Epilepsy & Behavior 27 (2013) 18–21

Contents lists available at SciVerse ScienceDirect

Epilepsy & Behavior

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

Post-seizure drug treatment in young rats determines clear incremental losses of frontal cortical and hippocampal neurons: The resultant damage is similar to very old brains

Lukasz M. Karbowski [a,b], Glenn H. Parker [a], Michael A. Persinger [a,b,c,*]

[a] Department of Biology, Laurentian University, Sudbury, Ontario, Canada P3E 2C6
[b] Behavioural Neuroscience Program, Laurentian University, Sudbury, Ontario, Canada P3E 2C6
[c] Biomolecular Sciences Program, Laurentian University, Sudbury, Ontario, Canada P3E 2C6

ARTICLE INFO

Article history:
Received 24 October 2012
Revised 17 December 2012
Accepted 19 December 2012
Available online 1 February 2013

Keywords:
Lithium/pilocarpine-induced seizures
Post-seizure drug treatments
Aging brain
Cerebral cortices
Hippocampus
Cortical layers

ABSTRACT

Loss of neurons occurs with aging and following lithium/pilocarpine-induced epileptic seizures. In the present study, the numbers of neurons within the layers from sample areas of the four lobes of the neocortices and the hippocampus were counted by light microscopy in brains of rats that had been administered lithium or pilocarpine and then injected immediately or shortly after seizure onset with either acepromazine, ketamine, or prazosin. The mean numbers of neocortical and hippocampal neurons were lowest in rats treated with acepromazine or prazosin 1 h after seizure onset, while those of rats immediately treated with ketamine displayed the least decrements and were most similar to normal rats. The largest loss of neurons occurred within the CA1 field and layers 5 and 6 of the frontal cortices. The mean numbers of neurons within the cortices in rats whose treatments had been delayed for 1 h were similar to those of normal rats over 700 days of age. These results support the hypothesis that neuronal loss from cumulative effects of seizure-induced brain damage simulates aging.

1. Introduction

A sustained epileptic seizure, *status epilepticus*, is associated with significant loss of neurons and accentuated risk of mortality. This is evident in the display of overt rearing, rapid forelimb clonus and electroencephalographic patterns of paroxysmal activity [1] within about 30 min in rats after a single subcutaneous injection of lithium (3 mEq/kg) and 4 to 24 h after a 30-mg/kg injection of pilocarpine. There is significant mortality unless post-seizure pharmacological treatments are administered [1,2].

The injections of different classes of post-seizure drugs that promote survival are associated with markedly different outcomes for subsequent behaviors. For example, seized rats injected with ketamine rather than acepromazine respond similarly to non-seized controls in various spatial memory tasks [3–5]. Even the onset of delayed extreme obesity, following the induction of these seizures in prepubescent female rats, is determined by a single post-seizure injection of acepromazine but not of ketamine [6].

In our original approach to this problem, we appreciated an opportunity to study the synergistic interactions between different receptor subtypes on different populations of neurons and their pharmacological ligands as a potential model to study differential patterns of epilepsy-induced brain damage and how treatments might be developed to ameliorate these patterns as well as the anomalous behaviors that followed. Even those seized rats that received compounds such as ketamine and displayed no conspicuously abnormal behaviors were vulnerable to influence from other stimuli to which the normal rats or the seized rats receiving other compounds were oblivious [7].

One of the most conspicuous observations noted in the learning and memory of seized rats administered acepromazine was their marked similarity to very old rats. Consequently, we decided to compare the brains of rats that had received various single post-seizure treatments to our population of aged rats to discern if this intrinsic hypothesis was supported. If the numerical values for seizure-induced neuronal loss in young brains are comparable to those associated with very old (geriatric) brains, then different approaches to both pharmacological treatment and strategies for adaptation might be considered.

There have been many qualitative and semi-quantitative studies regarding the regions of the rat brain that are damaged as revealed by necrosis, gliosis, and the formation of cystic lesions over time [8–12]. The most devastated areas involve the piriform and entorhinal cortices, the hippocampus, the substantia nigra reticulata, specific amygdaloid nuclei, and a variety of thalamic nuclei such as the reuniens, lateral posterior groups, and subnuclei of the medial geniculate and dorsomedial thalamus [13]. The CA1 field of the hippocampus is obviously decimated.

* Corresponding author at: Department of Biology, Laurentian University, Sudbury, Ontario, Canada P3E 2C6. Fax: +1 705 671 3844.
E-mail address: mpersinger@laurentian.ca (M.A. Persinger).

1525-5050/$ – see front matter
http://dx.doi.org/10.1016/j.yebeh.2012.12.021

However, the changes within the cerebral cortices are often considered, qualitatively at least, minor.

In the present study, we focused upon the layers of various regions of the cerebral cortices and the hippocampus. Unlike previous approaches, direct quantitative measurement of the numbers of neurons rather than nominal estimates or rank order of missing neurons was utilized. We report here that a single injection of different drugs immediately or 1 h after overt indicators of epileptic seizures produced conspicuous quantitative decrements in the density of neurons. The more severe changes are comparable to neuronal densities observed in old (>700 days) rats.

2. Method

2.1. Subjects

Brains (n = 35) from rats that had been employed in a previously unpublished experiment were obtained from our library. Brains (n = 5) from age-matched female normal rats were also obtained. The cerebrums of all the brains (fixed in ethanol–formalin–acetic acid solution and embedded in paraffin) had been sectioned by a microtome such that 50- and 10-μm sections between the posterior commissure and the anterior commissure had been obtained. There were 7 brains in each of the 5 conditions that were available from our unpublished study. At around 90 days of age, all of these female rats had been injected subcutaneously with 3 mEq/kg of lithium in the left flank and then, 4 h later, with 30 mg/kg of pilocarpine in the right flank.

After the onset of a type IV seizure (rearing and rapid forelimb clonus), the rats were injected intraperitoneally with either acepromazine (30 mg/kg) or ketamine (100 mg/kg) immediately or after 1 h with one of the two drugs. A fifth group was injected with prazosin (5 mg/kg) after 1 h. (The brains of the group injected immediately with prazosin were not available.) The rats had been killed by decapitation about 50 days later (150 days of age). The brains were quickly fixed in ethanol–formalin–acetic acid solution.

2.2. Procedure

Sections from the same level of the occipital, temporal, and parietal lobes and sections from two different regions of the frontal lobes for each rat were stained with toluidine blue O. The two frontal regions were considered specific motor (Fp1) compared with an analog of the prefrontal (Fp2) cortices found in human beings. For each brain, for each area, and for each layer of the cortices, the numbers of neurons were counted at 400× in four different areas of the same layer. The mean value was used as the measure for that layer. A 6×6-grid system was employed to insure accuracy. The area within the 6×6 grid at 400× was 0.09 mm^2. Because there is no discernable layer 4 in the prefrontal cortices, the numbers of neurons in the lower portion of layer 3 and the upper portion of layer 5 were averaged to allow symmetry in the analyses. For the hippocampus, the numbers of neurons within the CA1, CA2 and CA3 fields were also enumerated in a similar manner.

2.3. Statistics analyses

The basic design for the cerebral cortices was a three-way analysis of variance with one between (5 post-seizure drug treatments and normal reference brains) and two within (layer and lobe) factors. For the hippocampus, the design was a two-way analysis of variance (treatment vs CA field). Post hoc analysis involved various combinations of t-tests for both main effects and interactions. Factor analyses and other ancillary procedures were also explored to isolate sources of variance. All analyses involved SPSS PC 16.

2.4. Comparison to age-span brains

To test the hypothesis of the aging simulation from epileptic neuronal damage, the curves for the mean neurons within the cerebral cortices and the hippocampus were obtained from our database. The age-span database [14] involved 32 brains from rats that had been between 30 and 940 days of age at the time of death.

3. Results

3.1. Post-seizure drug treatment effects

The most salient relationship associated with the numbers of neurons still viable about 50 days after lithium/pilocarpine-induced seizures and the different post-seizure treatments is reflected by the scattergram in Fig. 1. It shows the correlation between the mean numbers of cells per unit area for all of the cerebral cortical areas measured and the numbers of cells in the CA fields of the hippocampus. The different treatments are indicated within the figure. All seized rats, regardless of treatment, displayed fewer neurons than their age-matched controls. Ketamine treatment immediately after seizure onset resulted in the least loss of neurons while prazosin treatment 1 h after seizure onset produced the most loss. The discontinuity of groups (below the horizontal line) illustrates the effect.

The more formal analyses of variance for the three CA fields as a function of treatment (5 drugs and control) demonstrated a statistically significant [$F(5,34) = 321.93$, $p < .001$; $eta^2 = 0.98$] difference between groups for the average numbers of neurons in all fields. Post hoc analyses indicated that all groups were significantly different from each other. The statistically significant interaction [$F(10,68) = 36.96$, $p < .001$; partial $eta^2 = .84$] between treatment and CA field was due primarily to the greater loss of neurons within the CA1 field for the prazosin treatment ($M = 54$, $SD = 6$) compared with the controls ($M = 109$, $SD = 4$), whereas this difference was not as evident in the CA2 ($M = 50$, $SD = 3$; $M = 76$, $SD = 1$) and CA3 ($M = 56$, $SD = 3$; $M = 74$, $SD = 2$, respectively) fields. Other smaller group differences contributed in a smaller manner but displayed the same pattern. Compared with the CA1 field that showed a significant (Tukey's $p < .05$) difference between each group, there were no significant differences in the numbers of neurons for the 1-h-delayed acepromazine- or prazosin-treated brains. For the CA3 field, there was no significant difference between the 1-h-delayed acepromazine- and prazosin-treated

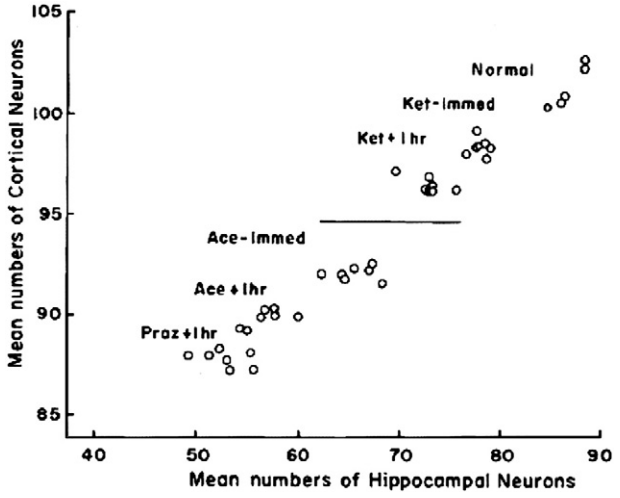

Fig. 1. Mean numbers of neurons per 0.09 mm^2 (400×) for all areas and layers within the cerebral cortices, and mean numbers within the CA1 to CA3 fields of the hippocampus as a function of post-seizure drug treatment. The means of all groups were significantly different from each other.