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Increased expression of gamma-aminobutyric acid transporter-1 in the forebrain of infant rats with corticotropin-releasing hormone-induced seizures but not in those with hyperthermia-induced seizures

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Abstract

High affinity, gamma-aminobutyric acid (GABA) plasma membrane transporters (GATs) influence the availability of GABA, the main inhibitory neurotransmitter in the brain. Recent studies suggest a crucial role for GATs in maintaining levels of synaptic GABA in normal as well as abnormal (i.e., epileptic) adult brain. However, the role of GATs during development and specifically changes in their expression in response to developmental seizures are unknown. The present study examined GAT-1-immunolabeling in infant rats with two types of developmental seizures, one induced by corticotropin-releasing hormone (CRH) lasting about 2 h and the other by hyperthermia (a model of febrile seizures) lasting only 20 min. The number of GAT-1-immunoreactive (ir) neurons was increased in several forebrain regions 24 h after induction of seizures by CRH as compared to the control group. Increased numbers of detectable GAT-1-ir cell bodies were found in the hippocampal formation including the dentate gyrus and CA1, and in the neocortex, piriform cortex and amygdala. In contrast, hyperthermia-induced seizures did not cause significant changes in the number of detectable GAT-1-ir somata. The increase in GAT-1-ir somata in the CRH model and not in the hyperthermia model may reflect the difference in the duration of seizures. The brain regions where this increase occurs correlate with the occurrence of argyrophyllic neurons in the CRH model.

Keywords: GABA transporter; Corticotropin-releasing hormone; Febrile seizures; Infant rat

1. Introduction

The majority of seizures occurring in the developing human are not spontaneous; i.e., they are

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not related to inherent abnormalities in the balance of neuronal excitation and inhibition. Instead, most developmental seizures are provoked by injurious or stressful stimuli (Baram and Hatalski, 1998). Thus, seizures during development may be induced by hypoxia (Jensen et al., 1998) or fever (see Baram et al., 1997b for review). Febrile seizures represent an important type of developmental seizure. They are extremely common in the human and have been modeled in the immature rat (Baram et al., 1997b; Toth et al., 1998; Chen et al., 1999; Dube et al., 2157). The mechanisms by which these stressful stimuli cause seizures are not clear. However, recently a role for the stress-activated excitatory neuropeptide, corticotropin-releasing hormone (CRH)(Vale et al., 1981), has been suggested in developmental seizures (Baram and Hatalski, 1998). Stress activates the expression of CRH in several brain regions that are involved in the circuitry for stressful stimuli (Hatalski et al., 1998). The limbic system, particularly the amygdala and hippocampus, is rich in neuronal populations that either synthesize CRH or possess CRH receptors (Swanson et al., 1983; De Souza et al., 1985; Avishai-Eliner et al., 1996). CRH functions as an excitatory neuromodulator (Aldenhoff et al., 1983; Ehlers et al., 1983; Hollrigel et al., 1998). In fact, picomolar amounts of CRH induce severe and prolonged seizures within minutes in young rats (Baram and Schultz, 1991; Baram et al., 1992; Baram and Schultz, 1995). Furthermore, repeated administration of CRH doses (150×10^{-12} mole), which results in status epilepticus, leads to enhanced excitability of the limbic circuit (Baram and Hatalski, 1998) and to excitotoxic injury in select hippocampal and amygdala neurons in the infant rat (Baram and Ribak, 1995; Ribak and Baram, 1996). This information suggests that some stressors (i.e., hypoxia and hyperthermia) may provoke seizures early in life by increasing the levels of CRH in limbic structures. CRH-induced seizures are a model of developmental seizures because they are far more robust during the second posnatal week in rats than in adults (Baram et al., 1992; Baram and Hatalski, 1998). This may be partially due to high levels of the CRH receptor in limbic regions at this time (Avishai-Eliner et al., 1996).

Many previous neurophysiological studies have indicated that a failure of inhibition is a principal reason for neuronal hyperexcitability during epileptic seizures (Gean et al., 1989; Kamphuis et al., 1987; Kapur et al., 1989; Morimoto et al., 1987a,b; Ribak, 1991). Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Extracellular GABA concentrations in the synaptic cleft are regulated by the activity of GABAergic neurons and by specific Na^+ dependent transporter proteins on presynaptic terminals and glial cells involved in GABA uptake (Iversen and Kelly, 1975). Molecular studies have revealed at least four subtypes of GABA transporters: GAT-1, GAT-2, GAT-3 and BGT-1 (Borden et al., 1992; Guastella et al., 1990; Clark et al., 1992). These transporters have several possible functional roles, including the inactivation of GABA's action in synaptic transmission by removing GABA from the vicinity of its receptors (Iversen and Kelly, 1975; Schousboe, 1981; Isaacson et al., 1993).

GAT-1 has been demonstrated within neurons and astrocytes in various regions of the rat brain, including the olfactory bulb, neocortex, hippocampus, ventral pallidum and cerebellum (Durkin et al., 1995; Minelli et al., 1995; Ribak et al., 1996). The neuronal localization of GAT-1 in the adult hippocampus is exclusively within axon terminals forming symmetric synapses, including those from GABAergic basket and chandelier cells (Ribak et al., 1978, 1996). In young rats at 10–30 postnatal days (PND) of age, GAT-1 is also localized to the somata and dendrites of GABAergic interneurons in the neocortex and hippocampus (Yan et al., 1997). The number of labeled somata varied with the age of the rat during this period. A role for

GATs in adult seizures has been established (Akbar et al., 1998; During et al., 1995; Hirao et al., 1998; Mathern et al., 1999). Here we tested the hypothesis that seizures influence the normal distribution of GAT-1-immunolabeling in the immature brain. Thus, the present study addresses the question of whether an alteration in transporter distribution occurred after two types of developmental seizures: CRH-induced and hyperthermia-induced seizures. Immunocytochemical techniques were used to assess regional differences in the number of GAT-1 labeled neurons in rats with both types of seizure, and those data were compared to that of vehicle-treated controls.

2. Materials and methods

2.1. Animals

Rats were the offspring of time-pregnant, Sprague-Dawley dams. Rats were born in our federally approved animal facility and were kept on a 12 h light/dark cycle. The time of birth of the pups were determined every 12 h, and the day of birth was considered day 0. Litters were culled to 12 pups and mixed among experimental groups. Cages were maintained in a quiet room, and were undisturbed for 24 h prior to experiments.

2.2. CRH-infusions

Pups [10 postnatal days of age (PND)] were implanted with cannulae 24 h prior to experiments, and the position of the cannulae was verified histologically in all cases (see below). Briefly, stainless steel cannulae were implanted into the lateral ventricles under halothane anesthesia, using an infant rat stereotaxic apparatus as described elsewhere (Baram et al., 1992, 1997a; Baram and Schultz, 1995; Brunson et al., 1998). Infant rats ($n = 9$) were injected once with CRH i.c.v. on 11 PND. The infusions were made while the pups were freely moving in a heated Plexiglas chamber. CRH (5 μg in 1 μl) was infused via the chronic cannula using a micro-infusion pump. Cannula-carrying control animals ($n = 7$) were given a dye vehicle (Baram et al., 1992, 1997a). Subsequent to CRH infusion, seizure latency and duration were monitored: animals were scored for behavioral limbic seizures occurring within 5-min epochs (Baram et al., 1997a; Baram and Schultz, 1995). The concordance of limbic automatism and epileptic discharges induced by CRH has been established previously (Baram et al., 1992, 1997a).

2.3. Hyperthermia induction paradigm

The hyperthermic seizure paradigm has been described previously (Baram et al., 1997b; Toth et al., 1998; Dube et al., 2157). Briefly, the core temperature of 5 pups (PND 10) was raised using a regulated stream of moderately heated air. Pups were placed on the floor of a 3 l glass container and the air stream was directed ≈ 50 cm above them. Rectal temperatures were measured at baseline, at 2-min intervals, and at the onset of hyperthermia-induced seizures. Hyperthermia was maintained for 30 min, aiming for a core temperature of 41°C, and the presence and duration of seizures for each pup were noted at 2-min intervals. After the hyperthermic period, rats were placed on a cool surface, monitored for 15 min, and then returned to their home cages for rehydration by the mothers. The behavioral seizures in this paradigm are stereotyped and easily monitored. They were previously shown to correlate with EEG rhythmic epileptiform discharges from the hippocampus and amygdala (Baram et al., 1997b; Dube et al., 2000). These seizures consist of complete arrest of the heat-induced hyperkinesis, unilateral body flexion and biting of an extremity, occasionally

followed by clonic or swimming motions. Another group of rats ($n = 4$) received pentobarbital (PB) (30 mg/kg) just before induction of hyperthermia.

2.4. Tissue preparation

At 4 h ($n = 3$) or 24 h ($n = 6$) following CRH treatment and at 24 h following hyperthermia the experimental and age-matched control groups were anesthetized with sodium pentobarbital and perfused transcardially with 0.12 M phosphate buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde and 0.2% glutaraldehyde in phosphate buffer (pH 7.4). Brains were then removed from the rats, placed in fixative for 24 h at 4°C, and transferred to cold PBS for several hours to a few days. The brains were sectioned in the coronal plane at 60 μm with a vibratome. Sections through both hemispheres, including those at the injection site, were collected in tissue-culture wells in cold PBS.

2.5. Immunocytochemistry

Free-floating sections from both control and experimental animals were immunostained for GAT-1 using a standard avidin-biotin complex (ABC) method. Sections were processed simultaneously using the same reagents and incubation times. Endogenous peroxidase activity was blocked with a 20-min incubation in 0.1% H_2O_2. Then, the sections were incubated in 5% normal horse serum (NHS) at room temperature for 2 h. Sections were then placed in a PBS solution containing 5% NHS, 0.5% Triton X-100 and rabbit anti-GAT-1 serum (1:2500) at 4°C for 48 h. After several rinses with PBS containing 0.1% Triton X-100, sections were incubated in 1% anti-rabbit IgG with 5% NHS for 2 h at room temperature. Then, sections were rinsed again and incubated in 1% ABC solution for 1 h. After several rinses in PBS, bound antibody was visualized by incubating sections in 0.05% DAB and 0.005% H_2O_2. Sections were mounted on gelatin-subbed slides and allowed to air-dry for at least 30 min. Then, sections were dehydrated in ascending ethanols, cleared in xylene and coverslips were applied with DPX mounting medium.

2.6. Quantitative analysis

Sections from the dorsal hippocampus were used for counting immunoreactive neurons in the hippocampal formation. The number of cells in each hippocampal subregion was expressed as the mean number of immunoreactive cells per section, after manual counting at a magnification of $\times 200$ with the aid of an ocular grid (Pitkanen et al., 1999). Sections were divided into equivalent septotemporal levels, between 2.0 and 2.3 mm rostral to Bregma (Sherwood and Timiras, 1970). Five to 10 sections from these levels were counted for each rat, and only sections with consistent immunostaining were used. Immunoreactive neurons were counted in all parts of the hippocampus and dentate gyrus.

The number of GAT-1-ir somata in the neocortex, piriform cortex and amygdala was expressed as the number of GAT-1-ir cell bodies per unit area. A grid reticule of 100 frames covering an area of 250×250 μm^2 was centered over the parietal cortex to obtain data for the neocortex and between the dorsal endopiriform nucleus and ventral endopiriform cortex for the piriform cortex. A similar method was used to count GAT-1-ir somata in the basal amygdala nucleus.

2.7. Statistical analysis

Data were obtained from both hemispheres. Results were analyzed for statistical significance ($P < 0.05$) using a one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison posthoc test (Sigma Stat Program; Jandel Scientific, USA). The number of GAT-1-ir somata in the experimental groups was also expressed as a percentage of the control group.

3. Results

3.1. Distribution and localization of GAT-1-immunoreactivity in the control group

3.1.1. Hippocampal formation

The regional immunolabeling pattern for GAT-1 in control brains was similar to that described previously for the hippocampal formation and neocortex in young rats (Yan et al., 1997). GAT-1-immunoreactivity (ir) in the dentate gyrus was dense in the molecular layer and lighter in the hilus where mainly unlabeled mossy fibers were found. The somata of unlabeled hippocampal principal neurons, granule and pyramidal cells, were outlined by small immunolabeled puncta (Fig. 1A and C, Fig. 2A and B). The CA3 area showed lighter GAT-1-ir

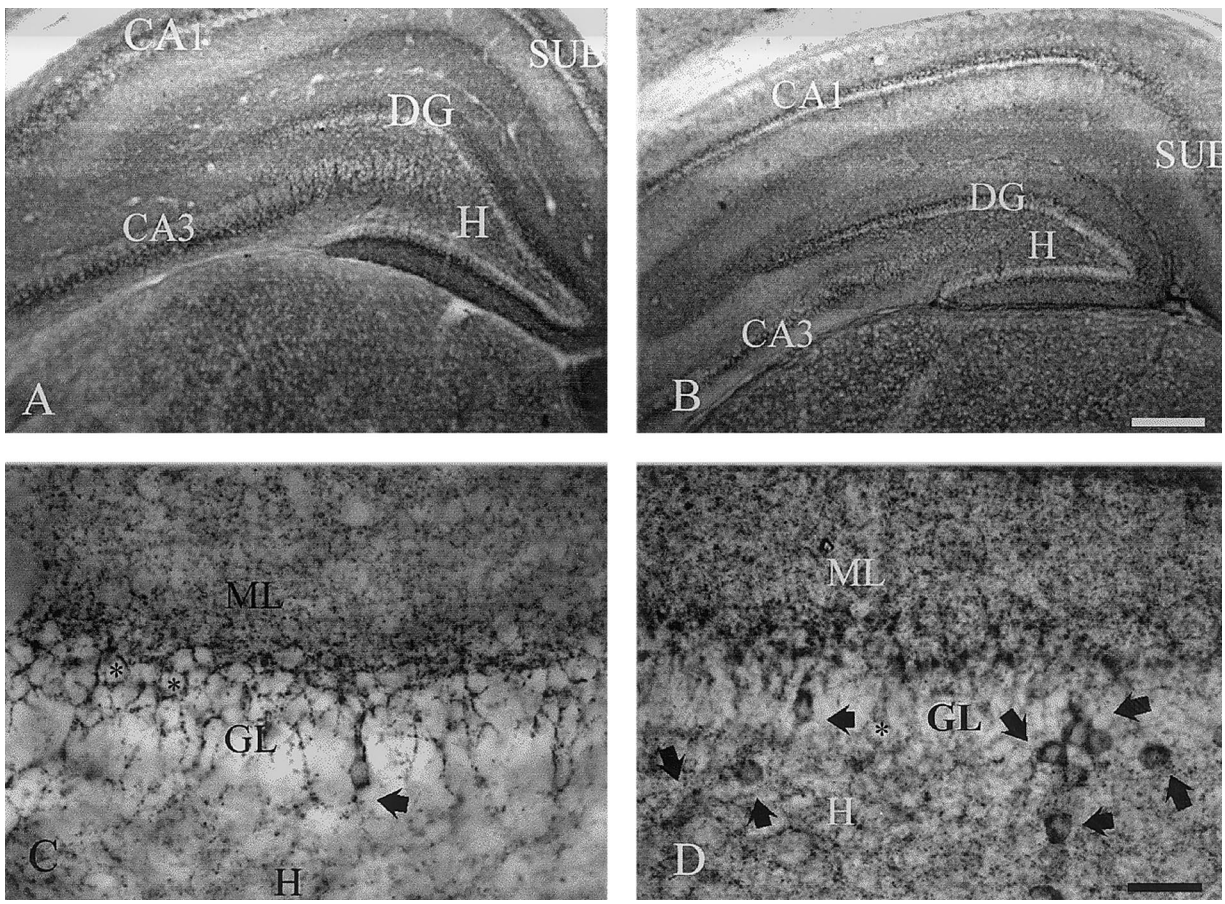

Fig. 1. Photomicrographs of GAT-1-ir in the rat hippocampus. (A) and (B): Low magnifications of the control group (A) and the 24 h CRH group (B). Note the intense GAT-1-ir in the supragranular layer of the dentate gyrus (DG) and pyramidal cell layer (CA3, CA1). (C) and (D) show details of GAT-1-ir puncta and somata in the dentate gyrus. (C): Control group and (D): The 24 h CRH group show several immunopositive somata (arrows) within or close to the granule cell layer. Note that many more are shown in (D) than in (C). GL; granule cell layer, ML; molecular layer, H; hilus and SUB; subiculum. Scale bars = 100 μm in (A) and (B); 20 μm in (C) and (D).

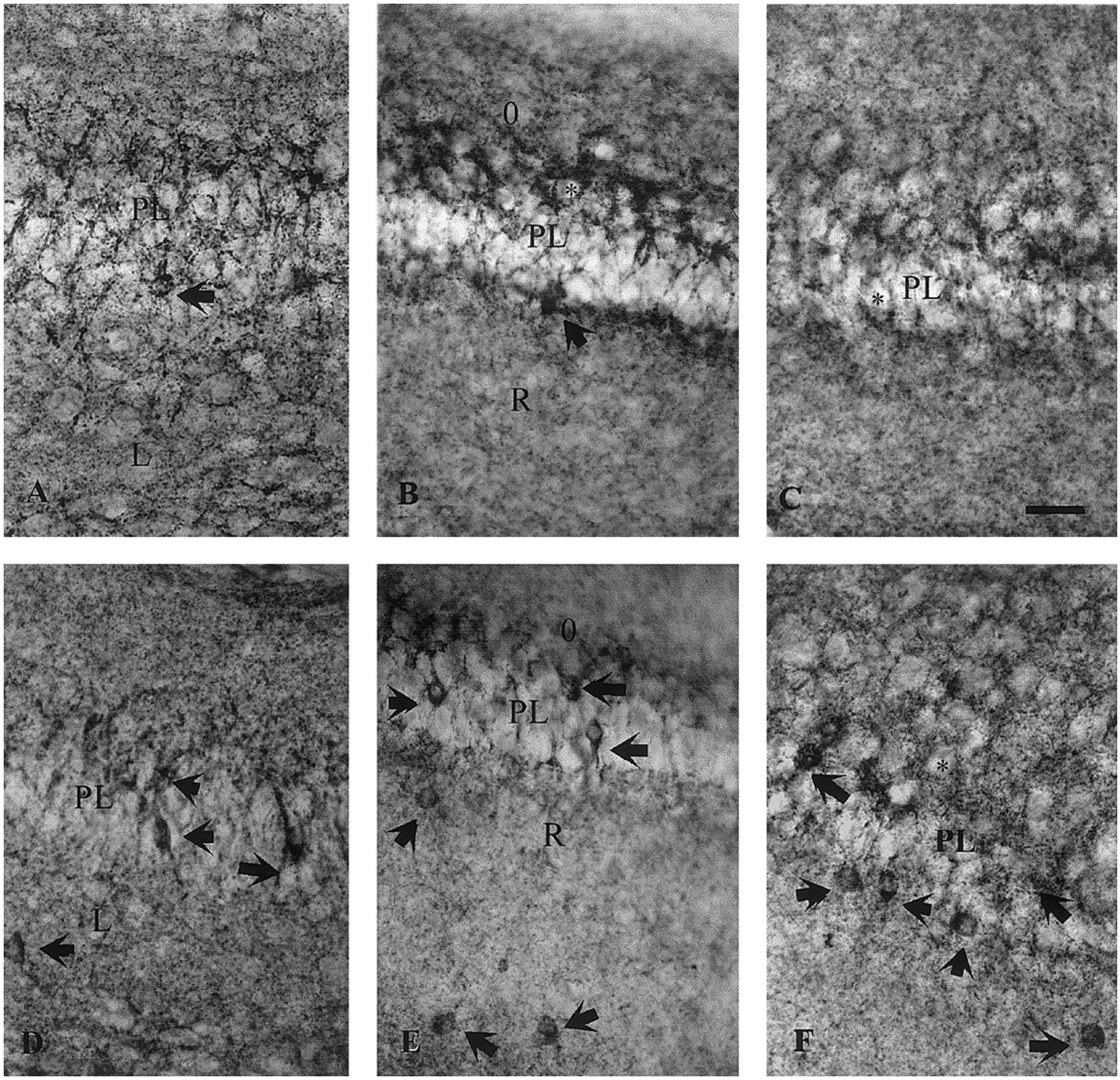

Fig. 2. Details of GAT-1-ir puncta and somata in several regions of the hippocampal formation. (A–C): Control group. Numerous puncta and fibers are shown in CA3 (A), CA1 (B) and the subiculum (C) and some unlabeled somata (arrows). (D–F): The 24 h CRH group. Several immunopositive somata (arrows) are in strata pyramidale, lucidum and radiatum in CA3 (D) and CA1 (E). They have the features of interneurons (see text). (F) Numerous GAT-1-ir somata (arrows) appear in the subiculum. O; stratum oriens, R; stratum radiatum; L; stratum lucidum, PL; stratum pyramidale. Scale bar = 20 μm.

in stratum lucidum, the termination zone of mossy fibers. In contrast, strata radiatum and lacunosum-moleculare of CA3 displayed denser GAT-1-ir (Fig. 1A). The strata oriens, lucidum and radiatum showed a few GAT-1-ir cell bodies (Fig. 2A). The CA1 area showed lighter GAT-1-ir

in stratum pyramidale as compared to CA3 (cf., Fig. 2A and B). Some fusiform GAT-1-ir cell bodies were found in stratum pyrami- dale. The subiculum showed a similar GAT-1 labeling pattern to that found in CA1 in that the somata of unlabeled pyramidal cells were outlined by small immunolabeled puncta. However, GAT-1-ir cell bodies were absent in the subiculum (Fig. 2C).

3.1.2. Neocortex

The pattern of GAT-1-ir in the neocortex was similar in all sections examined and consisted of labeled punctate structures, radially or obliquely oriented fibers and somata (Fig. 3A and Fig. 4B). GAT-1-ir puncta were relatively sparse in layer I. In the other layers, GAT-1-ir appeared to be distributed homogeneously, although it was slightly increased in layer IV and the supragranular layers (Fig. 3A). In layers II–VI, numerous GAT-1-ir puncta outlined the somata and proximal dendrites of pyramidal and nonpyramidal cells (Fig. 4A). Small, round or oval GAT-1-ir somata were found in all cortical layers but mostly in layers IV and VI (Fig. 4B). GAT-1-ir fibers were also observed in the underlying white matter.

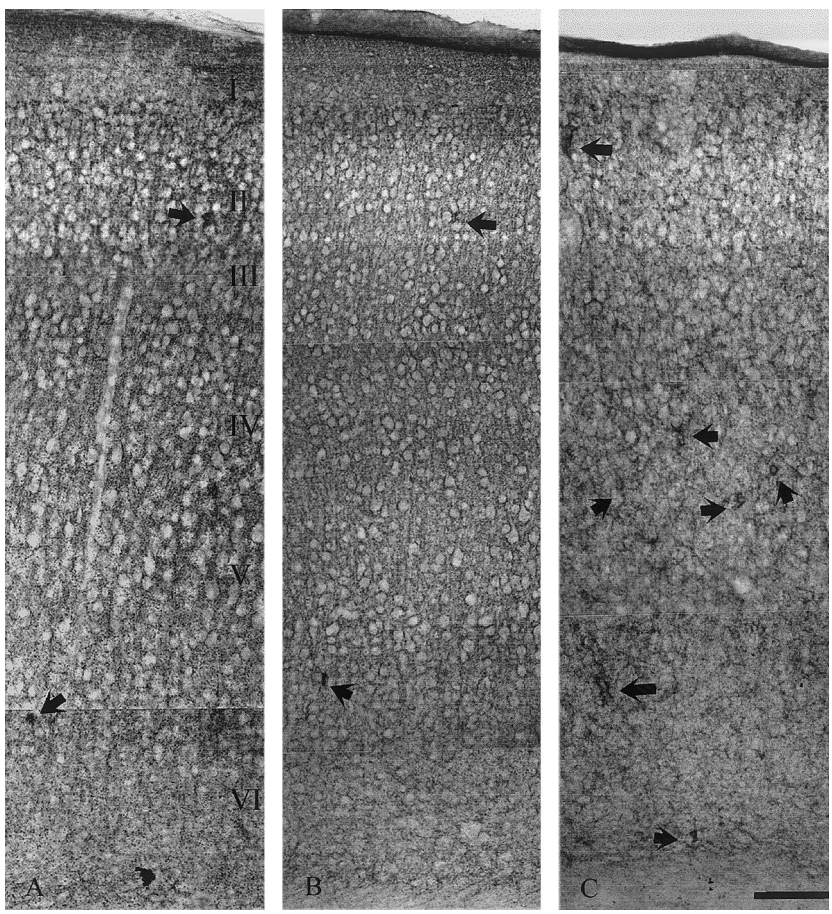

Fig. 3. GAT-1-ir in the neocortex. (A): Control group. (B): The 4 h CRH group is similar to the control group. (C): The 24 h CRH group shows several more GAT-1-ir somata (arrows) throughout the cortex than in either (A) or (B). Layers are indicated by Roman numerals I–VI. Scale bar = 100 μm.

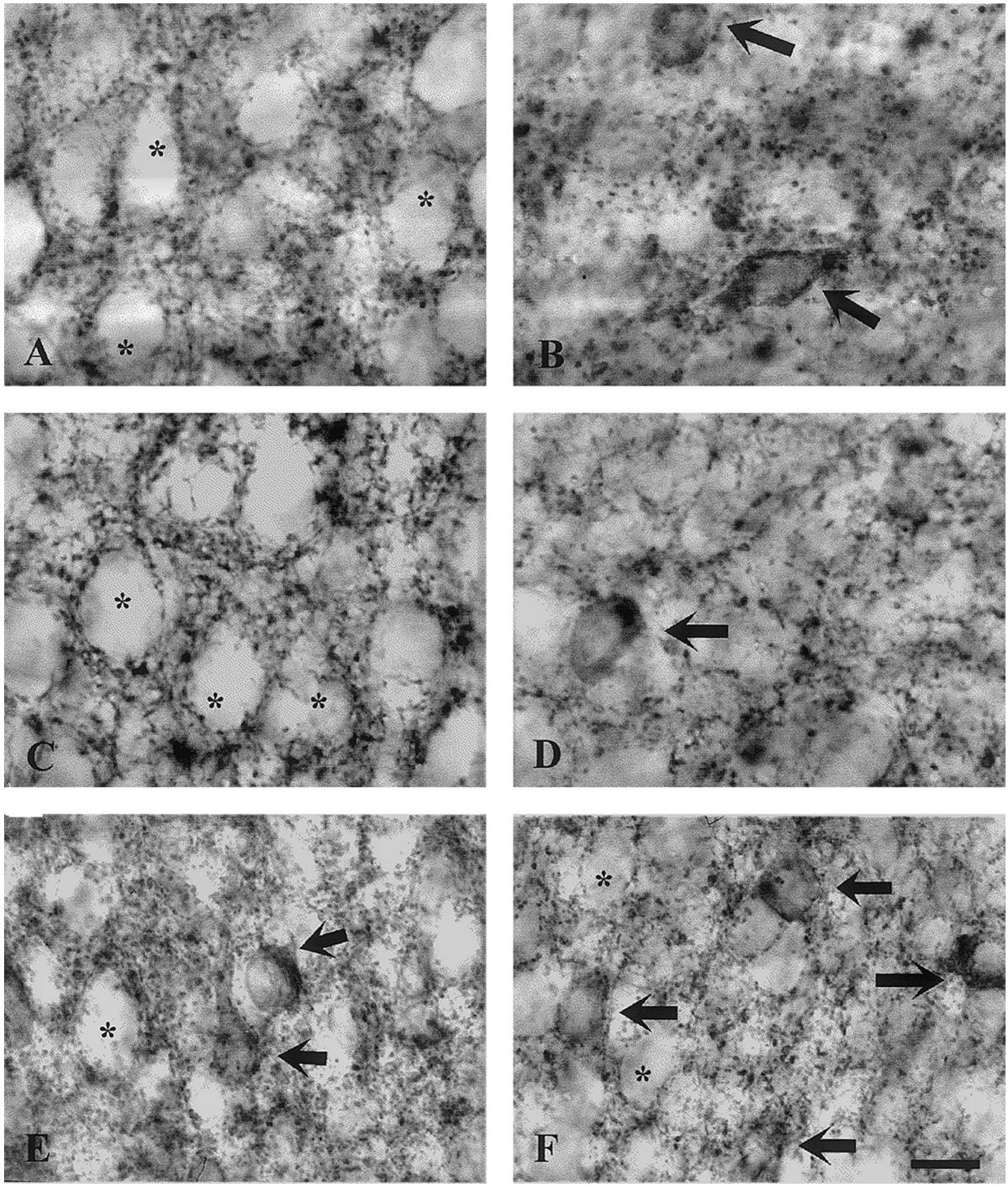

Fig. 4. Details of GAT-1-ir puncta and somata in the rat neocortex. In all panels (A–F), GAT-1-ir puncta are apposed to the somata and proximal dendrites of unlabelled pyramidal neurons (asterisks). (A) and (B): The control group showed some GAT-1-ir somata in layers II and VI, respectively. (C) and (D): The 4 h CRH group also showed some GAT-1-ir somata in the same respective layers. (E) and (F): The 24 h CRH group shows several more GAT-1-ir somata (arrows) and the quantitative data indicated a significant increase. Layer II in panels (A), (C) and (E); Layer VI in panels (B), (D) and (F). Scale bar = 10 μm.

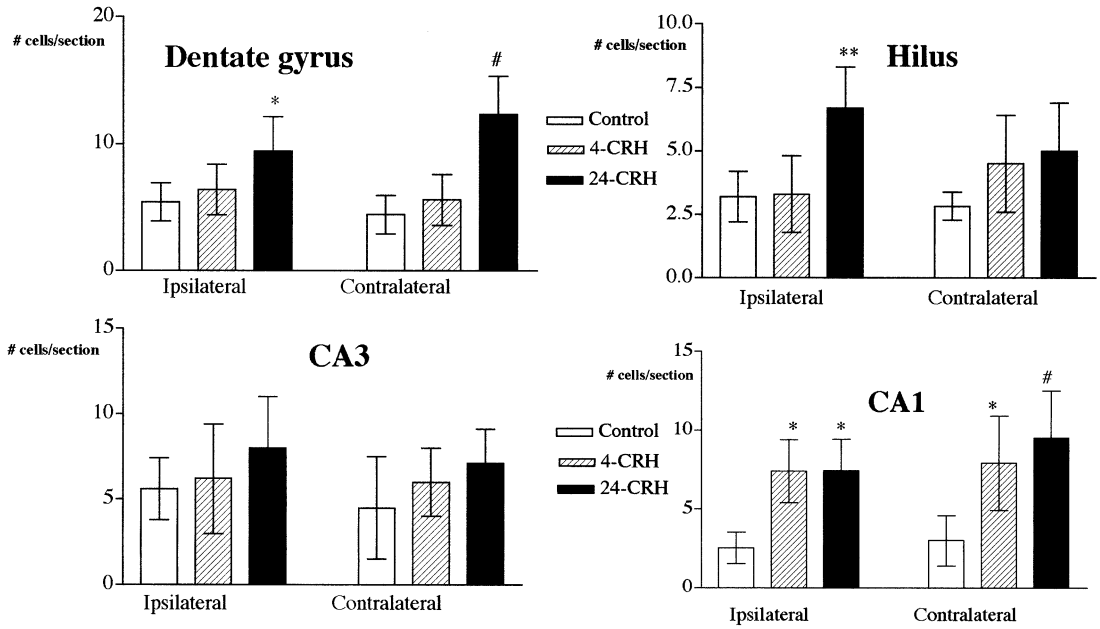

Fig. 5. Histograms of the number of GAT-1-ir somata in the examined subfields of the hippocampal formation (mean ± S.D.) compared to the control group in each hemisphere (one-way ANOVA with Bonferroni's posthoc test) $*P < 0.002$, $**P < 0.05$ and $\#P < 0.001$.

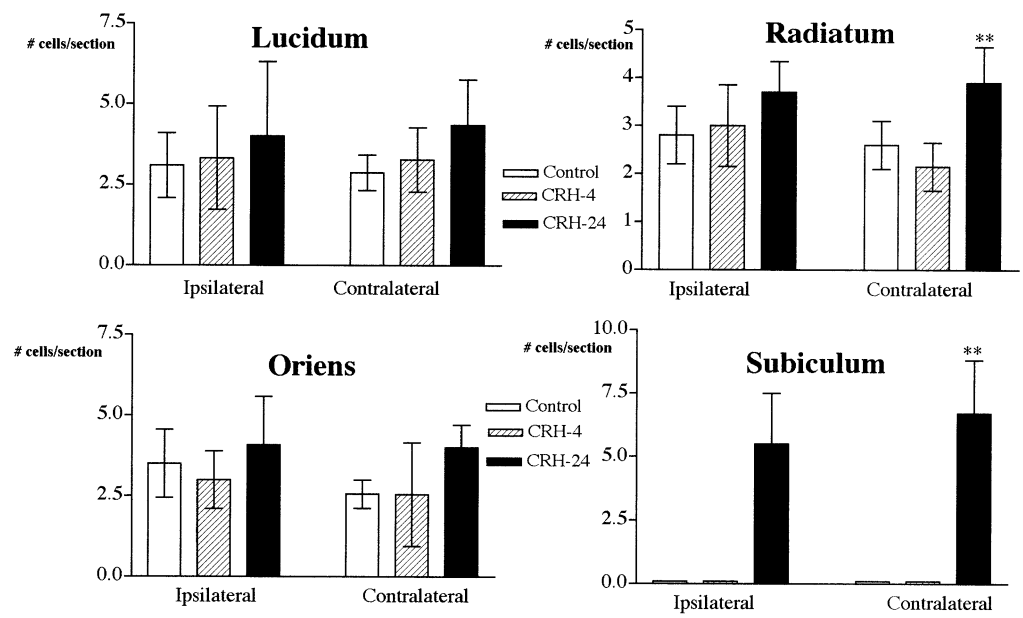

Fig. 6. Histograms of the number of GAT-1-ir somata in some of the strata of the hippocampal formation and the subiculum (mean ± S.D.) as compared to the control group in each hemisphere (one-way ANOVA with Bonferroni's posthoc test) $**P < 0.05$.

3.2. Effects of CRH-induced seizures on the distribution of GAT-1-ir

3.2.1. Hippocampal formation

Increased numbers of detectable GAT-1-ir cell bodies and more intensely labeled puncta were found throughout the hippocampal formation in rats examined 24 h after CRH-induced seizures compared with controls. In the dentate gyrus, GAT-1-ir puncta were more concentrated in the granule cell layer and hilus than in those structures in control rats (cf., Fig. 1B and D). Numer-ous round and fusiform GAT-1-ir cell bodies were located in the lower border of the granule cell layer (Fig. 1D) and were probably basket cells (Ribak and Seress, 1983). The number of detectable GAT-1-ir cell bodies in the granule cell layer of the dentate gyrus was significantly increased in both hemispheres; ($P < 0.002$) in the ipsilateral side and ($P < 0.001$) in the contralateral side as compared to the control group (Fig. 5). Significantly increased numbers of immunolabeled somata were also observed in the hilus ($P < 0.05$) of the ipsilateral hemisphere as compared to the control group (Fig. 5). More immunoreactive neurons were found in the rostral sections than in the caudal ones, and this variation could arise from a delayed development of the caudal hippocampus (Bayer, 1980).

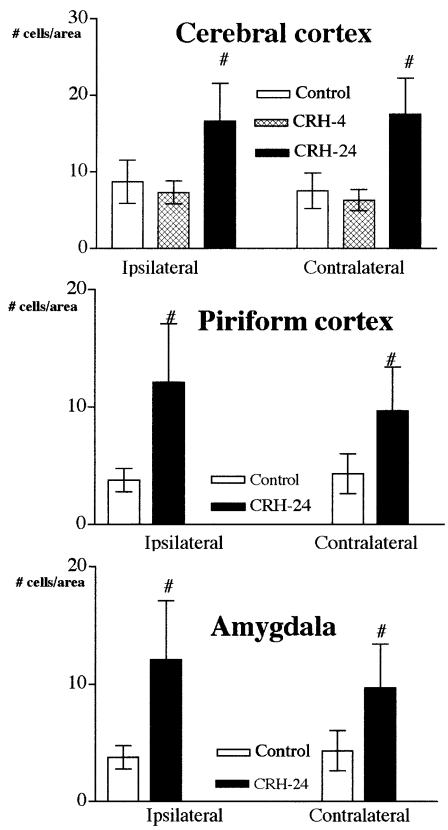

Fig. 7. Histograms of the number of GAT-1-ir somata in the cerebral cortex (neocortex), piriform cortex and amygdala (mean ± S.D.) as compared to the control group in each hemisphere (one-way ANOVA) # $P < 0.001$.

A normal pattern of GAT-1-ir puncta with vertically oriented rows of boutons was found in Ammon's horn (Fig. 2D). Numerous GAT-1-ir cell bodies were also observed, but their number in CA3 was not significantly different than that of the control group for both sides (Fig. 5). However, the number of detectable GAT-1-ir cells in stratum pyramidale of CA1 was significantly increased on both the ipsilateral ($P < 0.002$) and contralateral ($P < 0.001$) sides as compared to the control group (Fig. 5). The GAT-1-ir cell bodies were also counted in strata lucidum, oriens and lacunosum-moleculare but no significant dif-ferences were found (Fig. 6). These latter im-munolabeled somata were mostly small and round (Fig. 2D and E).

GAT-1-ir puncta in the subiculum from the 24 h CRH group were present around unlabeled pyramidal cells and appeared similar to the control group (cf., Fig. 2 C and F). However, the former preparations also showed numerous GAT-1-ir somata (Fig. 2F) while the control group had none. These round and fusiform immunolabeled cells were smaller than pyramidal cells indicating that they were probably interneurons (Swanson et al., 1987). The quantitative data indicated a highly significant increase in the number of detectable GAT-1-ir somata in the 24 h group as compared to the control group (Fig. 6).

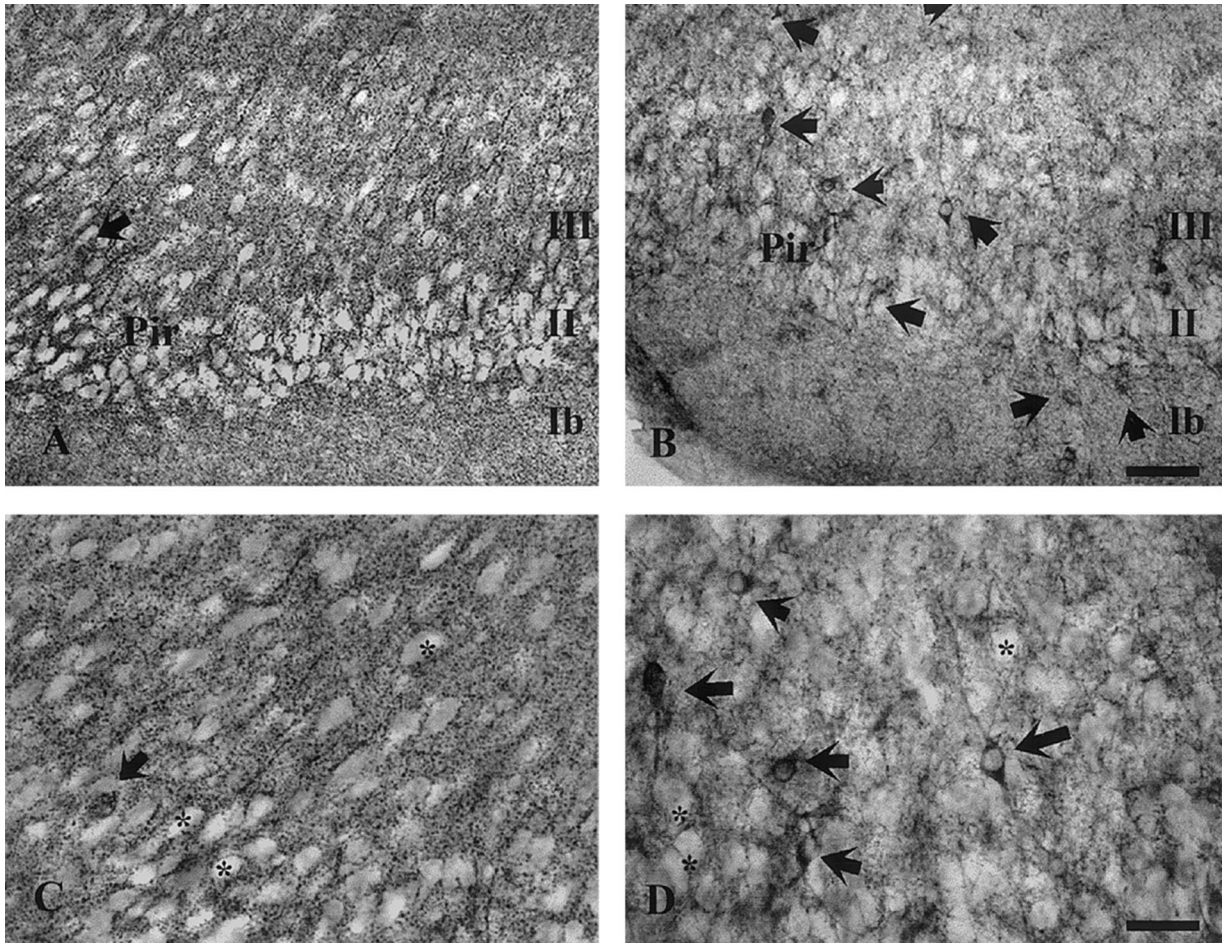

Fig. 8. Photomicrographs of piriform cortex. (A): Low magnification of the control group. (C): Details of the GAT-1-ir puncta and GAT-1-ir negative somata (asterisks) and their dendrites in layers II and III from the left side of panel (A). (B): Low magnification of the 24 h CRH group that shows numerous GAT-1-ir somata (arrows) in all layers. (D): Enlargement of the GAT-1-ir somata (arrows) from the center of panel (B). Pir, piriform cortex. Scale bar = 100 μm (A and B) and 20 μm (C and D).

Findings in the 4 h group were indistinguishable from those of controls throughout the hippocampal formation. GAT-1-ir cell bodies were present in all strata of the hippocampus and dentate gyrus. The only region where the number of immunolabeled cells was significantly increased ($P < 0.02$) was in stratum pyramidale of CA1 (Fig. 5).

3.2.2. Neocortex

GAT-1-ir in the 24 h CRH-group was localized throughout the layers and the underlying white matter. GAT-1-ir puncta were found in all neocortical layers and observed around the somata of pyramidal cells and non-pyramidal cells (Fig. 3C and Fig. 4E) as shown previously for controls. Furthermore, all layers of neocortex had

GAT-1-ir non-pyramidal cell bodies (Fig. 3C and Fig. 4F). The immunolabeled somata were of various sizes and shapes. The great majority of the labeled somata were bipolar with small to medium sizes (Fig. 4F). More labeled neuronal somata were found in the 24 h CRH group (Fig. 7) and statistical tests showed a significant increase as compared to the control group ($P < 0.001$).

The 4 h CRH group showed the same distribution pattern of GAT-1-ir puncta as that observed in the control group (Fig. 3B and Fig. 4D). Also, some non-pyramidal cells were immunolabeled for GAT-1 but their number was similar to that of the control group ($P > 0.05$; ANOVA).

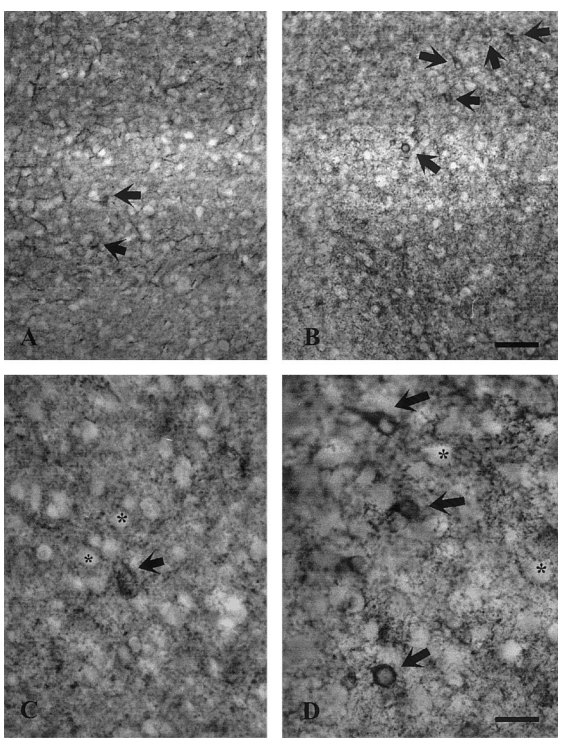

Fig. 9. GAT-1-ir in the amygdala. (A): Low magnification of the control group showing GAT-1-ir puncta and GAT-1-ir somata (arrows). (C): Details of the morphology of GAT-1-ir somata (arrow) and GAT-1-ir negative somata (asterisk). (B): Low magnification of the 24 h CRH group and (D): an enlargement to show the morphology of GAT-1-ir somata. Note an increase in their number as compared to (C). Scale bar = 100 μm (A and B) and 20 μm (C and D).

3.2.3. Other forebrain regions

The previous study by Yan et al. (1997) described GAT-1-immunolabeled somata in the neocortex and hippocampus of young rats. During the course of the present study, numerous GAT-1-ir somata were observed in other forebrain regions of the 24 h CRH group, specifically the piriform cortex and amygdala (Fig. 8B and Fig. 9B). Only small numbers of immunolabeled somata were found in the control group in these brain regions (Fig. 8A and Fig. 9A). The labeled somata in the 24 h CRH group were found in the outer layers (layers II and III) of the piriform cortex (Fig. 8B and D). These cells were of vari-ous sizes and shapes. GAT-1-ir puncta were also found around the somata and dendrites of neurons in the piriform cortex similar to that in the neocortex, and the 24 h CRH and control groups had similar distributions of GAT-1-ir puncta (Fig. 8A–D). The 24 h group also showed GAT-1-ir processes that were thick, and extended from layer II into layer I near the surface of the brain (Fig. 8D). Only a few such processes were observed in the piriform region from the control group (Fig. 5C). The number of detectable GAT-1-ir cell bodies in piriform cortex was significantly increased ($P < 0.001$) in both hemispheres as compared to the control group (Fig. 7).

GAT-1-ir puncta in the amygdala of the 24 h CRH group resembled the distribution of that present in the control group. However, numerous small and round GAT-1-ir somata were present in the 24 h group (Fig. 9B and D) while the control group had only a few GAT-1-ir somata (Fig. 9A and C). The number of GAT-1-ir cells in the amygdala was significantly increased ($P < 0.001$) in both hemispheres as compared to the control group (Fig. 7).

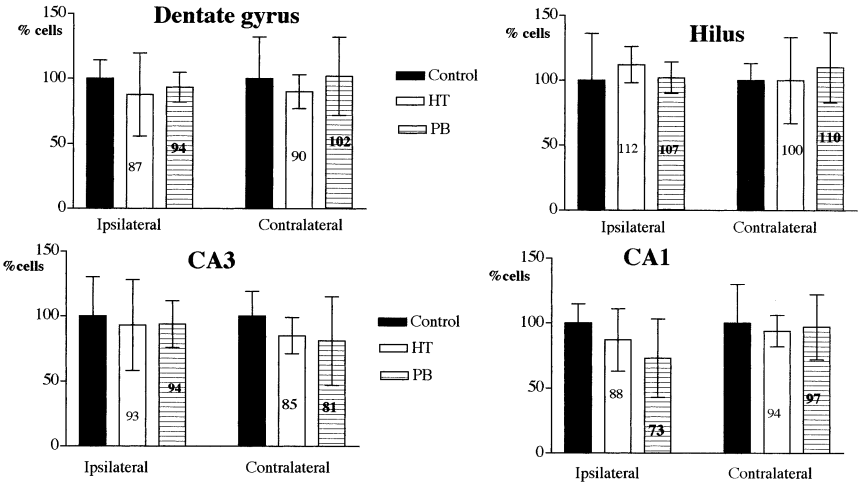

Fig. 10. Histograms of the number of GAT-1-ir somata in the dentate gyrus and hippocampal formation for both hyperthermic groups (HT-hyperthermia-induced seizures and PB-pentobarbital treated rats prior to hyperthermia). The data represent mean ± S.D. The results were analyzed for statistical significance ($P < 0.05$) using a one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison posthoc test. The raw data were expressed as a percentage of the control group.

3.3. Effects of hyperthermia-induced seizures on the distribution of GAT-1-ir

The regional labeling pattern for GAT-1-ir in the neocortex and hippocampus was not influenced by hyperthermic seizures or hyperthermia alone (i.e., when seizures were eliminated with PB). GAT-1-ir cell bodies occurred in all strata of the hippocampus and dentate gyrus, and they showed similar morphological features as those in the control group. The number of detectable GAT-1-ir somata did not vary significantly among the three groups (Figs. 10 and 11). It should also be noted that no immunolabeled somata were observed in the subiculum.

4. Discussion

The results of this study indicate that GAT-1-ir is altered by CRH-induced seizures but not by hyperthermia or hyperthermia-induced seizures. Changes induced by CRH seizures appear to depend on the time following CRH infusion and are region specific. Our data showed significant increases in the number of detectable GAT-1-ir neuronal somata in specific forebrain areas in the 24 h CRH group as compared to the control group. However, the number of neurons with GAT-1-ir was not altered in either the 4 h CRH group or hyperthermia-induced seizure groups.

GAT-1 Immunolabeling of GABAergic Somata. GAT-1-ir in control infant rats was found within the cell bodies and dendrites of GABAergic interneurons in the cerebral cortex. This finding is consistent with the results of a previous study by Yan et al. (1997); showing that GAT-1-ir appeared in the somata and dendrites of 5-day old rats, but the rats 45 days old and older had GAT-1-ir limited to axon terminals of GABAergic neurons. These authors hypothesized that the transient immunolabeling of GABAergic interneurons for GAT-1 may be involved in releasing GABA from somal and dendritic membranes through a non-vesicular

mechanism during development as part of the neurotrophic role of GABA in this period.

The data from the 24 h CRH group suggest that the significant increase in the number of detectable GAT-1-ir neurons in the hippocampal formation, neocortex, piriform cortex and amygdala reflects increased expression of GAT-1 by GABAergic interneurons. First, labeled cells were likely interneurons because the morphology of the immunolabeled somata was nonpyramidal. Second, the immunolabeled neurons in the 24 h CRH group were distributed in the laminar location of GABAergic interneurons (Freund and Buzsaki, 1996; Jones, 1993). Although granule cells in the dentate gyrus continue to be generated at the age of rats in this study, hippocampal interneurons are generated prior to this age (Amaral and Kurz, 1985). Therefore, the increase in the number of GAT-1-immunolabeled neurons in the 24 h group is interpreted as an increase in labeling of GABAergic interneurons that are present in the brain at the time of seizure induction.

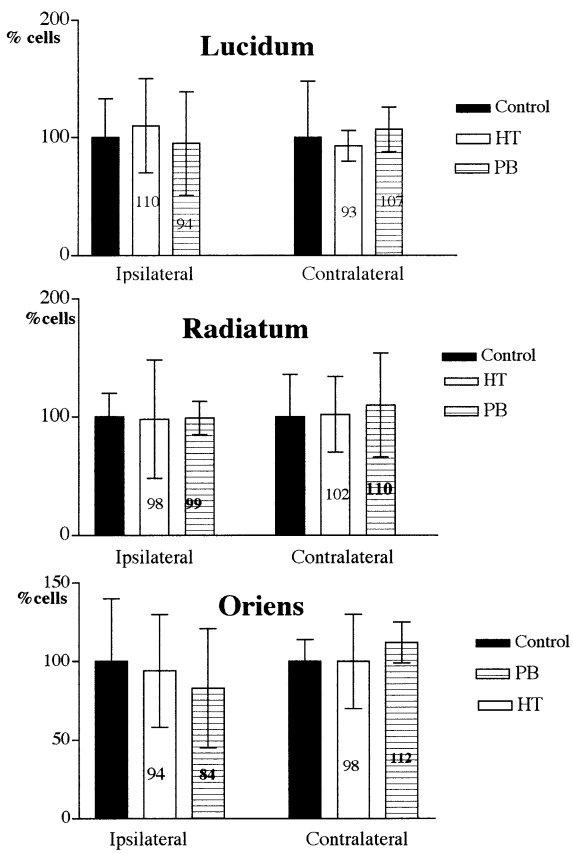

Fig. 11. Histograms of the number of GAT-1-ir somata in several strata of the hippocampal formation for both hyperthermic groups (HT and PB as defined in Fig. 10). The data represent mean ± S.D. The results were analyzed for statistical significance ($P < 0.05$) using one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison posthoc test. The cell counts were expressed as a percentage of the control group.

What is the reason for the increased number of detectable GAT-1-immunolabeled somata following CRH-induced seizures? An intriguing possibil-ity is that the increase may provide the basis for increased release of GABA via a non-vesicular mechanism following the excitatory surge caused by CRH-induced seizures (Baram and Hatalski, 1998). Thus, the GAT-1-ir increase and the proposed concommitant increase in non-vesicular release of GABA may provide a compensatory mechanism that may counteract the hyperexcitability in the amygdala–hippocampal limbic circuit following CRH-induced seizures (Brunson et al., 1998). Induction of GAT-1, at least at the level of protein expression, requires more than 4 h because it was not observed in the 4 h group. Therefore, our study indicates that CRH-induced seizures can trigger increases in GAT-1 levels. Future studies are needed to determine the persistence of this change.

The present results also showed increased numbers of detectable GAT-1-ir somata in piriform cortex and the basal nucleus of the amygdala in the 24 h CRH group. Numerous GAT-1-ir somata were observed in layers II and III of piriform cortex as compared to only a few in the control group. Also, the 24 h CRH group had enlarged and more numerous GAT-1-ir processes in this brain region. Some of these processes were thick and could be dendrites. It is conceivable that the increased expression of

GAT-1 may be a non-specific response to seizures or stress. However, the remarkably selective distribution of the changes in GAT-1 levels indicates that it correlates with brain areas that displayed argyrophillic neurons in a previous study (Baram and Ribak, 1995). It needs to be noted that the basal nucleus of the amygdala was incorrectly labeled in that paper. The fact that the number of GAT-1 labeled somata was increased by CRH-induced seizures but not by hyperthermia-induced seizures indicates that it is not seizures per se (or seizure-associated stress, per se) that determines GAT-1 expression. A selective effect mediated by activity of CRH receptors cannot be excluded (Hatalski et al., 1998).

Hyperthermia-Induced Seizures. Febrile seizures in the immature rat did not increase the number of detectable GAT-1-ir somata in any forebrain structure analyzed compared to controls. These findings indicate that the hyperthermic seizure model is different from the CRH model in regard to the short-term response (24 h) of GABAergic neurons. First, the lack of a change in the number of detectable GAT-1-ir somata could be due to the difference in the duration of seizures. CRH-induced seizures last for about 2 h while those induced by hyperthermia are only about 20 min in length. Another basis for this difference comes from the recent study by Chen et al. (1999) elucidating the nature of the modulation of the hippocampal circuit in the hyperthermia model. Their in vitro studies revealed a selective increase in inhibitory synaptic transmission in hippocampal CA1 neurons (Chen et al., 1999). This finding is consistent with increased GABA in the synaptic cleft, and would not be expected to trigger mechanisms for further enhancement of synaptic GABA levels. In summary, GAT-1 protein expression in the immature brain may be upregulated by developmental seizures. The specific determinants of this upregulation remain to be delineated.

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