0.2 0 ± 0 $0.7\pm 0.2^{*}$ Substantia nigraPars compacta 0 ± 0 0.0 ± 0.0 0 ± 0 0 ± 0	Reuniens nucleus	0±0	2.3±0.2***	0±0	2.3±0.2***	
Septal nuclei 0 ± 0 $0.8 \pm 0.2^{**}$ 0 ± 0 $1.0 \pm 0.2^{**}$ Caudate-putamen 0 ± 0 0.3 ± 0.2 0 ± 0 $0.7 \pm 0.2^{*}$ Substantia nigra $Pars$ compacta 0 ± 0 0.0 ± 0.0 0 ± 0 0 ± 0		0 ± 0	$0.8 \pm 0.2^{**}$	0±0	0.5 ± 0.0	
L $O \pm 0$ $O \pm 0.2^*$ Substantia nigraPars compacta $O \pm 0$ $O \pm 0$ $O \pm 0$ $O \pm 0$		0+0	08+02**	0+0	10+02**	
Substantia nigra Pars compacta 0±0 0.0±0.0 0±0 0±0	*					
Pars compacta 0 ± 0 0.0 ± 0.0 0 ± 0 0 ± 0		0.10	0.5±0.2	010	0.7 ± 0.2	
*	•	0±0	0.0 ± 0.0	0±0	0±0	
	•	0±0	2.2±0.0***	0±0	2.8±0.2***,†	

p*<0.05, *p*<0.01, ****p*<0.001, comparing control to corresponding SE group. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, comparing the 6 h to the 24h SE group (results are mean±SEM). A 0-3 grading scale was used, as previously published (Fujikawa et al., 1994, 1999, 2000, 2002; Fujikawa, 1995, 1996). See the Experimental procedures section for details. In the 6-h SE group (control and SE groups, n=3), 15 brain regions showed significant numbers of acidophilic neurons, whereas 19 brain regions had significant numbers of acidophilic neurons in the 24-h SE group (control group n=3, SE group n=4). Neuronal damage worsened from 6 h to 24 h following SE in 6 brain regions (ventral hippocampal CA1-3 pyramidal cell layers, piriform cortex, dorsal endopiriform nucleus and substantia nigra pars reticulata), but in the lateroposterior thalamic nucleus, damage was worse at 6 h. On the other hand, the maximal extent of neuronal injury was reached early and did not increase from 6 to 24 h after SE in 10 other brain regions, including the dorsal and ventral dentate hilus, amygdala, rhinal and entorhinal cortices, three thalamic nuclei, neocortex and septal nuclei.

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