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Journal
DEVELOPMENTAL BRAIN RESEARCH, 23(2)

ISSN
0165-3806

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Publication Date
1985-12-01

DOI
10.1016/0165-3806(85)90050-1

Developmental Brain Research, 23 (1985) 277–281
Elsevier

BRD 60105

Short Communications

Increased numbers of neurons occur in the inferior colliculus of the young genetically epilepsy-prone rat

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(Accepted August 13th, 1985)

Key words: GABAergic neuron — inferior colliculus — audiogenic seizure — development

To determine if the increase in the number of neurons observed in the inferior colliculus (IC) of the adult genetically epilepsy-prone rat (GEPR) as compared to the Sprague–Dawley rat was present in the young GEPRs prior to the time at which seizure activity commences, brains from both types of rats 4–10 days of age were studied. A statistically significant increase in the numbers of small neurons occurred in the IC of the young GEPR. At 4 days of age, a 55% increase in the number of small neurons was found in the GEPR as compared to the Sprague–Dawley rat and at 10 days of age this increase was 105%. The numbers of the medium and large neurons were similar in the older group of rats. These data suggest that the increase in cell number observed in the adult GEPR is not compensatory to the seizure activity, but is genetically programmed.

Previous work from our laboratory has shown that the inferior colliculus (IC) of the adult genetically epilepsy-prone rat (GEPR) has an increase in the total number of neurons (mainly the small cells) and an increase in the number of GABAergic neurons[18]. This increase of GABAergic neurons is probably related to the seizure activity in GEPRs because: (1) other brain regions did not display such a difference[18], (2) the IC is thought to be an important site for epileptogenesis[1,8,9,20] and (3) the IC shows electrophysiological abnormalities[2,3]. Although previous studies on focal models of epilepsy show a loss of GABAergic neurons[13–16], genetic models of epilepsy display increases in the number of GABA neurons in specific brain regions associated with the analysis of seizure stimuli[12,18]. If the increase in number of GABAergic neurons causes an increased inhibition of GABAergic neurons, then projection neurons would be disinhibited[12]. This hypothesis has been proposed to explain epilepsy in genetic models which exhibit spontaneous seizures without the introduction of exogenous agents into the brain with concomitant glial scars. The present study was undertaken to determine if this increase in cell number was present in young GEPRs prior to the seizure state because the increased number of neurons in the adult IC of the GEPR may be a cause of the seizures or a compensatory mechanism for the seizure activity.

The GEPRs used in this study were from the same colony used in our previous study[18]. The parents of the young GEPRs were tested and displayed maximal seizures[7]. Four GEPRs and 5 Sprague–Dawley (SD) rats ranging in age from 4 to 10 days of age were deeply anesthetized with Nembutal and intracardially perfused with 0.9% saline followed by a 4% paraformaldehyde solution in phosphate buffer (pH 7.4). The brains were dehydrated in alcohol, embedded in paraffin and sectioned in the coronal plane on a rotary microtome at a thickness of 10 μm. Every tenth section throughout the midbrain was stained with cresyl violet and analyzed.

The somata of neurons were counted from a repre-

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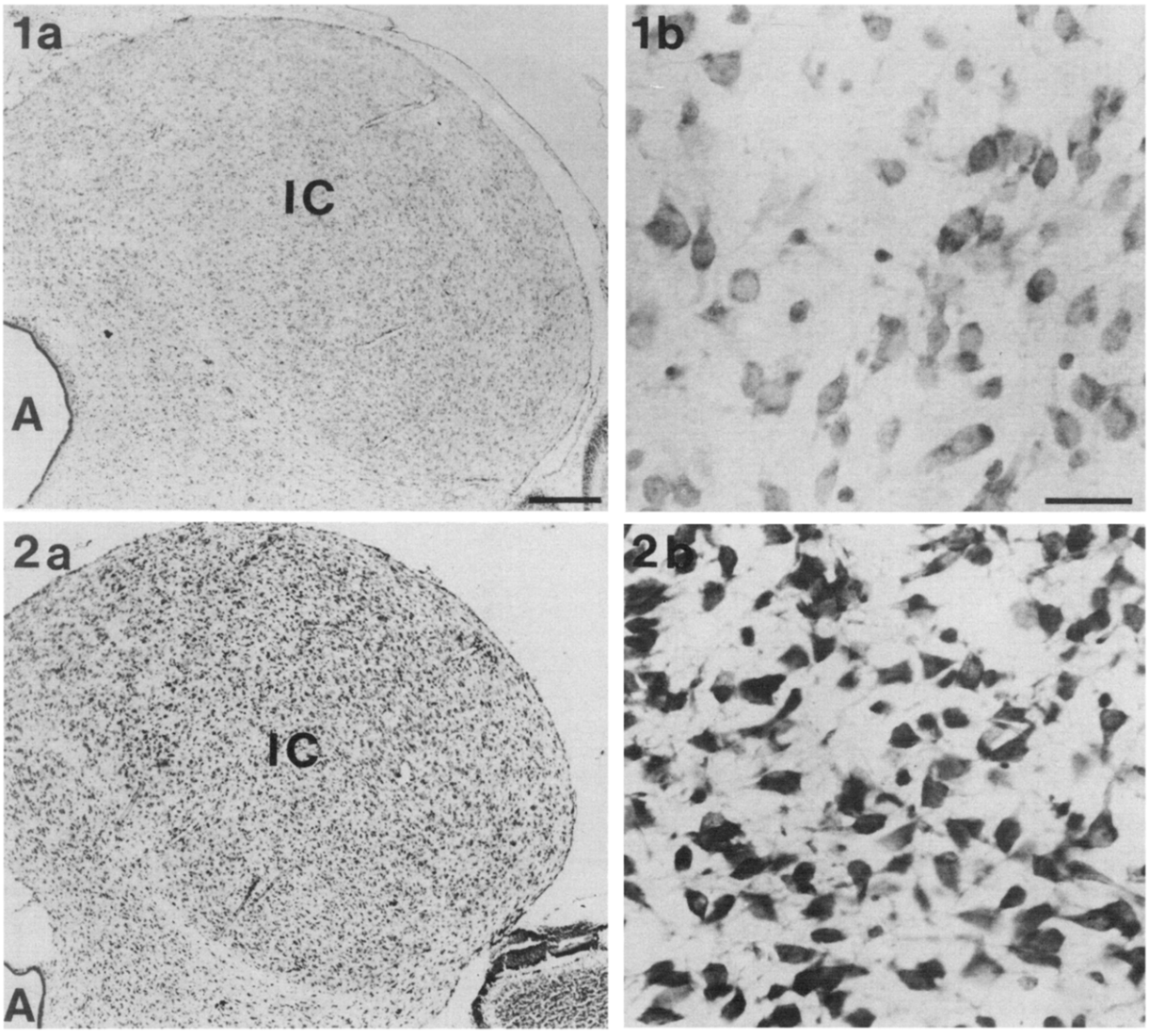
sentative 62,500 μm^2 grid in the ventral lateral portion of the central nucleus of the inferior colliculus (ICCN) throughout its rostrocaudal extent. Somata were classified as small (less than 15 μm in diameter), medium (15–25 μm in diameter) or large (greater than 25 μm in diameter) and the average cell number in each size group was tabulated. A grid reticule that was divided into 100 small squares was used to categorize the sizes of neurons. With the use of a 40× objective lens, the length of one side of a square was determined to be 25 μm. Thus, neurons that had a longer diameter of half the size of a square or were classified as small; neurons that had diam one-half to a whole square were classified as n um; and neurons that had diameters longer th square were classified as large. The criteria use the identification of neurons included distinct plasmic boundaries, lucid nuclei and basophilic plasm and nucleoli. These features were used to tinguish neurons from glia. For example, oligo drocytes are small (less than 10 μm) with deeply sophilic nuclei. Although astrocytes are simila



Figs. 1 and 2. Photomicrographs of Nissl preparations of the IC. Fig. 1 shows the IC of an 8–10-day-old SD rat at low and high mag cations, respectively. Fig. 2 shows age-matched preparations obtained from a GEPR at the same magnifications. The areas of the tire IC are similar for both the SD and the GEPR (cf. Figs. 1a and 2a). However, the GEPR displays a dramatic increase in the nun of neuronal somata as compared to the SD (cf. Figs. 1b and 2b). A, cerebral aqueduct. Scale bar, 250 μm for 1a and 2a and 25 μm fc and 2b.

size as the small neurons, they lack a stained perikaryal cytoplasm and have stippled nuclei. Quantitation of neuron number was made by the same individual in a single-blind study where all slides were coded and this code was broken only after all sections were counted. To determine if changes in the number of neurons were present in other brain regions, neuronal somata were also counted from every 10th section in the oculomotor nucleus (cnIII) and a portion of the ventral cochlear nucleus in both groups of animals.

To determine if the size of the IC was similar in the two types of rats at matched ages, the area of the IC was determined by tracing the outlines of selected sections on a digitizing tablet connected with an Apple II computer with an R&M Biometrics software package. The area of the IC was calculated rather than the area of the central nucleus alone because the subnuclear boundaries are unclear at young ages. Data were statistically analyzed using a Student's *t*-test.

The Nissl preparations revealed a heterogenous population of small, medium and large neurons in the IC of both groups of animals (Figs. 1 and 2). However, many more neuronal somata occurred in the GEPR as compared to the SD rat at comparable ages. This increase in neuron number appeared to be due to a selective increase in the small-sized neurons in both 4–6 and 8–10 days of age (Figs. 1b and 2b). The density of the Nissl substance appeared to be greater in the GEPR preparations than in the SD preparations. Therefore, the somata in the GEPR material were darker than those in the SD.

Quantitative analysis of the preparations confirmed these visual observations. At 4 days of age there were 116 ± 24 small, 20 ± 14 medium and 0.8 ± 1.1 large neurons per analyzed grid in the SD (Fig. 3a). In contrast, age matched GEPRs displayed 180 ± 50 small, 18 ± 14 medium and 0.1 ± 0.3 large neurons in the same area (Fig. 3a). Thus, the 4-day-old GEPRs had 55% more small neurons and 40% fewer medium-sized neurons than the SD rats. The large neurons which are infrequent at this age did not display a significant difference in number between these two strains of rats. Nevertheless, the GEPR displayed more total numbers of neurons than the SD rat (198 ± 44 vs 147 ± 11). The difference in the number of both small and medium-sized neurons was statistically significant ($P < 0.001$).

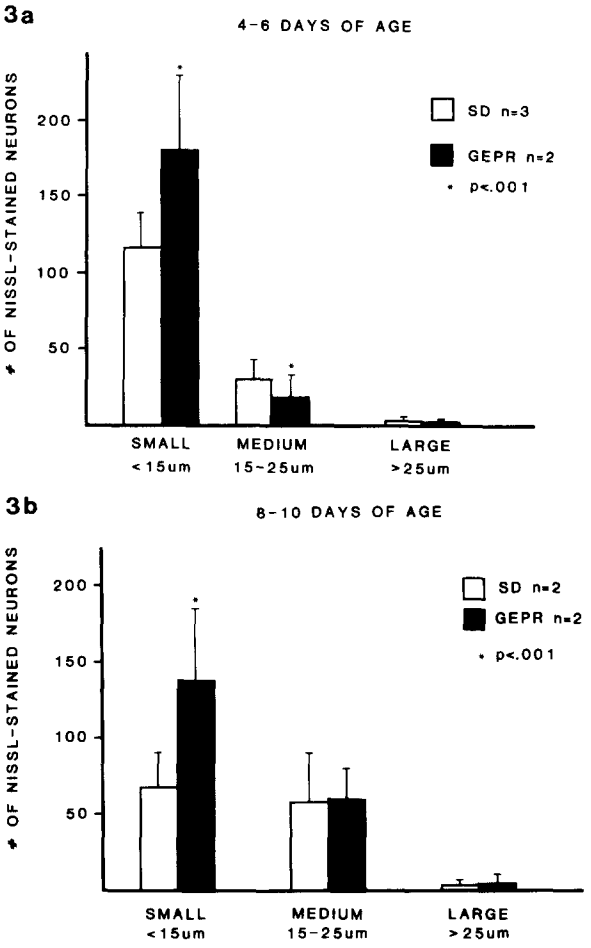


Fig. 3. Histograms showing the average number of small, medium and large Nissl-stained neurons counted from 62,500 μm^2 areas in the ICCN, ventrolateral portion. a: at 4–6 days of age the GEPRs display a 55% increase in the number of small neurons and a 40% decrease in the number of medium-sized neurons relative to the SD rats. There are very few large-sized neurons in both these types of animals. b: at 8–10 days of age the GEPRs display a 105% increase in the number of small neurons relative to the SD. At this age there are similar numbers of medium-sized and large neurons. Error bars indicate standard deviation; n = number of animals; 17–20 samples were counted from each animal.

The area of the IC was increased in size in the 8–10-day-old rats as compared to the 4–6-day-old rats. Concomitant with this increase was a decrease in cell density indicative of the growth and maturation of neuronal processes. At this age, the total number of neurons in the GEPR was greater than that of the SD (203 ± 27 vs 127 ± 27). The data for different size categories were 67 ± 22 small, 57 ± 31 medium and 2.5 ± 2.8 large neurons for the SD rat and 138 ± 48 small, 60 ± 21 medium and 5.3 ± 5.6

TABLE I

Nissl counts of neurons in the IC of epileptic and non-epileptic rats

The data from the present study are summarized under categories for 4–6 and 8–10 days of age. For comparison the data from adults[18] are included to show the similarities and differences.

	Small neurons	*Medium neurons*	*Large neurons*
4–6-days-old			
SD rats	116.7 ± 24.5	30.4 ± 14.0	0.8 ± 1.2
GEPRs	180.3 ± 50.6	18.4 ± 14.2	0.1 ± 0.3
Statistics	$t = 7.61$	$t = 3.89$	$t = 3.26$
	df = 82	df = 82	df = 82
	$P < 0.001$	$P < 0.001$	
8–10-days-old			
SD rats	67.2 ± 22.3	57.4 ± 31.6	2.5 ± 2.8
GEPRs	138.3 ± 48.4	60.5 ± 20.9	5.3 ± 5.6
Statistics	$t = 8.35$	$t = 0.50$	$t = 2.79$
	df = 74	df = 74	df = 74
	$P < 0.001$		
Adult			
SD rats	26.6 ± 10.9	15.9 ± 5.1	6.7 ± 2.4
GEPRs	50.8 ± 18.5	21.3 ± 7.8	6.2 ± 2.5
Statistics	$t = 7.00$	$t = 3.659$	$t = 0.889$
	df = 73	df = 73	df = 73
	$P < 0.001$	$P < 0.001$	

large neurons for the GEPR (Fig. 3b). These findings represent a 105% increase in the number of small neurons in the GEPR as compared to the SD. Note that at this age the number of the medium-sized and large neurons was similar in both groups of animals. At both ages there were no significant differences in the numbers of neurons in cnIII or the ventral cochlear nucleus between the two strains of rats.

These data suggest that a developmental defect occurs in the generation of small neurons which results in an increase in the number of these neurons in the ICCN of the GEPR. This increase in small neurons is present prior to the time at which seizure activity commences. Therefore, small neurons are not generated as a result of the seizure activity in an attempt to compensate for the increased activity. The situation is somewhat different for the medium-sized neurons because in comparison to SD rats the 4-day-old GEPRs had fewer neurons of this size, the 10-day-old GEPRs had similar numbers, and the adults displayed an increase (see Table I). Such data suggest that a developmental lag in the growth of this size neuron may occur in the GEPR. Large neurons did not show any difference in number between the two strains. Since the area of the IC is similar in both groups of animals, the increases in total neuron and small neuron numbers observed in the GEPR are real and not simply due to an equal amount of neurons concentrated into a smaller sized structure.

Previous work has reported that: (1) most GABAergic neurons in the IC are small although some are medium and a few are large[17–19] and (2) increased numbers of GABAergic neurons occur in the IC of the GEPR and the major increase occurs in the small neurons[18]. Since the increase of small GABAergic neurons was so great, it was reflected in a greater percentage of the total number of neurons being GABAergic in the adult GEPR as compared to the SD and suggested that the defect may be preferential for the GABAergic system. The present study did not examine glutamic acid decarboxylase (GAD) immunocytochemical preparations in these young GEPRs because GAD is not present at detectable levels in this structure at such young ages (Ribak, unpublished observations). However, the increase in the number of small GABAergic neurons in the adult GEPR suggests that the increased numbers of small neurons observed in the young, preseizure GEPRs is probably correlated with an increase in GABA neurons.

How is this defect related to epileptogenesis in the GEPR? One possibility is that this defect results from the noradrenergic deficit that is found in the brains of the GEPR rats[4–7,10]. Norepinephrine (NE) has been shown to potentiate the action of GABA in the cerebellum of normal rats, yet NE is ineffective in augmenting GABA-mediated inhibition in the GEPR[21]. Perhaps the defect in the NE system of GEPRs triggers a compensatory increase in the generation of increased numbers of GABAergic neurons.

A second possibility, the one which we favor, is that the additional GABAergic neurons in the IC of GEPRs may be inhibiting the tonically active GABAergic neurons, thereby releasing excitatory projection neurons of their tonic inhibition. This hypothesis was originally proposed by Peterson et al.[12] to explain how an increase in the number of GABAergic basket cells in the hippocampal dentate gyrus of the seizure-sensitive gerbil may be causing seizure activity. A preliminary ultrastructural analy-

sis of gerbil preparations supports this hypothesis because more symmetric synapses appear to be present on the somata of basket cells in seizure-sensitive gerbils[11]. We plan to examine the IC of the GEPR to see if more symmetric synapses occur with GABAergic neurons to add further support for the disinhibition hypothesis in models of genetic epilepsy.

The authors wish to thank Dr. Phillip Jobe for his initial supply of the GEPRs whose offspring were used in this study and Yashoda Jhurani and Margot Brundage for technical assistance. This project was supported by a Klingenstein Fellowship (C.E.R.), NIH Grant NS-15669 and a grant from the Epilepsy Foundation of America.

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