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Temporal and spatial characterization of neuronal injury following lateral fluid-percussion brain injury in the rat

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Abstract The pattern of neuronal injury following lateral fluid-percussion (FP) brain injury in the rat was systematically characterized at sequential time points to identify selectively vulnerable regions and to determine the temporal contribution of primary and delayed neuropathological events. Male Sprague-Dawley rats (n = 28) were killed 10 min, 2 h, 12 h, 24 h, 4 days, and 7 days following a lateral FP brain injury of moderate severity (2.2 atm), or 24 h after a sham injury. Brain sections were stained and analyzed using Nissl, acid fuchsin, and silver staining methods to identify regions with injured neurons or with visible lesions. Extensive numbers of acid fuchsin or silver-stained neurons were observed as early as 10 min after the FP brain injury in regions extending from the caudate/putamen to the pons. The frequency of injured neurons was greatest in the ipsilateral cortex, hippocampus, and thalamus, and a visible loss of Nissl-stained neurons was observed in these regions beginning at 12 h after the FP brain injury. Acid fuchsin-stained neurons were restricted to the same brain regions for all of the survival periods and gradually decreased in numbers between 24 h and 7 days after injury. These findings suggest that lateral FP brain injury in the rat produces a combination of focal cortical contusion and diffuse subcortical neuronal injury, which is present within minutes of the impact, progresses to a loss of neurons by 12 h, and does not markedly expand into other brain regions with survival periods up to 7 days. Furthermore, the acute onset and rapid evolution of the neuronal injury process may have important implications when considering a window of opportunity for pharmacological intervention.

Key words Traumatic brain injury · Contusion

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Introduction

Although databases characterizing the neuropathology observed following non-missile human head injury have proven extremely valuable, much remains unknown about the pathological sequelae of traumatic brain injury (TBI). For example, the clinical picture which has emerged following a severe, fatal TBI includes cortical contusion, subcortical hemorrhage, diffuse edema, and diffuse axonal injury [1, 7, 18, 46]. However, other than diffuse axonal injury [46], the temporal evolution and underlying mechanisms associated with these pathological findings are poorly understood. Furthermore, the neuropathology of non-fatal mild and moderate clinical head injuries, which comprise approximately 75–90% of all cases [32, 33], has been difficult to investigate in the human.

Several brain injury models have recently been developed in rodents and other mammals. The lateral fluid-percussion (FP) brain injury model is one of the most widely used and well characterized of these models. This device has been shown to reproduce many clinically relevant features, i.e., alterations in intracranial pressure and EEG activity [38], regional alterations in cerebral blood flow [69, 70, 72], disruption of the blood-brain barrier [8], both focal and global alterations in cerebral metabolism [23, 71], regional cerebral edema [64], alterations in hippocampal seizure thresholds [36], expression of neuronal stress proteins and immediate-early genes [52], neuromotor deficits [38], and cognitive dysfunction [44, 62]. In addition, the lateral FP injury device is capable of reproducing mild, moderate or severe levels of brain damage in response to the magnitude of the impact [36, 38, 62].

The specific temporal and regional neuronal injury associated with the lateral FP brain injury has only been partially characterized. Injured neurons have been observed

in the ipsilateral cortex, hippocampus, and lateral thalamus between 1 and 24 h after a moderate lateral FP injury with Nissl and acid fuchsin staining [8, 11]. Bilateral neuronal injury was observed in the hilus of the dentate gyrus within 4 h of impact with silver methods [36]. Frank cavitation in the ipsilateral cortex is present by 2 weeks post-injury [8, 38]. None of these studies have investigated whether the onset of neuronal injury varies for different brain regions, as has been reported in rodents following transient ischemia. Within hours of an ischemic insult, the neurons in the hilus of the dentate gyrus appear injured, whereas the CA1 pyramidal cells do not appear injured until 1–4 days post-ischemia [28–31, 51, 54]. Thus, in the rodent model of ischemia, the neuronal injury evolves over a period of hours and days, which suggests that the potential for therapeutic intervention may be prolonged. It is hypothesized that delayed injury may also be important following TBI, since as many as 40% of those with fatal injuries have exhibited a period of normal consciousness followed by a progressive decline in status at some time before death [5, 17].

The purpose of the present study was to characterize the temporal evolution of the regional neuronal injury from 10 min to 7 days following a lateral FP injury of moderate severity. Neuronal injury was evaluated with a combination of histochemical markers, including Nissl staining (thionin), a modification of the Gallyas silver method, and acid fuchsin staining. Nissl stains have been used to identify injured neurons following CNS insults based on morphological changes [11, 13, 27, 31, 54, 55] and to identify regions with a loss of neurons based on an absence of Nissl staining [8, 34]. Silver staining methods [4, 10, 15, 16, 25, 36, 39, 40, 68] and acid fuchsin staining [2, 3, 6, 8, 42, 50, 56, 61, 73] have also been used in numerous studies of CNS injury to identify injured neurons.

Materials and methods

Fluid-percussion injury

Adult male Sprague-Dawley rats (330–400 g; $n = 25$) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), placed in a stereotaxic frame, and the scalp and temporal muscle were reflected. A hollow female Luer-Lok fitting was rigidly fixed with dental cement to a 4.8-mm craniotomy centered between bregma and lambda and 2.5 mm lateral to the sagittal sinus. Experimental lateral FP brain injury of moderate severity (2.2 atm) was induced in anesthetized animals using a lateral (parasagittal) model of FP brain injury which has been previously described in detail [38]. Briefly, the FP injury device is connected to the animal via the Luer-Lok fitting. The device produces a pulse of increased intracranial pressure of 21- to 23-ms duration through the rapid injection of saline into the closed cranial cavity. This results in a brief displacement and deformation of neural tissue. The pressure pulse is measured extracranially by a transducer (Gould) housed in the injury device, and recorded on a computer oscilloscope emulation program (RC Electronics). A subset of animals ($n = 3$) underwent surgery and anesthesia but were not injured (sham treatment).

Histopathological analysis

After predetermined survival times (10 min, $n = 5$; 2 h, $n = 4$; 12 h, $n = 4$; 24 h, $n = 3$; 4 day, $n = 4$; 7 day, $n = 5$; sham, 24 h survival, $n = 3$), animals were anesthetized and perfused with 4% paraformaldehyde in phosphate buffer (pH 7.6). The brains were fixed in situ for 24 h at 4° C, then removed from the skulls and fixed for another 24 h. Following fixation the brains were embedded in paraffin. Serial 8-μm coronal sections were taken every 250 μm over the length of the brain from the frontal pole to the mid-cerebellum. One series of sections was stained with thionin (Nissl stain) for neuronal morphology and to determine cytoarchitectural boundaries. Another series was stained with acid fuchsin to identify injured neurons and the location of contusions and hemorrhage [34]. Sections were deparaffinized, rehydrated through alcohols, stained in acid fuchsin (1 mg/ml, Sigma) and 0.1% glacial acetic acid in H_20 for 2 min, washed in H_20 for 2 min, dehydrated and placed in xylene prior to coverslipping. A modification of the Gallyas silver impregnation method [40] was used to confirm the location of injured or degenerating neurons in additional sections.

Detailed maps of injured neurons in sections taken from the rostral pole to the level of the mid-cerebellum were made for all cases. Camera lucida drawings of Nissl-stained sections were made at a magnification of × 15 using a microprojector, depicting all visible landmarks, as well as hemorrhagic areas. Cytoarchitectonic borders, nomenclature, and terminology were based on the most recently available atlas of the rat brain [43]. Injured neurons and hemorrhagic regions were then labeled on the maps during microscopic examination of the adjacent acid fuchsin- or silver-stained sections at a magnification of × 100. Neuronal damage was plotted in selected cases with an x-y plotter attached to a microscope at a magnification of ×100.

The number of damaged neurons was graded for various brain regions as either none (0–10 damaged cells), few (10–50 damaged cells), many (> 50 damaged cells), or most (majority of cells are damaged) based on acid fuchsin staining. Neurons with normal and abnormal morphologies (dark, shrunken, and elongated, or light and swollen) were counted along a 1-mm length of the pyramidal cell layer CA3 at the level of the mid-hippocampus (4.8 mm posterior to bregma) in Nissl-stained sections. One-way analysis of variance followed by Fisher's least significant difference test was used to determine if the number of CA3 neurons was significantly different among the groups ($P < 0.05$).

Results

Neuronal injury, 10 min

A specific and consistent pattern of neuronal injury was observed as early as 10 min after lateral FP injury in the ipsilateral neocortex, hippocampus, and thalamus, with only minor variations noted between cases (Table 1). Neuronal injury was also evident, although to a lesser extent and with considerably more variation, in regions farther away from the impact site (e.g., the brain stem, hypothalamus, and contralateral cortex and hippocampus). A similar pattern of neuronal injury was observed with acid fuchsin and silver staining methods for the 10-min survival period (Fig. 1). The number of acid fuchsin- and silver-stained neurons present in adjacent sections in the CA3 region of the hippocampus was virtually identical (224 ± 14 vs 227 ± 26). Although the silver method deposited a black precipitate over shrunken cell bodies, with longer survival periods this technique also stained microglial, astrocytes, and cellular debris, making it more difficult to identify injured neurons. Since acid fuchsin se-