Title
Alteration of GABA transporter expression in the rat cerebral cortex following needle puncture and colchicine injection

Journal
Brain Research, 816(2)

ISSN
1385-299X

Authors
Yan, Xiao-Xin
Ribak, Charles E

Publication Date
1999

DOI
10.1016/s0006-8993(98)01103-2

ELSEVIER

Brain Research 816 (1999) 317–328

BRAIN RESEARCH

Research report

Alteration of GABA transporter expression in the rat cerebral cortex following needle puncture and colchicine injection

Xiao-Xin Yan, Charles E. Ribak *

Department of Anatomy and Neurobiology, University of California at Irvine, Irvine, CA 92697-1275, USA

Accepted 13 October 1998

Abstract

In the adult cerebral cortex, GABA transporters (GATs) are expressed by both neurons and astrocytes. GAT-1 immunoreactivity is found in axon terminals of GABAergic neurons and astrocytes, while GAT-3 immunolabeling occurs only in the latter. The present study was designed to determine whether the expression of GAT-1 and GAT-3 in the adult rat cerebrum changes after needle lesion and colchicine infusion. Following a needle puncture or a saline injection, immunolabeling for GAT-1 and GAT-3 was slightly increased in an area around the needle track. Not only was the neuropil labeling for both GATs increased, but also a few neuronal somata were found to be immunoreactive for GAT-1. Colchicine injections induced a striking increase in immunolabeling for both GATs in the neuropil in an area adjacent to the needle path and surrounding it. A homologous region of the contralateral hemisphere also showed a moderate increase of immunoreactivity in the neuropil for both GATs. Furthermore, this contralateral site showed many neuronal somata immunolabeled for GAT-1. These changes were mainly detected during the first 5 days following intracortical lesions. These results indicate that (1) the upregulation of GAT-1 and GAT-3 in cortical interneurons and astrocytes is caused by both mechanical and chemical factors associated with the injections; (2) increased GAT-1 and GAT-3 expression contralateral to the site of colchicine injection is mediated by transcellular signaling across the corpus callosum; and (3) the lesion-induced GAT expression may play a protective role by helping to balance excitatory and inhibitory neuronal activities.

Keywords: GABA; Interneuron; Astrocyte; Neocortex; Hippocampus

1. Introduction

GABA transporters (GATs) are recently identified translemmal proteins in the brain and peripheral tissue [4,9,17,32]. They are found in neurons and glial cells in the nervous system, and functionally have been shown to clear GABA from the synaptic cleft and extracellular space [23,25,26,51], though they may also be involved in the secretion of GABA from neurons in a Ca^{2+}-independent, nonvesicular manner in certain circumstances [2,3,31]. Thus, it is suggested that GATs play an important role in determining the magnitude and duration of GABA's neurotransmission as well as other activities. To date, four GATs have been cloned and they are classified as GAT-1, GAT-2, GAT-3 and GAT-4 according to their differential amino acid sequences and pharmacological properties [4,9,17,32,35]. For example, GAT-1 displays the pharmacological features typical of a 'neuronal transporter' in that its GABA uptake is strongly inhibited by *cis*-3-aminocyclohexane carboxylic acid (ACHC) but not by β-alanine. On the other hand, GAT-2 and GAT-3 are considered to be 'glial transporters' because GABA uptake via these two GATs is inhibited by β-alanine but not ACHC. GABA uptake by GAT-4 is inhibited by neither agent [4,9,25,32].

The cellular and subcellular localization of GATs in the brain and several sensory organs has been recently studied, and it appears that GAT-1 and GAT-3 are the major GATs in the nervous system [5,9,11,21–24,36–39,42,43,45, 46,51,53,54]. In the adult mammalian cerebral cortex, GAT-1 mRNA is expressed in the cell bodies of GABAergic interneurons and astrocytes as determined by in situ hybridization [36,42,43], whereas its protein as shown in immunocytochemical preparations is mainly found in axon terminals of these neurons and astrocytic processes [36,45], though sparse immunoreaction products are detectable on the Golgi complex of some interneurons and in the somata of astrocytes at the electron microscopic level [45]. Unlike GAT-1, GAT-3 is only found in glial elements, i.e., the

* Corresponding author. Fax: +1-949-824-8549; E-mail: ceribak@uci.edu

0006-8993/99/$ - see front matter
PII: S0006-8993(98)01103-2

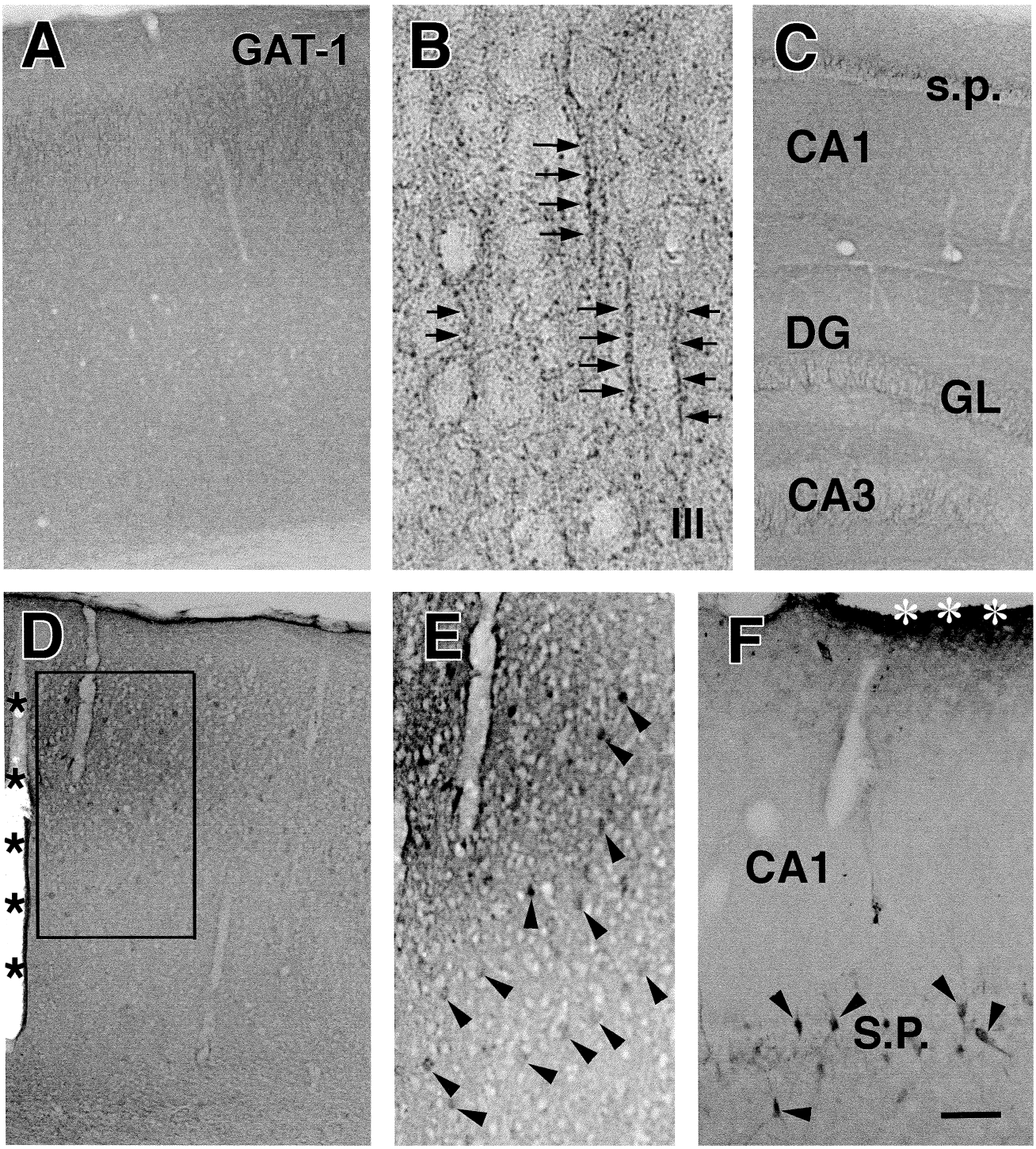


Fig. 1. Light micrographs showing the immunoreactivity for GAT-1 in homologous areas of the contralateral (A–C) and ipsilateral (D–F) neocortex and hippocampal formation from a rat that survived 2 days after an intracerebral saline injection. The immunolabeling in the contralateral side shows the normal distribution pattern of this GABA transporter in the adult rat cerebral cortex, with the immunoreactivity exclusively localized to neuropil. (A) the laminar pattern in the neocortex. (B) is an enlargement of a cortical area in layer III to show that immunonegative pyramidal cell bodies and their axon initial segments are contacted by basket and chandelier cell terminals (arrows). (C) shows the normal pattern of GAT-1 immunoreactivity in the neuropil of the hippocampal formation. Following the injection, the cortical areas near the needle track (indicated with stars in D and white asterisks in F) show a slight increase of immunoreactivity over the neuropil, and a few lightly stained cell bodies (arrowheads) in both the neocortex (E) and hippocampus (F). (E) is an enlargement of the boxed region in (D) to show that the labeled somata (arrowheads) are small, round, and have features of cortical interneurons. CA1 and CA3: CA regions of the hippocampus; s.p.: stratum pyramidale; DG: dentate gyrus; GL: granule cell layer. Scale bar = 200 μm for (A, C, D), 100 μm for (E, F) and 75 μm for (B).

astrocytic somata and processes [37,45]. It is important to note that GAT-1 immunoreactivity is transiently expressed in the somata and dendrites of interneurons in the neocortex and hippocampus of infant and young rats, aged 5–30 days [53].

While the distribution of GATs in the normal brain has been extensively studied, little is known about how the expression of GATs is regulated [13,15,20,41]. We have recently shown an increase in immunoreactivity for GAT-1 and GAT-3 in the superior colliculus following deafferentation from a distant lesion of the optic nerve, suggestive of an upregulation of these GATs following terminal degeneration [54]. In the present study, we analyzed whether the immunoreactivity for GAT-1 and GAT-3 in the cerebral cortex changes following colchicine infusion, based upon several considerations. First, since colchicine is commonly used to block axonal transport [47], we were interested in determining whether it enhances GAT-1 immunolabeling in the somata of mature cortical interneurons. Second, colchicine is somewhat neurotoxic and has been used to create chemical lesions in certain brain regions [19,34]. Thus, we wanted to determine whether colchicine neurotoxicity alters the immunoreactivity of GAT-1 and GAT-3 in cortical neurons and glial cells. Sterile saline was used as the vehicle for colchicine and the first control for this experiment examined the possible effect of this vehicle. In addition, as needle penetration can also cause tissue damage, a second control was used to verify if and how the mechanical lesion associated with the needle puncture contributed to cortical expression of GATs. Unilateral injections were made to the cerebral cortex, using the contralateral hemisphere as an intrinsic control for the injected cortex. A preliminary report of this study was presented elsewhere [48].

2. Materials and methods

2.1. Animals and surgery

Experiments were performed on male and female adult albino rats (Sprague–Dawley) weighing 250–350 g. The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed on a stereotaxic frame. A hole

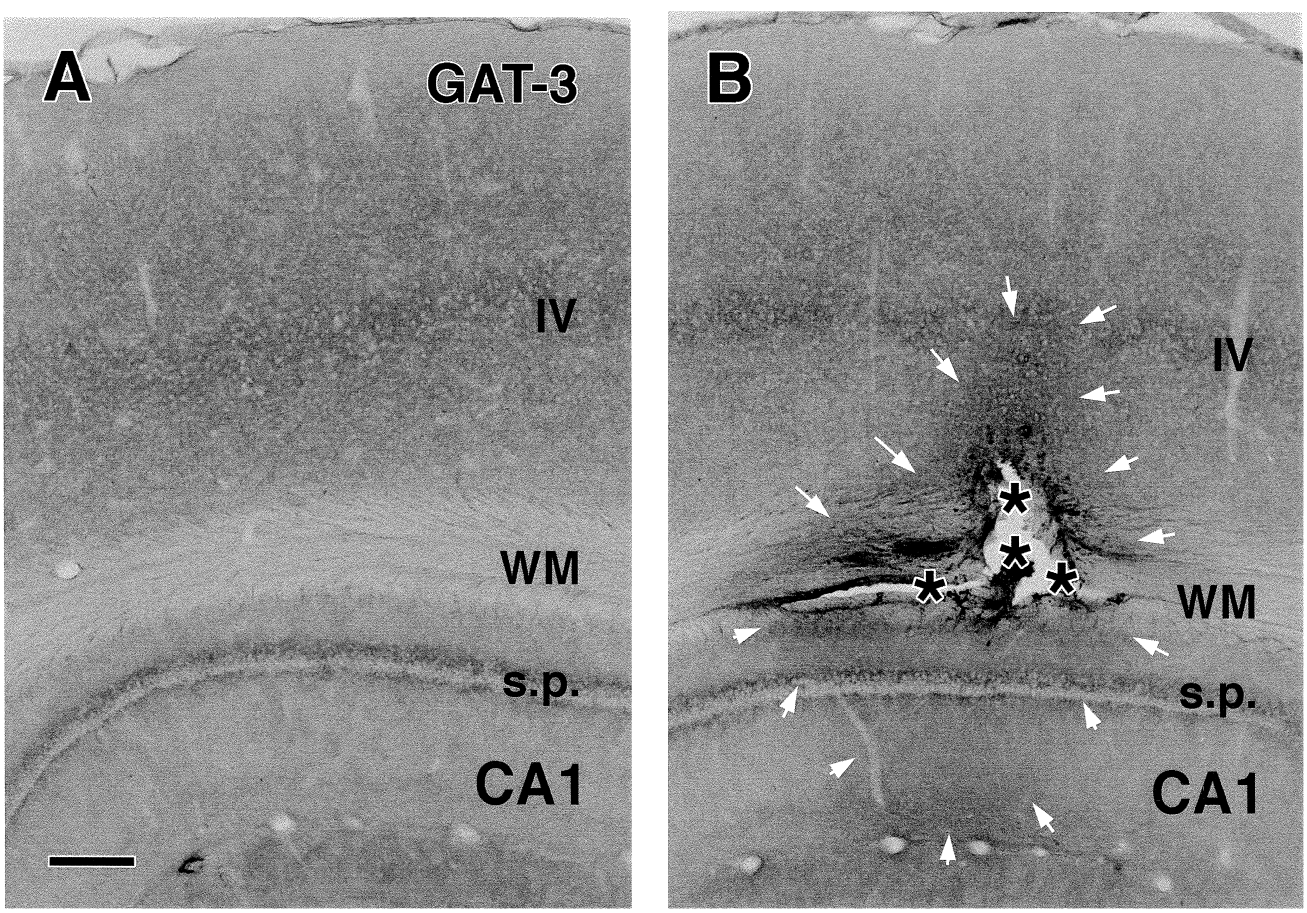


Fig. 2. GAT-3 immunoreactivity in homologous areas of the contralateral (A) and ipsilateral (B) neocortex and hippocampus from a rat that survived for 2 days after an intracerebral saline injection (stars). (A) represents a normal distribution pattern of this GAT in the adult rat cerebral cortex with immunolabeling occurring exclusively in the neuropil. (B) shows a slight increase of immunoreactivity in the neuropil of the neocortical and hippocampal area surrounding the needle track (white arrows). IV: layer IV; WM: white matter. Other abbreviations see Fig. 1. Scale bar = 200 μm.

was drilled on the left side of the skull at 4.5 mm lateral and 4.5–5.5 mm posterior to the bregma. A microliter syringe (#705, Hamilton, NV) that was empty, or loaded with 7 μl sterile saline, or with 7 μg colchicine (Sigma, St. Louis) diluted in 7 μl saline, was positioned above the predrilled hole and then advanced into the cerebrum 4.0 mm [40]. For colchicine injections, the solution was infused at a constant rate for 3 min and the needle remained in position for another 5 min before it was withdrawn. The skin incision was sutured and cleaned. After recovery, the operated animals were returned to the vivarium, and were allowed to survive for 1 ($n = 3$), 2 ($n = 3$), 5 ($n = 3$), 10 ($n = 2$), or 15 ($n = 2$) days before perfusion.

2.2. Tissue preparation

Operated animals were deeply re-anesthetized at the above-indicated time points with sodium pentobarbital and perfused transcardially with 0.02 M phosphate buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde and 0.2% glutaraldehyde in phosphate buffer (pH 7.4). The brains were then removed and postfixed in the perfusion solution for 4–8 h at 4°C, and transferred to cold PBS for several hours to a few days. Brain blocks were prepared to contain the posterior cerebrum and diencephalon. For orientation purposes, a portion of the ventrolateral cortex contralateral to the lesion side was removed. The brain blocks were then sectioned coronally at 40 μm with a vibratome, and the sections that included the injection site were collected in tissue-culture wells in cold PBS. They were further processed for immunocytochemistry and Nissl staining with cresyl violet.

2.3. Immunocytochemistry

Free-floating sections were stained for GAT-1 and GAT-3, respectively, using the standard avidin–biotin complex (ABC) method. At first, endogenous peroxidase activity was bleached with a 20-min rinse in 0.05% H_2O_2 in PBS. Non-specific background staining was lowered with a 2-h incubation in 5% normal horse serum (NHS) at room temperature. The sections were further incubated in a PBS solution containing 5% NHS, 0.3% Triton X-100 and rabbit anti-GAT-1 or GAT-3 (1:5000) at 4°C for 24 h and with agitation. After several rinses with PBS, the tissue was reacted in 1% anti-mouse/rabbit IgG with 5% NHS for 1 h at room temperature, followed by 1% ABC solution for another hour. The bound antibodies were visualized with 0.05% DAB and 0.005% H_2O_2. Three 10-min rinses with PBS were used between all incubations.

The primary antibodies were kindly provided by Dr. N.C. Brecha [36,37] and their specificity was verified in previous studies. Other immunoreactive reagents were purchased from Vector Laboratories. In the present experiments, processing the sections with the primary antibodies omitted, or replaced by normal horse serum, yielded no specific immunolabeling.

3. Results

3.1. Injection site and overview of immunolabeling

Pilot experiments were performed to determine the appropriate volume of vehicle and the amount of colchicine to be used. Briefly, 5–10 μl of vehicle were found to be enough to carry the needed amount of colchicine. The response of the cortex that was injected with this amount of saline was almost identical to that in the cortex punctured with an empty needle, which in both cases appeared as a regional and slight increase in immunostaining for GAT-1 and GAT-3. Injections of 3–7 μg colchicine induced a much greater increase in immunoreactivity in a larger area than that caused by the needle puncture alone or with saline injections. In all of these three conditions, cell loss was only observed within the needle track. Higher doses of colchicine often resulted in structural distortion and cell loss in a larger area within and outside the needle track. As a result, 7 μl vehicle and 7 μg colchicine were chosen to be used for most animals in our experiments.

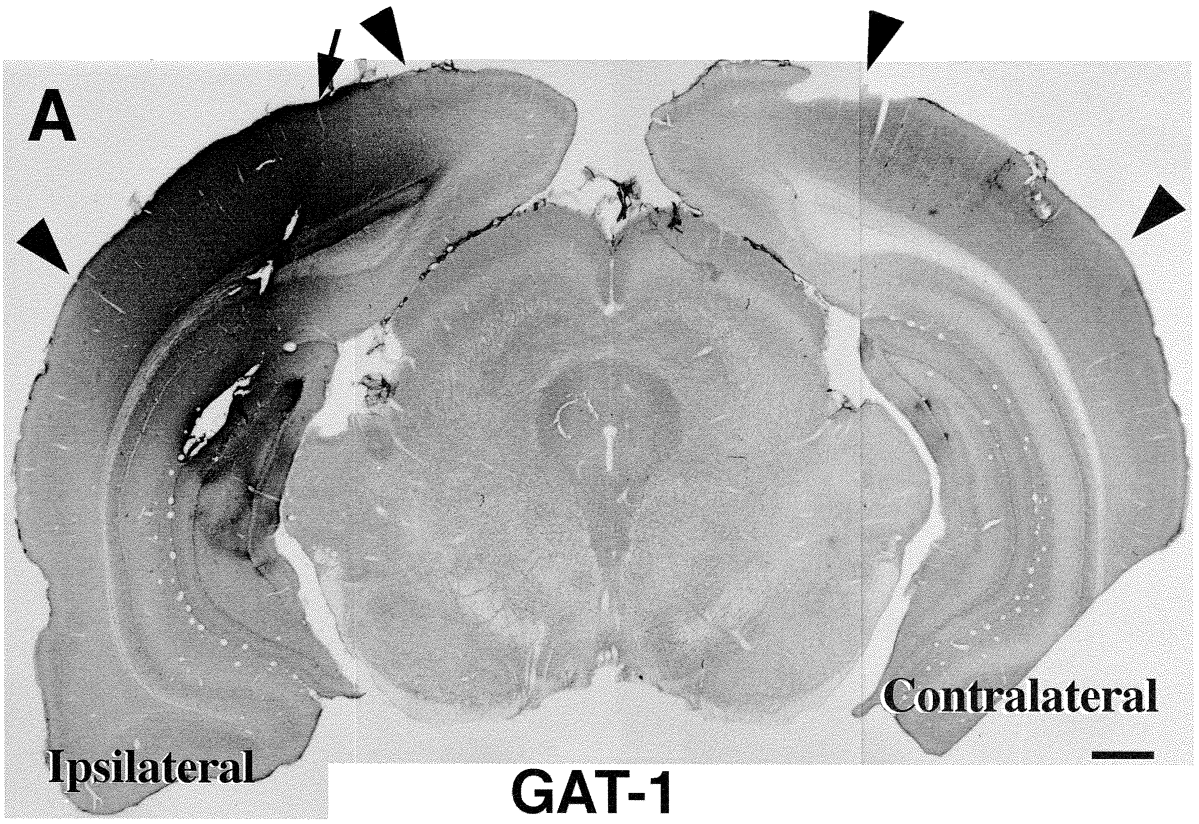
The pattern of immunoreactivity in the cerebral cortex was basically similar for the three short time points (1, 2 and 5 days postoperation) examined in the present study. We will first describe the pattern of immunolabeling observed in the rats with 2-day survival time. Observations from the rats survived for 10 and 15 days will be described at the end of this section.

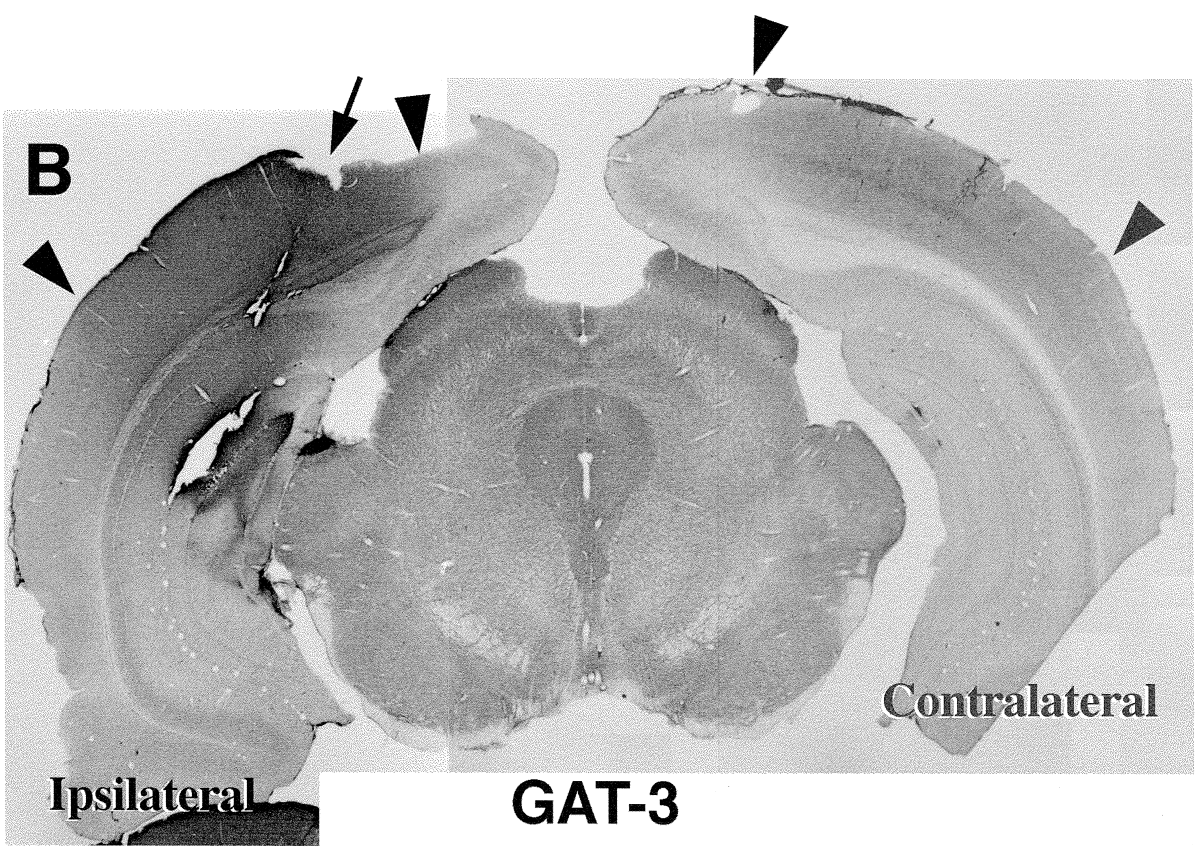
3.2. Immunoreactivity in the unaffected cortex: normal distribution pattern

The normal distribution pattern of immunoreactivity for GAT-1 and GAT-3 in the adult rat cerebral cortex including the hippocampal formation has been described in detail by ourselves and other investigators in previous studies [36,37,42,43,45,53]. In the present study, cortical regions with a normal pattern of immunolabeling were observed in most cortical areas from rats receiving a puncture lesion or saline injection. In both cases, the unaffected regions included the entire contralateral hemisphere and most parts

Fig. 3. Low magnification light microphotographs showing an altered pattern of immunoreactivity for GAT-1 (A) and GAT-3 (B) in both hemispheres following an unilateral intracortical colchicine injection, 2 days postoperation. The needle track in the ipsilateral hemisphere is indicated with arrows. The cortical regions with changes in immunoreactivity are bordered by arrowheads. Note the remarkable upregulation of GAT-1 and GAT-3 within and outside the needle track in the ipsilateral side, and a noticeable increase in a contralateral neocortical region that is symmetric to the affected area on the injected side. Scale bar = 1 mm.

of the ipsilateral cortex except for a zone surrounding the needle path. In the rats injected with colchicine, the unaffected cortical regions were smaller than those in other animals, and were medial and ventral to the affected areas (see below) in both the ipsilateral and contralateral hemispheres.





For GAT-1, a normal pattern of immunostaining is shown in Fig. 1A–C, which was obtained from the contralateral cortex of a rat with a saline injection at this same level. In the neocortex, GAT-1 immunoreactivity was present in the neuropil of all six layers, with little immunolabeling in the white matter. Unlabeled cell bodies were outlined by immunolabeled puncta in layers II–VI, and were most distinct in the supragranular layers (Fig. 1B). Axonal plexuses surrounding the somata and initial segments, respectively, of neocortical pyramidal neurons were

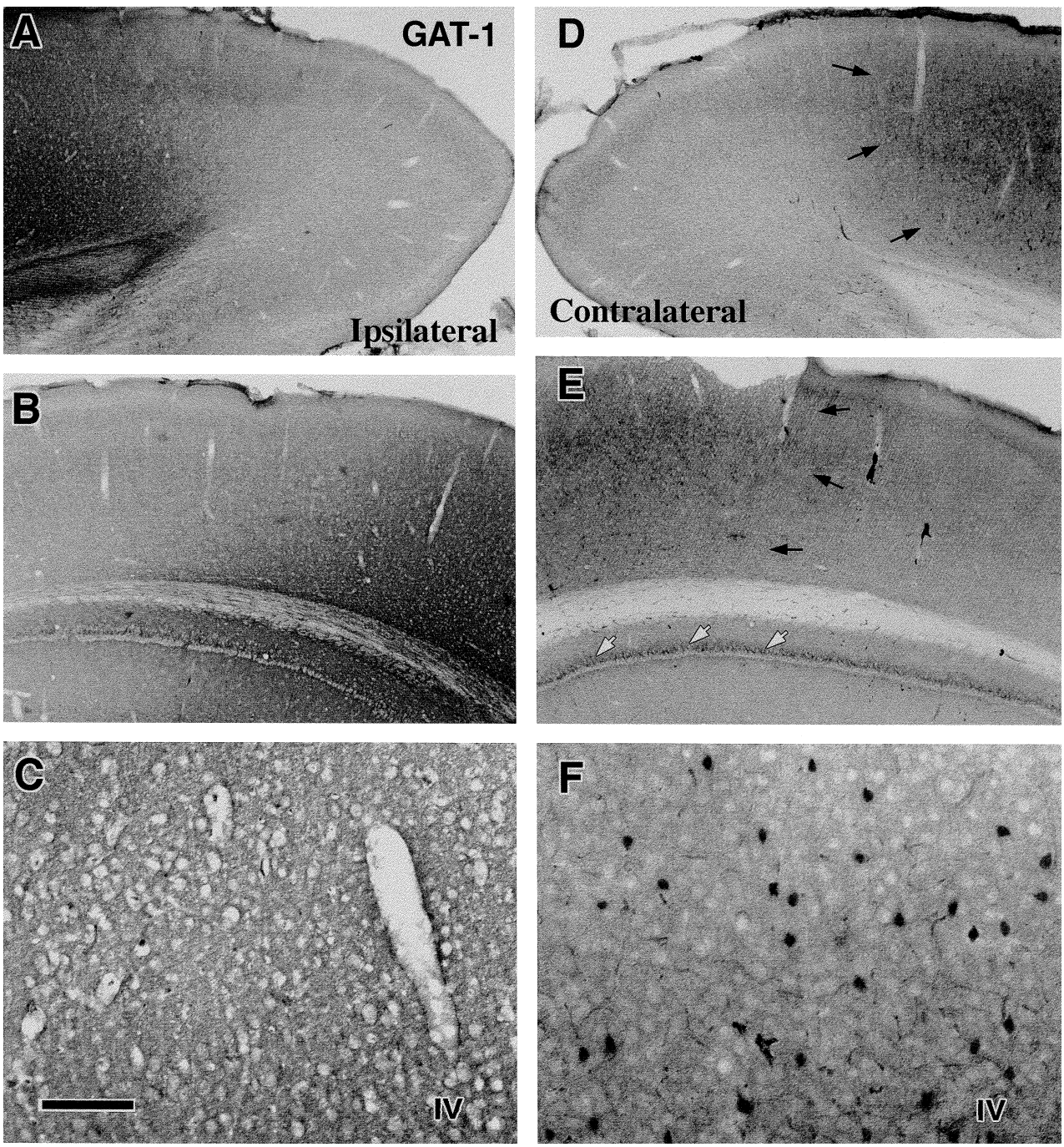


Fig. 4. Higher power views of GAT-1 immunoreactivity in the ipsilateral (A–C) and contralateral (D–F) hemispheres 2 days postinjection of colchicine. (A, B, D, E) illustrate the transitional areas of increased GAT-1 expression from the affected areas to the medial (A, D) and ventrolateral (B, E) unaffected regions. The general staining intensity is much greater in the affected area of the ipsilateral side than the contralateral side (indicated by small arrows in D, E). The upregulated immunostaining is confined to the neuropil in the affected neocortical and hippocampal areas in the ipsilateral side (C), whereas that in the contralateral side involves both neuropil and cell bodies in the neocortex (F). The CA1 region (small white arrows) deep to the affected neocortex shows a slight increase of neuropil immunolabeling as compared to other hippocampal regions, but no cell bodies are found (E). IV: layer IV. Scale bar = 500 μm for (A, B, D, E) and 100 μm for (C, F).

found to be GAT-1 immunoreactive in layers II/III (Fig. 1B). In the hippocampal formation, the cell bodies of pyramidal and granule cells were unlabeled, whereas axon terminals on them and their axon initial segments were immunoreactive for GAT-1 (Fig. 1C). GAT-3 immunoreactivity in the unaffected cortex was diffusely distributed in the neuropil in both neocortical and hippocampal areas (Fig. 2A).

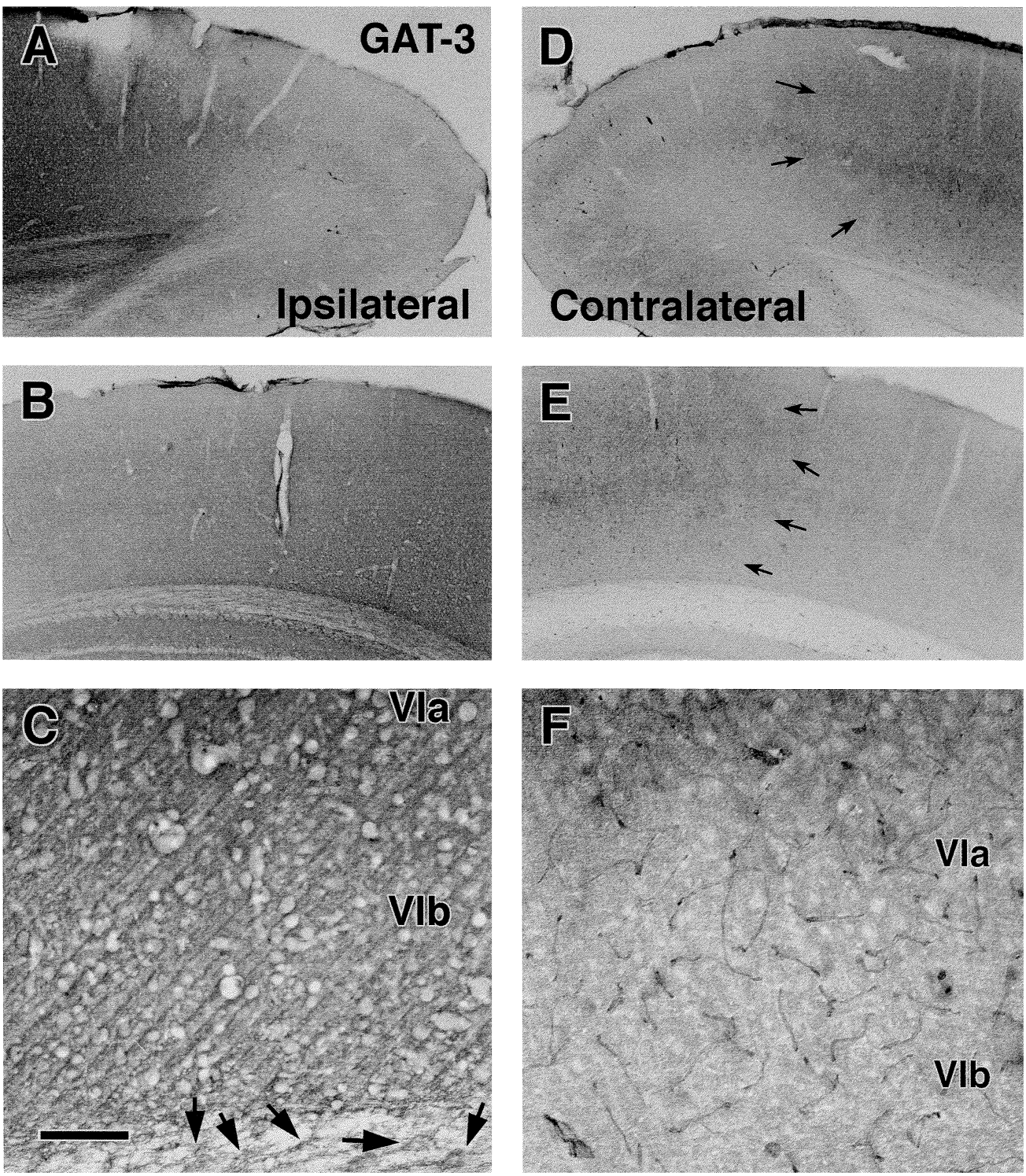


Fig. 5. Higher magnification light microphotographs showing GAT-3 immunoreactivity in the affected cortex of ipsilateral (A–C) and contralateral (D–F) hemispheres 2 days after colchicine injection. Note that the increase of immunolabeling is much greater in the ipsilateral side, and that the elevated staining is exclusively localized to the neuropil on both sides. Deep to the affected neocortex on the ipsilateral side, some small and flat cell bodies with long, thin processes (arrows in C) are recognizable in the white matter. They represent immunoreactive astrocytes. VIa and VIb: sublamina of layer VI. Scale bar = 200 μm for (A, B) and 75 μm for (B, D).

3.3. Immunoreactivity in the ipsilateral cortex of control rats

Both the needle puncture and saline-injected control rats showed similar changes in immunoreactivity for GAT-1 and GAT-3 in the injected hemisphere. A cortical area ranging from 100 μm to 500 μm in diameter surrounding the needle track showed a slight increase in immunostaining for both GATs. The degree of the upregulation was generally greater for GAT-1 (Fig. 1D–F) than GAT-3

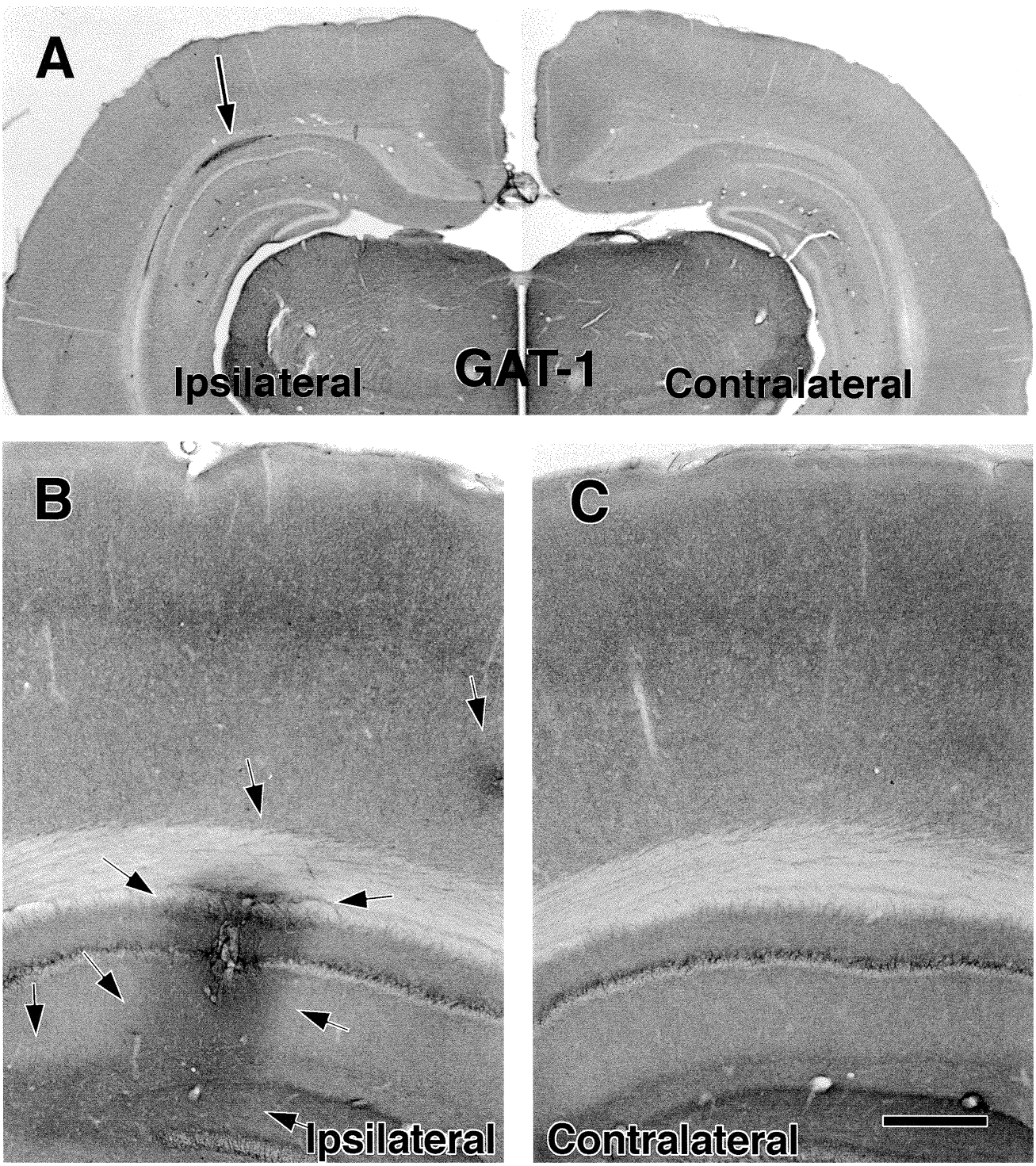


Fig. 6. GAT-1 immunoreactivity in the cerebral cortex of a saline-injected (A) and a colchicine-injected (B, C) rats 10 days postlesion. (A) shows that following a saline injection, the upregulation of GAT-1 is only present in the injection site at the corticohippocampal border (arrow). With colchicine application, the upregulation of GAT-1 remains in the neuropil of a small area (arrows) surrounding the needle track (B), while the corresponding cortical region in the contralateral hemisphere demonstrates a normal distribution pattern (C). Scale bar = 1.5 mm for (A), 300 μm for (B, C).

(Fig. 2B). Both GAT-1 and GAT-3 showed increased immunostaining of the neuropil. However, for GAT-1 but not GAT-3, a few immunoreactive cell bodies were detected within the affected region of the neocortex and hippocampus (Fig. 1D,E, Fig. 2B). In the neocortex, GAT-1 immunoreactive neuronal somata were found in all layers except for layer I and the white matter (Fig. 1D). They were round, small to medium size (10–15 μm), and lacked identifiable dendritic or axonal processes (Fig. 1E). In the hippocampus, GAT-1 immunoreactive cell bodies were often observed in stratum pyramidale of CA1, and some of them had immunolabeled dendrites (Fig. 1F).

3.4. Immunoreactivity in the ipsilateral cortex of colchicine-injected rats

The neocortical and hippocampal regions injected with colchicine displayed a striking increase in the immunoreactivity for GAT-1 and GAT-3 (Fig. 3). The affected area was much larger and the amount of expression was much greater than that in the region around the injected cortex of the control rats. Thus, the affected region in colchicine-injected rats was as much as 1/4 to 1/3 of the cortical area of the entire ipsilateral hemisphere (Fig. 3A,B). The strongest immunolabeling was present at the needle track and its vicinity, and the intensity of immunoreactivity reduced gradually from this central zone toward the periphery.

At higher magnification, the cellular distribution of this intense immunolabeling was confined to the neuropil for both GAT-1 (Fig. 4A–C) and GAT-3 (Fig. 5A–C). Thus, no neuronal somata were observed in the injection site and adjacent areas. The immunonegative cell bodies of pyramidal cells were distinctly outlined by strong neuropil immunolabeling for GAT-1 and less so for GAT-3. However, the size and laminar distribution pattern of these somal profiles were basically the same as those in the unaffected cortex, though the increase of neuropil immunoreactivity resulted in an elevated contrast in the affected region (Fig. 4C, Fig. 5C). In the underlying white matter, some GAT-1 and GAT-3 immunolabeled profiles had long, slender processes that resembled the morphological features of astrocytes (Fig. 5C).

3.5. Immunoreactivity in the contralateral cortex of colchicine-injected rats

In contrast to both the needle puncture and saline-injected controls, rats with colchicine injections had a homologous region of the contralateral hemisphere that showed an increased expression of GAT-1 and GAT-3. This contralateral region was spatially symmetric to the region with intense GAT expression in the ipsilateral cortex (Fig. 3). The general staining intensity in this homologous zone was higher than that in the other portions of this hemisphere, but was much lighter than the regions around the injection site.

At higher magnification, it was found that the GAT-1 immunoreactive changes in the homologous area of the contralateral side involved both neuronal somata and neuropil (Fig. 4D–F). Thus, GAT-1 immunolabeled cell bodies were distributed across the cortex, but peaked in layers III and IV (parietal cortex at the injection level). The immunoreaction products were concentrated in the cytoplasm and the nucleus of the cell body. The processes of these cells were mostly unlabeled. If present, they were very short and probably the proximal dendrites. In contrast to GAT-1, the increased immunolabeling for GAT-3 in the homologous zone of the contralateral hemisphere was solely associated with neuropil (Fig. 5D–F).

The change in immunostaining in the hippocampal formation of the contralateral hemisphere was less than that of the neocortex. For GAT-1, a slight increase of neuropil immunostaining was detectable in the CA1 region deep to the cortical area that showed alterations in GAT expression (Fig. 4E), but no immunoreactive cell bodies were observed. For GAT-3, no immunoreactive changes were detectable over the hippocampal formation (Fig. 3B).

3.6. Immunoreactivity at later survival time points

At 10 days, the increased immunoreactivity in the needle puncture or saline-injected controls became very localized to the injection site at the white matter in the ipsilateral hemisphere (Fig. 6A). For the colchicine-injected rats, the immunostaining in the neuropil of the contralateral hemisphere became equivalent to that found in the unaffected regions, and the GAT-1 immunoreactive cell bodies became undetectable (Fig. 6C). However, in the injected side a small cortical area close to the needle track still expressed increased immunostaining for GAT-1 (Fig. 6B,C) and GAT-3 (not shown) in the neuropil as compared to other regions at this stage. By 15 days, it returned to baseline level (not shown).

4. Discussion

The present study revealed that the immunoreactivity for two GABA transporters, GAT-1 and GAT-3, is upregulated in the adult rat cerebral cortex following colchicine infusion. The increased expression appears not only in the injected cortical region, but also in its homologous area in the contralateral hemisphere. Needle punctures and saline infusions also cause a certain amount of upregulation for both GATs but it is limited to the area surrounding the injection site, suggesting that the mechanical lesion associated with needle penetration is causing a local alteration of GAT expression. For GAT-1, the elevated expression is found to be associated with both glia and neurons, and is most prominent for colchicine-injected rats. The possible mechanisms and biological significance for the postlesion upregulation of glial and neuronal GATs observed in the present study in the adult cerebral cortex are discussed below.

4.1. GAT upregulation in astrocytes as a response to neural injury

The first important finding of the present study is a localized alteration of GAT-1 and GAT-3 immunoreactivity in neocortical and hippocampal regions associated with the mechanical lesion made by needle penetration. A careful histological examination showed that neuronal damage was limited to the needle track, because the cortical thickness as well as the size, morphology and distribution pattern of the neurons in the surrounding cortex remained virtually normal in immunocytochemical and Nissl (not shown) preparations. However, the cortical area that displayed an increase of GAT immunolabeling extended beyond the needle track and was not limited to only the site with cell loss caused by the injection needle. Furthermore, this change in immunostaining was reversible with time. Thus, the lesion-induced upregulation of GAT-1 and GAT-3 immunoreactivity observed in the present study may be triggered by other factors than neuronal death. For example, the mechanical lesion caused by needle penetration may damage neuronal processes [6,12,18,30,33], resulting in degenerating axons and activated astrocytes in the area displaying increased GAT expression. In fact, early studies had shown that following a needle (2.4 mm external diameter) lesion in the brain, the astrocytes within a zone up to 1200 μm from the wound edge were activated and underwent proliferation [6]. The mitotic activity of the focal astrocytes was increased between 1–6 days after the needle lesion [6]. These activated astrocytes became phagocytes that could remove cell debris, including degenerating axon terminals [1]. It is possible that these activated astrocytes are involved in the upregulation of GAT-1 and GAT-3 in the neuropil of the cortex around the needle track.

Pertinent to this hypothesis are data from our recent study that showed increased immunolabeling for GAT-1 and GAT-3 in the rat superior colliculus after optic nerve transection [54]. This change in GAT expression was limited to the retinal-recipient layers of the superior colliculus and was temporally associated with axon terminal degeneration following retinal ganglion cell axotomy. Electron microscopic data indicated that the increased immunolabeling was found in hypertrophied astrocytes that were frequently associated with degenerating ganglion cell axons [54].

Why do colchicine injections induce a much greater upregulation of GAT-1 and GAT-3 expression in the neuropil than needle punctures or saline injections? First, it may be that colchicine is stressful to cortical neurons, and thus induces a more severe neuronal damage and a stronger glial response than needle puncture does. In fact, cell death occurs in certain vulnerable neuronal populations, such as dentate granule cells, following a similar dose of colchicine injection into the cortex [19,34]. Another possibility is that colchicine can promote cell division, especially of astrocytes [6,10]. For example, an intracerebral colchicine infusion increases astrocytic proliferation by 2-fold more than that caused by needle lesion alone [6]. Thus, the upregulation of GAT expression in the neuropil of the colchicine-injected cortex is probably enhanced due to astrocytic proliferation. In fact, immature astrocytes express concentrated GAT-1 and GAT-3 in the developing rat cerebral cortex [53] and cerebellar cortex [own unpublished data].

4.2. Differential regulation of GAT-1 expression in neuronal somata

A novel finding in the present study is that colchicine infusions caused an increased expression of GAT-1 and GAT-3 in a homologous region of the contralateral hemisphere. It is important to note that this region showed light microscopically detectable levels of GAT-1 immunoreactivity within the somata of many cortical neurons. Our results indicated that the laminar pattern, size and somal shape of these somata resembled the features of neocortical GABAergic interneurons [44,55]. This finding is consistent with the fact that GAT-1 mRNA occurs in GABAergic neurons in the mature cerebrum [36,42,43]. Neuropil immunolabeling is also enhanced in this selective contralateral cortical area. As GAT-3 is only contained in astrocytes [37,45], it is obvious that glial cells in this region also contribute to the increased neuropil immunostaining for GATs, including GAT-1.

The upregulation of GAT-1 and GAT-3 in interneurons and glial cells in the contralateral hemisphere cannot be explained by a direct effect of colchicine applied to the other hemisphere because of the regional selectivity of the immunoreactive change. Colchicine's effect on blocking axonal transport [10,47] cannot explain a glial upregulation of GATs and an occurrence of GAT-1 only in the cell bodies of interneurons, because only a few GABAergic neurons in the adult rat have callosal projections and they are mainly localized to the supragranular layers [16,27]. In the present study, GAT-1 immunoreactive somata are numerous and distributed throughout the cortical depth.

The increased neuropil immunolabeling in the contralateral side could be a response of the astrocytes in the vicinity of the callosally projecting axons undergoing retrograde degeneration caused by the injections. On the other hand, the 'reactive' upregulation of GAT-1 in the neocortical interneurons contralateral to colchicine injections is likely related to principal neurons in the ipsilateral hemisphere or those in the contralateral side that project across the corpus callosum. These neocortical pyramidal cells are probably activated by these injections, and then send a signal to GABAergic interneurons, perhaps astrocytes as well [13], to induce the alteration of GAT expression in these latter cells, which is mediated by activation of glutamate receptors and/or the cAMP signal pathway [13,15,20,41]. The reason why there is no upregulation of GATs in GABAergic somata of the contralateral *hip-*

pocampal formation could be because hippocampal pyramidal cells have a limited point-to-point commissural projection [8,29].

A similar explanation can be drawn for the expression of GAT-1 in the neuronal somata of the cerebral cortex with a needle puncture or saline injection because these treatments enhance neuronal activity around the needle path. This notion is supported by the finding of Fos expression in local neuronal and non-neuronal cells following needle puncture [12]. One result of the present study that puzzles us the most involves the lack of GAT-1 immunolabeling in interneurons in the colchicine-injected hemisphere. It is unlikely that GABAergic interneurons in this region are dying after colchicine infusion because previous studies used colchicine to label these neurons for GAD [47]. A possible explanation for the absence of GAT-1 upregulation in GABAergic neurons around the colchicine-injected cortex may be, again, because this chemical is somewhat toxic to local neurons. As a result, the GAT-1 regulatory/synthetic system in these interneurons may be somewhat distorted by this neurotoxicity.

4.3. Functional considerations for altered GAT expression

GABA is the major inhibitory neurotransmitter in the normal adult brain [28]. However, a body of evidence indicates that it exerts an excitatory effect during development and following neuronal trauma [7,50,52]. GABA's action can be regulated by its high affinity transporters, which take up GABA from the synaptic cleft and extracellular spaces into presynaptic axon terminals and astrocytes, so that inhibitory synaptic transmission is terminated in a timely manner and GABA can be recycled into presynaptic terminals [23,26]. In the present study, the upregulation of GAT-1 and GAT-3 is present mainly in the neuropil in the neocortical and hippocampal areas around the needle track. This appearance suggests an enhanced GABA uptake into astrocytes in the cortical regions with a direct lesion. If GABA acts as an excitatory substance in the lesioned cortex, an increased GABA uptake into glial cells would provide a significant neuroprotective effect for the cortical neurons.

On the other hand, the upregulation of GAT-1 in the somata of cortical interneurons in the ipsilateral (puncture) and contralateral (colchicine infusion) cortex may be to strengthen the GABA uptake ability for GABAergic neurons. The biological significance of this mechanism is perhaps to enhance synaptic and non-synaptic GABA release [2,3,31,41,49,56]. As a result, cortical inhibitory activity is elevated to antagonize neuronal overexcitation caused either by the stimulation from the needle puncture, or by transneuronal signaling from the transcallosally projecting pyramidal cells that are activated after colchicine injections. Furthermore, since excitation of cortical pyramidal neurons appears to be an inductive factor for GAT-1 expression [20,41], it is possible that a persistent overactivation or excitation of these cerebral principal neurons may somehow deplete GAT-1 or interfere with GAT-1 production in the interneurons. This suggestion may explain why there is a reduction of GABA transporters in human temporal lobe epilepsy [14].

Acknowledgements

This study was supported by the National Science Foundation (IBN 9422392) and National Institute of Health (NS 15669). We thank Dr. Nicholas C. Brecha for providing GAT antibodies, and Dr. Richard T. Robertson for a critical reading of the manuscript.

References

[1] S.Y. Al-Ali, S.M. Al-Hussain, An ultrastructural study of the phagocytic activity of astrocytes in adult rat brain, J. Anat. 188 (1996) 257–262.
[2] D. Attwell, B. Barbour, M. Szatkowski, Nonvesicular release of neurotransmitter, Neuron 11 (1993) 401–407.
[3] V.F. Balcar, I. Dammasch, J.R. Wolff, Is there a non-synaptic component in the K^+-stimulated release of GABA in the developing rat cortex, Dev. Brain Res. 10 (1983) 309–311.
[4] L.A. Borden, K.E. Smith, T.A. Branchek, R.L. Weieshank, Molecular heterogeneity of the γ-aminobutyric acid (GABA) transport system, J. Biol. Chem. 267 (1992) 21098–21104.
[5] N.C. Brecha, C. Weigmann, Expression of GAT-1, a high affinity gamma-aminobutyric acid plasma membrane transporter in the rat retina, J. Comp. Neurol. 345 (1994) 602–611.
[6] J.B. Cavanagh, The proliferation of astrocytes around a needle wound in the rat's brain, J. Anat. 106 (1970) 471–487.
[7] E. Cherubini, J.L. Gaiarsa, Y. Ben-Ari, GABA: an excitatory transmitter in early postnatal life, Trends Neurosci. 14 (1991) 515–519.
[8] R.B. Chronister, J.F. DeFrance, Organization of projection neurons of the hippocampus, Exp. Neurol. 66 (1979) 509–523.
[9] J.A. Clark, A.Y. Deutch, P.Z. Gallipoli, S.G. Amara, Functional expression and CNS distribution of a β-alanine sensitive neuronal GABA transporter, Neuron 9 (1992) 337–348.
[10] R.M. Dasheiff, L.F. Ramirez, The effects of colchicine in mammalian brain from rodents to monkeys, Brain Res. Rev. 10 (1985) 47–67.
[11] S. DeBiasi, L. Vitellaro-Zuccarello, N.C. Brecha, Immunoreactivity for the GABA transporter-1 and GABA transporter-3 is restricted to astrocytes in the rat thalamus: a light and electron-microscopic immunolocalization, Neuroscience 83 (1998) 815–828.
[12] M. Dragunow, D. de Castro, R.L. Faull, Induction of Fos in glia-like cells after focal injury but not during Wallerian degeneration, Brain Res. 527 (1990) 41–54.
[13] J. Drejer, E. Meier, A. Schousboe, Novel neuron-related regulatory mechanisms for astrocytic glutamate and GABA high affinity uptake, Neurosci. Lett. 37 (1983) 301–306.
[14] M.J. During, K.H. Ryder, D.D. Spencer, Hippocampal GABA transporter function in temporal lobe epilepsy, Nature 376 (1995) 171–177.
[15] J. Gomeza, C. Gimenez, F. Zafra, Cellular distribution and regulation by cAMP of the GABA transporter (GAT-1) mRNA, Mol. Brain Res. 21 (1994) 150–156.
[16] Y.A. Gonchar, P.B. Johnson, R.J. Weinberg, GABA-immunopositive neurons in rat neocortex with contralateral projections to S–I, Brain Res. 697 (1995) 27–34.

[17] J. Guastella, N.C. Brecha, H. Nelson, L. Czyzyx, S. Keynan, M.C. Davidson, H.A. Lester, B.I. Kanner, Cloning and expression of a rat brain GABA transporter, Science 249 (1990) 1303–1306.
[18] K.M. Guthrie, A.G. Woods, T. Nguyen, C.M. Gall, Astroglial ciliary neurotrophic factor mRNA expression is increased in fields of axonal sprouting in deafferented hippocampus, J. Comp. Neurol. 386 (1997) 137–148.
[19] V.R. Heale, K. Petersen, C.H. Vanderwolf, Effect of colchicine-induced cell loss in the dentate gyrus and Ammon's horn on the olfactory control of feeding in rats, Brain Res. 712 (1996) 213–220.
[20] T. Hirao, K. Morimoto, Y. Yamamoto, T. Watanabe, Time-dependent and regional expression of GABA transporter mRNAs following amygdala-kindled seizures in rats, Mol. Brain Res. 54 (1998) 49–55.
[21] S. Honda, M. Yamatomo, N. Saito, Immunocytochemical localization of three subtypes of GABA transporter in rat retina, Mol. Brain Res. 33 (1995) 319–325.
[22] N. Ikegaki, N. Saito, M. Hashima, C. Tanaka, Production of specific antibodies against GABA transporter subtypes (GAT-1, GAT-2, GAT-3) and their application to immunocytochemistry, Mol. Brain Res. 26 (1994) 47–54.
[23] L.L. Iversen, J.S. Kelly, Uptake and metabolism of γ-aminobutyric acid by neurons and glial cells, Biochem. Pharmacol. 24 (1975) 933–938.
[24] J. Johnson, T.K. Chen, D.W. Rickman, C. Evans, N.C. Brecha, Multiple γ-aminobutyric plasma membrane transporters (GAT-1, GAT-2, GAT-3) in the rat retina, J. Comp. Neurol. 375 (1996) 212–224.
[25] F. Jursky, S. Tamura, A. Tamura, S. Mandiyan, H. Nelson, N. Nelson, Structure, function and brain localization of neurotransmitter transporters, J. Exp. Biol. 196 (1994) 283–295.
[26] B.I. Kanner, S. Shuldiner, Mechanism of transport and storage of neurotransmitters, CRC Crit. Rev. Biochem. 22 (1987) 1–39.
[27] F. Kimura, R.W. Baughman, GABAergic transcallosal neurons in developing rat neocortex, Eur. J. Neurosci. 9 (1997) 1137–1143.
[28] K. Krnjević, S. Schwartz, The action of gamma-aminobutyric acid on cortical neurons, Exp. Brain Res. 3 (1967) 320–336.
[29] S. Laurberg, Commissural and intrinsic connections of the rat hippocampus, J. Comp. Neurol. 184 (1979) 685–708.
[30] D.L. Lei, D.L. Yang, H.M. Liu, Local injection of kainic acid causes widespread degeneration of NADPH-d neurons and induction of NADPH-d in neurons, endothelial cells and reactive astrocytes, Brain Res. 730 (1996) 199–206.
[31] G. Levi, M. Raiteri, Carrier-mediated release of neurotransmitters, Trends Neurosci. 16 (1993) 415–418.
[32] Q.-R. Liu, B. Lopez-Caarcuera, S. Mandiyan, H. Nelson, N. Nelson, Molecular characterization of four pharmacological distinct γ-aminobutyric acid transporters in the mouse brain, J. Biol. Chem. 268 (1993) 2106–2112.
[33] M.R. McElhaney, L.J. Chandler, W.J. Streit, Astrocytes but not microglia express NADPH-diaphorase activity after motor neuron injury in the rat, Neurosci. Lett. 180 (1994) 67–70.
[34] J.F. McGinty, S.J. Hendriksen, A. Goldstein, L. Terenius, F.E. Bloom, Dynorphin is contained within hippocampal mossy fibers: immunocytochemical alterations after kainic acid administration and colchicine induced neurotoxicity, Proc. Natl. Acad. USA 80 (1983) 589–593.
[35] S.L. McIntire, R.J. Reimer, K. Schuske, R.H. Edwards, Identification and characterization of the vesicular GABA transporter, Nature 389 (1997) 870–876.
[36] A. Minelli, N.C. Brecha, S. DeBiasi, F. Conti, GAT-1, a high-affinity GABA plasma membrane transporter, is localized to neurons and astroglia in the cerebral cortex, J. Neurosci. 15 (1995) 7734–7748.
[37] A. Minelli, S. DeBiasi, N.C. Brecha, L.V. Zuccarello, F. Conti, GAT-3, a high-affinity GABA plasma membrane transporter, is localized to astrocytic processes, and it is not confined to the vicinity of GABAergic synapses in the cerebral cortex, J. Neurosci. 16 (1996) 6255–6264.
[38] M. Nishimura, K. Sato, M. Mizuno, I. Yoshiya, S. Shimada, N. Saito, M. Tohyama, Differential expression patterns of GABA transporters (GAT1–3) in the rat olfactory bulb, Mol. Brain Res. 45 (1997) 268–274.
[39] H. Obata, K. Shimada, N. Sakai, N. Saito, GABAergic neurotransmission in rat taste buds: immunocytochemical study for GABA and GABA transporter subtypes, Mol. Brain Res. 49 (1997) 29–36.
[40] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic, New York, 1986.
[41] J.P. Pin, J. Bockaert, Two distinct mechanisms, differentially affected by excitatory amino acids, trigger GABA release from fetal mouse striatal neurons in primary culture, J. Neurosci. 9 (1989) 648–656.
[42] R. Radian, O.P. Ottersen, J. Storm-Mathisen, M. Castel, B.I. Kanner, Immunocytochemical localization of the GABA transporter in rat brain, J. Neurosci. 10 (1990) 1319–1330.
[43] M. Rattray, J.V. Priestley, Differential expression of GABA transporter-1 messenger RNA in subpopulations of GABA neurons, Neurosci. Lett. 156 (1993) 163–166.
[44] C.E. Ribak, Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxylase, J. Neurocytol. 7 (1978) 461–478.
[45] C.E. Ribak, W.M.Y. Tong, N.C. Brecha, The GABA plasma membrane transporters, GAT-1 and GAT-3, display different distributions in the rat hippocampus, J. Comp. Neurol. 367 (1996) 595–606.
[46] C.E. Ribak, W.M.Y. Tong, N.C. Brecha, Astrocytic processes compensate for the apparent lack of GABA transporters in the axon terminals of cerebellar Purkinje cells, Anat. Embryol. 194 (1996) 379–390.
[47] C.E. Ribak, J.E. Vaughn, K. Saito, Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport, Brain Res. 140 (1978) 315–322.
[48] C.E. Ribak, X.-X. Yan, Unilateral intracerebral colchicine injections induce upregulation of GABA transporters in interneurons and astrocytes in the neocortex and hippocampus of both hemispheres in adult rats, Soc. Neurosci. Abstr. 23 (1997) 2043.
[49] P. Saransaari, S.S. Oja, Enhanced GABA release in cell-damaging conditions in the adult and developing mouse hippocampus, Int. J. Dev. Neurosci. 15 (1997) 163–174.
[50] K.L. Staley, B.L. Soldo, W.R. Proctor, Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors, Science 269 (1995) 977–981.
[51] M. Swan, A. Najlerahim, R.E.B. Watson, J.P. Bennett, Distribution of mRNA for the GABA transporter GAT-1 in the rat brain: evidence that GABA uptake is not limited to presynaptic neurons, J. Anat. 185 (1994) 315–323.
[52] A.N. van den Pol, K. Obrietan, G. Chen, Excitatory actions of GABA after neuronal trauma, J. Neurosci. 16 (1996) 4283–4292.
[53] X.-X. Yan, W.A. Cariaga, C.E. Ribak, Immunoreactivity for a GABA plasma membrane transporter, GAT-1, in developing rat cerebral cortex: transient presence in the somata of neocortical and hippocampal interneurons, Dev. Brain Res. 99 (1997) 1–19.
[54] X.-X. Yan, C.E. Ribak, Increased expression of GABA membrane transporters, GAT-1 and GAT-3, in deafferented superior colliculus of the rat, Brain Res. 783 (1998) 63–76.
[55] X.-X. Yan, D.S. Zheng, L.J. Garey, Prenatal development of GABA immunoreactive neurons in the human striate cortex, Dev. Brain Res. 65 (1992) 191–204.
[56] A.M.A. Ylinen, R. Miettinen, A. Pitkanen, A. Gulyas, T. Freund, P.J. Riekkinen, Enhanced GABAergic inhibition preserves hippocampal structure and function in a model of epilepsy, Proc. Natl. Acad. Sci. USA 88 (1991) 7650–7653.