

N-ACETYLASPARTATE (NAA) is one of the most prevalent compounds in the mammalian nervous system. As such, NAA largely contributes to the major peak on water-suppressed proton magnetic resonance spectra. Highly specific antibodies to NAA demonstrate that this compound is discretely localized in a substantial number of neurons throughout the extent of the rat CNS. N-acetylaspartylglutamate (NAAG) is a structurally related neuronal dipeptide which is less widely distributed than NAA. NAAG and NAA immunoreactivities were extensively colocalized in many brainstem areas, where NAAG containing neurons were more numerous than in forebrain structures.

Key words: Aspartate, Magnetic resonance spectroscopy, NAAG, Excitatory neurotransmission

## Immunohistochemical localization of N-acetylaspartate in rat brain

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### Introduction

N-Acetylaspartate (NAA) is a ubiquitous compound in the mammalian nervous system, with an estimated concentration in human brain tissue of 13 to 18 mM.<sup>1</sup> Numerous functions have been proposed for NAA. Early reports suggested a role in the acetyl transport system for extramitochondrial fatty acid synthesis.<sup>2</sup> Recently, a congenital error in NAA metabolism has been associated with Canavan disease, a degenerative disorder characterized by leukodystrophy.<sup>3</sup> This condition results from a deficiency in the hydrolytic enzyme, aspartoacylase.<sup>4</sup> The enzyme that acetylates aspartate, aspartate N-acetyltransferase, has a regionally specific distribution in the nervous system.<sup>5</sup> NAA may act as a precursor for the synthesis of N-acetylaspartylglutamate (NAAG),<sup>6</sup> a neuron specific dipeptide which may be involved in neurotransmission.<sup>7</sup>

NAA has gained interest due to its prominent signature on water-suppressed proton magnetic resonance spectra of human brain. In patients with chronic localized encephalitis the locally decreased ratios of NAA to creatine (NAA/Cr) resonance intensities may reflect neuronal loss.<sup>8</sup> Also, magnetic resonance spectroscopy in patients with multiple sclerosis suggests that decreases in the NAA/Cr ratio occur only in plaques associated with permanent neuronal loss.<sup>9</sup> There are decreases in the ratio of NAA to choline in cases of herpes simplex encephalitis<sup>10</sup> and ischemic brain damage.<sup>11</sup> Determining which cell groups in the nervous system contain high levels of these acetylated compounds should aid interpretation of the changes in magnetic resonance spectra which are observed in a variety of CNS disorders. Here we report the specific immunohistochemical localization of NAA to neurons of the rat brain, and compare its observed neuronal distribution with that of NAAG in several brain regions.

### Materials and Methods

Polyclonal antisera to NAA were produced in rabbits as previously described for NAAG.<sup>6</sup> The serum was affinity purified as previously described for NAAG<sup>12</sup> with some modifications. The antisera were diluted ten-fold in PBS (pH 7.2) and preincubated with 100  $\mu$ g ml<sup>-1</sup> of NAAG, aspartate and glutamate, each coupled to bovine serum albumin (BSA). The serum was then applied to a NAA-coupled aminoalkyl-agarose gel and continuously circulated with a peristaltic pump for 18 h at 4°C. The column was washed with ten column volumes of 3M guanidine HCl in PBS, eluted with the same volume of 6 M guanidine HCl, and the eluate was dialysed against PBS. Polyclonal sera against NAAG were affinity purified in a similar way on a NAAG-coupled aminoalkyl-agarose gel, after preblocking with BSA conjugates of NAA, glutamate and aspartate.

Three adult male rats were anaesthetized with Nembutal® (50 mg kg<sup>-1</sup> i.p.) prior to transcatheterial perfusion with 4% carbodiimide in PBS. Coronal sections (20  $\mu$ m) were cut on a Bright cryostat. Immunohistochemistry was performed by the floating section technique.<sup>12</sup>

Specificity controls were two-fold. Working dilutions of the antibodies were incubated with nitrocellulose sheets previously adsorbed with serial dilutions of NAAG, NAA, aspartylglutamate, aspartate and glutamate conjugated to BSA. Also, working dilutions of the primary antibodies were incubated individually with the above protein-ligand conjugates in solution overnight at 4°C before application to sections.

### Results

Immunoblots indicated both purified antibodies had a high degree of specificity for the corresponding

protein coupled compounds. NAAG antibodies crossreacted approximately 1-2% with aspartylglutamate and NAA conjugates as indicated by similar staining intensity in spots with concentration differences of 50 to 100 times. Similarly, the purified NAA antibodies showed 1-2% crossreactivity with the BSA-NAAG conjugate. In blocking experiments, 10  $\mu\text{g ml}^{-1}$  of the NAA conjugate completely eliminated the immunoreactivity (IR) for NAA in tissue sections, while 50  $\mu\text{g ml}^{-1}$  for the other conjugates, failed to inhibit binding. The antibodies to NAAG were similarly inhibited by 10  $\mu\text{g ml}^{-1}$  of NAAG coupled to BSA, but were not blocked by 50  $\mu\text{g ml}^{-1}$  of the other conjugates.

NAA-IR was found in neurons in every major area of the rat brain. This staining was punctate, restricted to cell bodies and basal dendrites, and was excluded from cell nuclei. Cresyl violet counterstaining showed that many neurons throughout the brain were not immunoreactive for NAA. NAAG-IR was less widespread than that of NAA, particularly in forebrain, but was also punctate. Regions in which NAAG staining was low included neocortex, central thalamus, and striatum. NAA-IR was moderate to intense in each of these regions. *Neocortex.* In neocortex, neuronal NAA-IR was

extensive (Fig. 1A), but there were also non-immunoreactive neurons in all layers of cortex. Pyramidal neurons in layer V ranged from highly immunoreactive to non-immunoreactive (Fig. 1C). In contrast, NAAG-IR was much more restricted in neocortex (Fig. 1B). Scattered neurons were highly immunoreactive for NAAG, but they were only a small fraction of the total number of cortical neurons. While many pyramidal neurons were immunoreactive for NAA, the scattered NAAG-IR neurons in cortex appeared to be interneurons (based on size and morphology, Fig. 1D).

*Hippocampus.* The staining patterns for NAA and NAAG in the hippocampus were distinct (Fig. 2A and 2B). NAA-IR was more ubiquitously distributed, with staining in most pyramidal cells, granule cells, and polymorphic cells. Pyramidal cell staining was most intense in the CA3 region (Fig. 2E). Granule cells displayed moderate NAA staining, and many of the polymorphic cells were also immunoreactive (Fig. 2C). The pattern of NAAG-IR was quite restricted (Fig. 2B), encompassing the majority of polymorphic cells (Fig. 2D), and small numbers of scattered neurons in the pyramidal cell layer of all regions (Fig. 2F). *Brainstem.* NAAG-IR and NAA-IR had similar

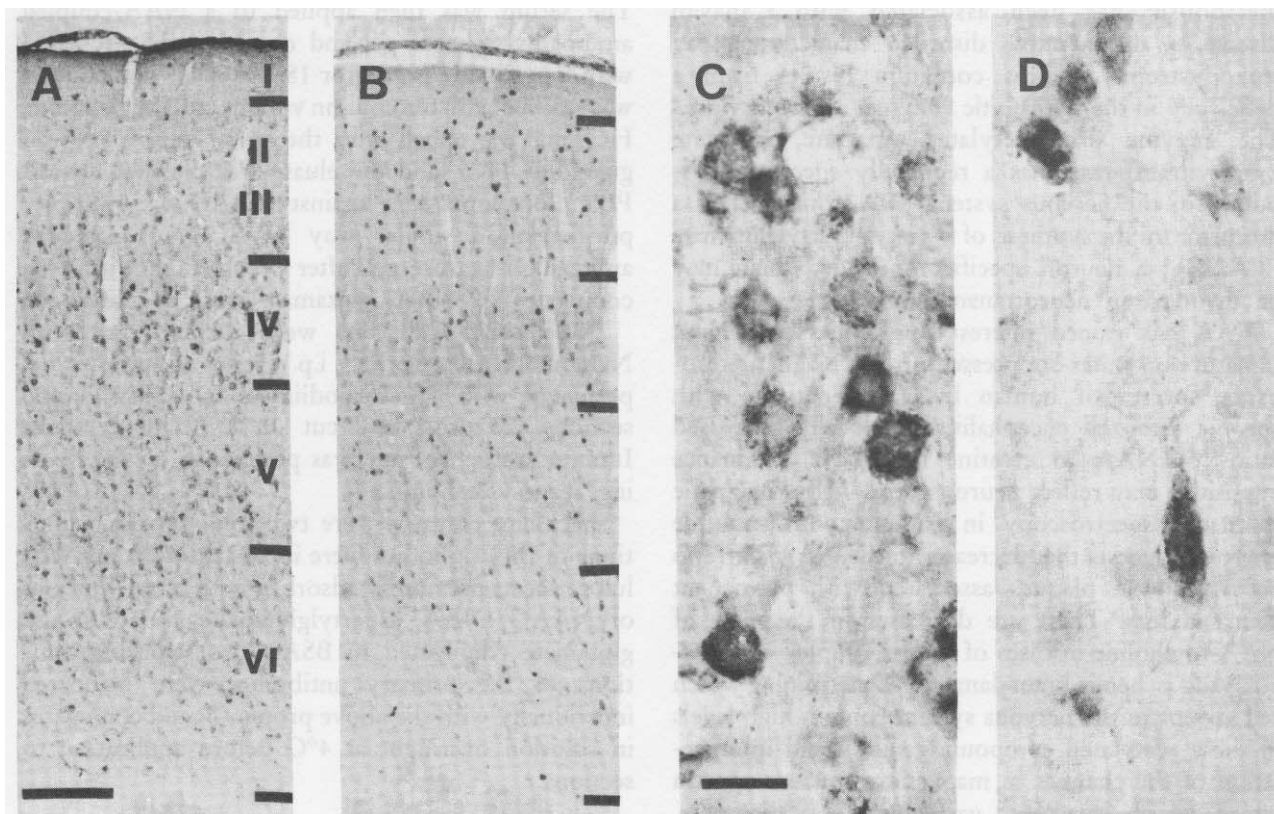


FIG. 1. The distributions of NAA (A) and NAAG (B) in temporal cortex. The distribution for NAAG was limited, being present in a relatively small number of neurons in layers I—IV in neocortex. The distribution for NAA was much more extensive, being present in the majority of cells in layers II—VI. Many neurons did not stain, however, indicating that NAA is not a ubiquitous compound in neocortical neurons. While many pyramidal neurons, such as those of layer V, were labelled for NAA (C), most neurons in this layer did not stain for NAAG, and those that did appeared to be interneurons (D). [Bars = 250  $\mu\text{m}$  A and B; 30  $\mu\text{m}$  C and D].

