Epilepsy Research (2009) **85**, 221—230

journal homepage: www.elsevier.com/locate/epilepsyres

# Effect of age on cognitive sequelae following early life seizures in rats

**Havisha B. Karnam, Qian Zhao, Tatiana Shatskikh, Gregory L. Holmes***

*Department of Neurology, Neuroscience Center at Dartmouth, Dartmouth Medical School, Hanover, New Hampshire, United States*

Received 15 November 2008; received in revised form 7 February 2009; accepted 12 March 2009
Available online 22 April 2009

**KEYWORDS**
Development;
Seizures;
Radial-arm water maze;
Open field;
Active avoidance;
Paired-pulse;
Long-term potentiation

**Summary**
*Purpose:* Clinical studies have suggested that seizures in newborns are more damaging than seizures occurring in older children. However, these studies are difficult to interpret for a variety of factors including differing etiologies of seizures across ages. Animal studies can provide insights into the question of whether age of seizure onset in children is a factor in cognitive outcome.
*Methods:* To evaluate the effect of age on seizure-induced cognitive impairment we subjected rats to 50 seizures from postnatal days P0—P10 or P15—P25. As adults the rats were studied in the Morris water maze, radial-arm water maze, open field, and active avoidance. To assess synaptic strength and network excitatory and inhibitory function animals were evaluated with long-term potentiation (LTP) and paired-pulse facilitation/inhibition.
*Results:* Compared to controls, both groups of rats with recurrent seizures were impaired in spatial memory in both water maze tests, had altered activity in the open field, and did not differ from controls in active avoidance. Rats with recurrent seizures had impaired LTP but showed no deficits in paired-pulse facilitation or inhibition. While rats with later onset showed a trend to worse performance than rats with earlier seizures, the differences were not substantial.
*Conclusions:* Recurrent seizures during development are associated with long-term behavioral deficits in learning, memory and activity level as well as impaired synaptic efficiency. Age of seizure onset was not a strong predictor of outcome.


## Introduction

Age plays a major role in virtually all aspects of epilepsy (Hauser, 1992). Children are at a substantially higher risk for epilepsy than young and middle aged adults (Hauser, 1994, 1995; Forsgren et al., 2005). In addition to the higher incidence of epilepsy in children than adults, precipitating factors such as fever are far more likely to

* Corresponding author at: Center for Neuroscience at Dartmouth, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, New Hampshire 03756, United States.
Tel.: +1 603 650 7610; fax: +1 603 650 7617.
*E-mail address:* Gregory.L.Holmes@dartmouth.edu (G.L. Holmes).

0920-1211/$ — see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.eplepsyres.2009.03.008

**Table 1** Behavioral tasks and primary brain area responsible for function.

| Test | Behavioral measure | Anatomical structures | References |
|---|---|---|---|
| Water maze | Working and reference memory | Hippocampus, prefrontal cortex | Morris (2007) |
| Radial-arm water maze | Working and reference memory | Hippocampus, prefrontal cortex | Bolhuis et al. (1985); Buresova et al. (1986) |
| Open field | Locomotor activity, hyperactivity, and exploratory behaviors. | Prefrontal, motor cortex | Walsh and Cummins (1976); Gewiss et al. (1989); Kalsbeek et al. (1989) |
| Active avoidance | Emotional memory | Amygdala | Grossman et al. (1975) |
| LTP | Synaptic plasticity | Multiple | Bliss et al. (2003); Cooke and Bliss (2006) |
| Paired-pulse inhibition/facilitation | Network excitability/inhibition | Multiple | Austin et al. (1989); Huang et al. (1999) |

induce a seizure in a young child than adult (Hauser, 1992; Fetveit, 2008). Age is also a determinant for prognosis. Intellectual impairment (Huttenlocher and Hapke, 1990; Glosser et al., 1997; Bulteau et al., 2000; Bjornaes et al., 2001; Hermann et al., 2002; Cormack et al., 2007), learning disabilities (Sillanpaa, 2004; Soria et al., 2007; Fastenau et al., 2008), social outcome (Lindsay et al., 1979; Sillanpaa, 1983) and medical refractoriness (Berg et al., 1996; Casetta et al., 1999; Camfield and Camfield, 2007) all appear to be influenced by age of onset. In a number of studies an early age of onset of seizures has been associated with more cognitive impairment than a later age of onset of seizures (Huttenlocher and Hapke, 1990; Glosser et al., 1997; Bulteau et al., 2000; Bjornaes et al., 2001; Hermann et al., 2002; Cormack et al., 2007). However, not all investigators have found a relationship between early onset of seizures and cognitive outcome (Sturniolo and Galletti, 1994; Bailet and Turk, 2000; Jokeit and Ebner, 2002).

Determining if age of onset of childhood epilepsy is a factor in outcome is important since it could alter how aggressively seizures are treated at various ages. However, interpreting clinical studies is difficult due to different seizure variables across different age groups. Etiology of the seizures, seizure type, frequency and duration of seizures, genetics, and antiepileptic drugs are but a few of these variables. The use of animal models allows the investigator to stringently control many of these variables and provides insight into the behavioral consequences of early life seizures (Huang et al., 1999; de Rogalski Landrot et al., 2001; Cha et al., 2002; Hoffmann et al., 2004; Zhao et al., 2005).

In this study we compared the effects of recurrent brief seizures at two developmental stages on subsequent learning and memory. We used the rat model of recurrent flurothyl seizures and then studied the rats during adulthood in a variety of tasks designed to assess hippocampal, prefrontal cortex, and amygdala function. We report here that rats subjected to seizures during early development have long-standing deficits in learning, memory, and activity level and have deficits in synaptic efficiency, as measured by long-term potentiation (LTP). The age of seizure onset was not a strong predictor of subsequent outcome.

## Methods

### Overview of experiments

Male Sprague—Dawley rats ($n = 56$) from Charles River Laboratories were used throughout the study and were treated in accordance to the guidelines set by the National Institute of Health and the Animal Care and Use Committee of Dartmouth College for the humane treatment of animals. Animals had access to food and water ad libitum and were group housed in plastic cages under diurnal lighting conditions, with lights on from 8.00 h to 20.00 h.

The animals underwent recurrent flurothyl-induced seizures or sham seizures between P0—P10 or P15—P25. The rats then underwent sequential testing in the Morris water maze, the radial-arm water maze, the open field test, and the active avoidance test. In an effort to restrict animal number we used the same rats for all of the studies. These tests were designed to evaluate a range of behavioral tasks mediated by hippocampus, prefrontal cortex, and amygdale (Table 1). We elected to test the two experimental groups at the same time rather than at a uniform interval following the last seizure. The interval between the last seizure and the Morris water maze was 15 days in the E2 group, a time sufficient to make any postictal effects unlikely (Boukhezra et al., 2003). We separated the water maze tasks to try to reduce carry-over learning from one maze to the other. Active avoidance was done last since this results in considerable stress to the animal which could alter the other behavioral studies. The behavioral studies were followed by two electrophysiological tests: (i) Long-term potentiation, a test of synaptic efficiency; and (ii) Paired-pulse facilitation/inhibition, a measure of the balance between excitation and inhibition in neuronal ensembles. Rats were then sacrificed and the brains examined for histological lesions. Table 2 summarizes the groups, animal numbers, and tests.

### Flurothyl-induced seizures

Male rat pups derived from 10 litters were divided into four groups and subjected to fluorthyl or sham seizures. The day