Pergamon

Neuroscience Vol. 92, No. 4, pp. 1177–1191, 1999

PII: S0306-4522(99)00025-1
0306-4522/99 $20.00+0.00

A LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE CB1 CANNABINOID RECEPTOR IN PRIMATE BRAIN

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Abstract—The immunohistochemical distribution and subcellular localization of the cannabinoid CB1 receptor was determined in the adult monkey using a polyclonal antiserum raised against the amino terminus of the rat CB1 receptor. At the level of light microscopy, our results generally parallel earlier studies investigating CB1 distribution in rodent brain with a few differences. In particular, high levels of receptor were found in the cortex, hippocampus, amygdala, cerebellum. However significant differences were also noted. The most striking differences were high levels of CB1 receptor in the monkey substantia nigra pars compacta, cerebellar Purkinje cells, and the principal cells of the hippocampus, while few receptors were found in the globus pallidus or substantia nigra pars reticulata. In contrast, in a previous study investigating the rat, using the same antibody, the opposite staining pattern was observed. At the electron microscopic level CB1 receptor was restricted to neurons. Here it was found both pre- and postsynaptically, particularly on dendritic spines and axon terminals.

The CB1 receptor is widely distributed in higher brain regions in the monkey. While its distribution is similar to that in the rat, there are major differences, some of which may be significant when extrapolating the behavioral effects of cannabinoids observed in rodents to primates (e.g., humans). The ultrastructural localization of the CB1 receptor suggests that it modulates neuronal excitability by both pre- and postsynaptic mechanisms. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: monkey, immunocytochemistry, dendrite, axon terminal, basal ganglia.

The marijuana plant (*Cannabis sativa*) contains about 60 psychoactive compounds termed cannabinoids that have profound effects on sensory perception, mood, thinking, and memory. These effects are primarily mediated by the brain (CB1) cannabinoid receptor.[19,28,29] This receptor is a member of the G protein-coupled receptor superfamily[17] and has previously been shown to couple in an inhibitory fashion to adenylyl cyclase[11] and both N-[1,14,27] and P/Q-type calcium channels.[15,37] An inwardly rectifying potassium conductance has also been shown to be activated by the cannabinoids.[15] Inhibition of presynaptic N or P/Q-type calcium channels by cannabinoids has been proposed to lead to decreased release of neurotransmitters from axon terminals. Indeed, presynaptic inhibition of glutamate release by cannabinoids is observed in cultured hippocampal neurons.[32] Presynaptic inhibition and decreased neurotransmitter release could explain many of the behavioral effects of cannabinoids, including those of the endogenous brain cannabinoids, anadamide (arachidonyl ethanolamide) and 2-arachidonyl glycerol.[3,35] However, as the channels (e.g., see Refs 2 and 15) and other effectors modulated by cannabinoids are not exclusively located presynaptically, it is logical to expect that the effects of cannabinoids are not restricted to the presynaptic terminal.

Cannabinoid receptors have been localized in the rat brain by binding assays using the synthetic cannabinoid ligand [^{3}H]CP55,940[8,9] and [^{3}H]WIN 55,212-2[12] *in situ* hybridization histochemistry using mRNA probes for the cloned CB1 receptor,[16,17] and immunocytochemistry using a highly purified polyclonal antibody to CB1.[36] In the autoradiography studies, very dense receptor binding was found in the lateral part of the caudate nucleus and putamen, cerebellar molecular layer, innermost layers of the olfactory bulb, and the CA3 and molecular layers of the dentate gyrus of the hippocampus. The rest of the forebrain was moderately densely labeled, while the brainstem and spinal cord were sparsely labeled.[8,9] These results were mostly similar to those of *in situ* hybridization studies, although some areas were high in receptor binding but low in cannabinoid mRNA expression and vice versa.[16,18] The molecular layer of the dentate gyrus, for instance, contained high levels of receptor binding but low mRNA levels.[16,18] Conversely, mRNA levels are high, but receptor binding relatively low, in the hilus of the dentate gyrus. The discrepancies between receptor binding and mRNA studies have

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Abbreviations: mGluR, metabotropic glutamate receptor; PBS, phosphate-buffered saline.

been attributed to presence of cannabinoid receptors on dendrites, axons and nerve terminals distant from the cell bodies that contain most of the mRNA.[16,18] Immunocytochemical studies showed intensely stained neurons in cortical structures including the hippocampus, and the olfactory bulb, and moderately or lightly stained neurons in the caudate–putamen and cerebellum.[36] A striking finding in the hippocampus was the strong labeling of a subpopulation of interneurons.

In contrast to the rat brain, relatively little is known about the distribution of CB1 in primates. Quantitative autoradiography using the [^{3}H]CP55,940 ligand and *in situ* hybridization have been carried out in parts of the monkey and human brain.[5,10] At the level of resolution possible with these techniques, the distribution of CP55,940 binding sites in the primate rougly parallels that of the rat. However, immunocytochemical localization of CB1 in the primate has, thus far, not been performed. Furthermore, even less is known about the ultrastructural localization of cannabinoid receptors, even though these receptors have been hypothesized to perform an important function in presynaptic modulation of neurotransmission.[14,32] The present study was therefore performed to elucidate the light and electron microscopic features of these receptors in the monkey brain. In view of the profound effects that cannabinoids have on mood, perception and memory, emphasis was placed on the cerebral neocortex and limbic areas such as the entorhinal cortex, amygdala, and hippocampus.

EXPERIMENTAL PROCEDURES

Animals and fixation

Two adult male and two adult female monkeys (*Macaca fascicularis*), weighing 1.5–8 kg were used in this study. These were deeply anesthetized with an intraperitoneal injection of pentobarbital (30 mg/kg), and transcardially perfused with normal saline, followed by a fixative, consisting of 4% paraformaldhyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were bisected, and a series of coronal slices of approximately 5 mm thickness were made from each hemisphere. The slices were kept in the same fixative overnight.

Immunocytochemistry

The blocks were sectioned at 100 μm thickness using an Oxford Vibratome. The free-floating sections were washed in five to six one-hourly changes of phosphate-buffered saline (PBS), and incubated for 45 min in a solution of 1% (w/v) non-fat dry skimmed milk in PBS (PBS–milk) to block non-specific binding of the antibody. The sections were then incubated overnight, in rabbit polyclonal antibodies to CB1,[36,37] diluted (1:3 000) in PBS–milk. This antibody was generated using the N-terminal 77 amino acid residues of the cloned rat CB1 receptor fused to glutathione S-transferase as the antigen.[37] (These residues are highly conserved between the rat and human CB1 receptors. The antibody used in these studies appropriately stains cells expressing the human CB1 receptor [data not shown]. But it will not recognize the CB1A receptor, which is a possible splice variant of the CB1 receptor[33]). They were then washed in three changes of PBS, and incubated for 1 h at room temperature in a 1:200 dilution of biotinylated goat anti-rabbit IgG (Vector). This was followed by three changes of PBS to remove unreacted secondary antibody. The sections were then reacted for 1 h at room temperature with an avidin–horseradish peroxidase complex. The reaction was visualized by treatment for 5 min in 0.05% 3,3-diaminobenzidine tetrahydrochloride solution in Tris buffer containing 0.05% hydrogen peroxide. The color reaction was stopped with several washes of Tris buffer, followed by PBS. Sections were then mounted on gelatin-coated glass slides and lightly counterstained with Methyl Green before coverslipping. As a control, sections from each brain region studied were incubated with PBS or pre-immune rabbit serum instead of primary antibody, or with immune serum and the immunizing protein (1 μg/ml), and showed a complete absence of immunostaining. For the cerebral cortex this is shown in Fig. 1A.

Electron microscopy

Electron microscopy was carried out by subdissecting the immunostained sections that included the cerebral neocortex, hippocampus, amygdala, cerebellar cortex and substantia nigra. These were osmicated, dehydrated in an ascending series of ethanol and acetone, and embedded in Araldite. Thin sections were obtained from the first 5 μm of the immunostained sections, mounted on copper grids coated with Formvar, and stained with lead citrate. They were viewed using a Philips EM400T or CM120 electron microscope.

RESULTS

Light microscopy

Cerebral cortex and subcortical white matter. A similar pattern of staining was observed in the frontal (Fig. 1B), temporal (Fig. 1C), parietal (Fig. 1D) and occipital neocortex, and the entorhinal cortex (Fig. 2A). Many densely labeled neuronal cell bodies were observed in layers II, III, V and VI. Most of these contained a prominent apical dendrite and had features of pyramidal neurons (Fig. 1C, D; Fig. 2A). Others lacked an apical dendrite, and had feature of non-pyramidal neurons (Fig. 1D). Spiny stellate neurons in layer IV[13] were unlabeled or very lightly labeled, and this layer was very lightly stained.

In contrast to the gray matter, no labeled cells, and only very occasional labeled axons were present in the subcortical white matter, and these and other white matter tracts such as the corpus callosum, internal and external capsule, were almost completely unlabeled for CB1.

Amygdala. The amygala was densely labeled for cannabinoid receptors. Label was observed in large diameter (16–22 μm), putative pyramidal or projection neurons[21] with a single prominent dendrite or several large dendrites, as well as putative non-projection neurons that lacked a large stem dendrite (Fig. 2B).

Hippocampus. Dense staining was observed in the cell bodies and stem dendrites of nearly all

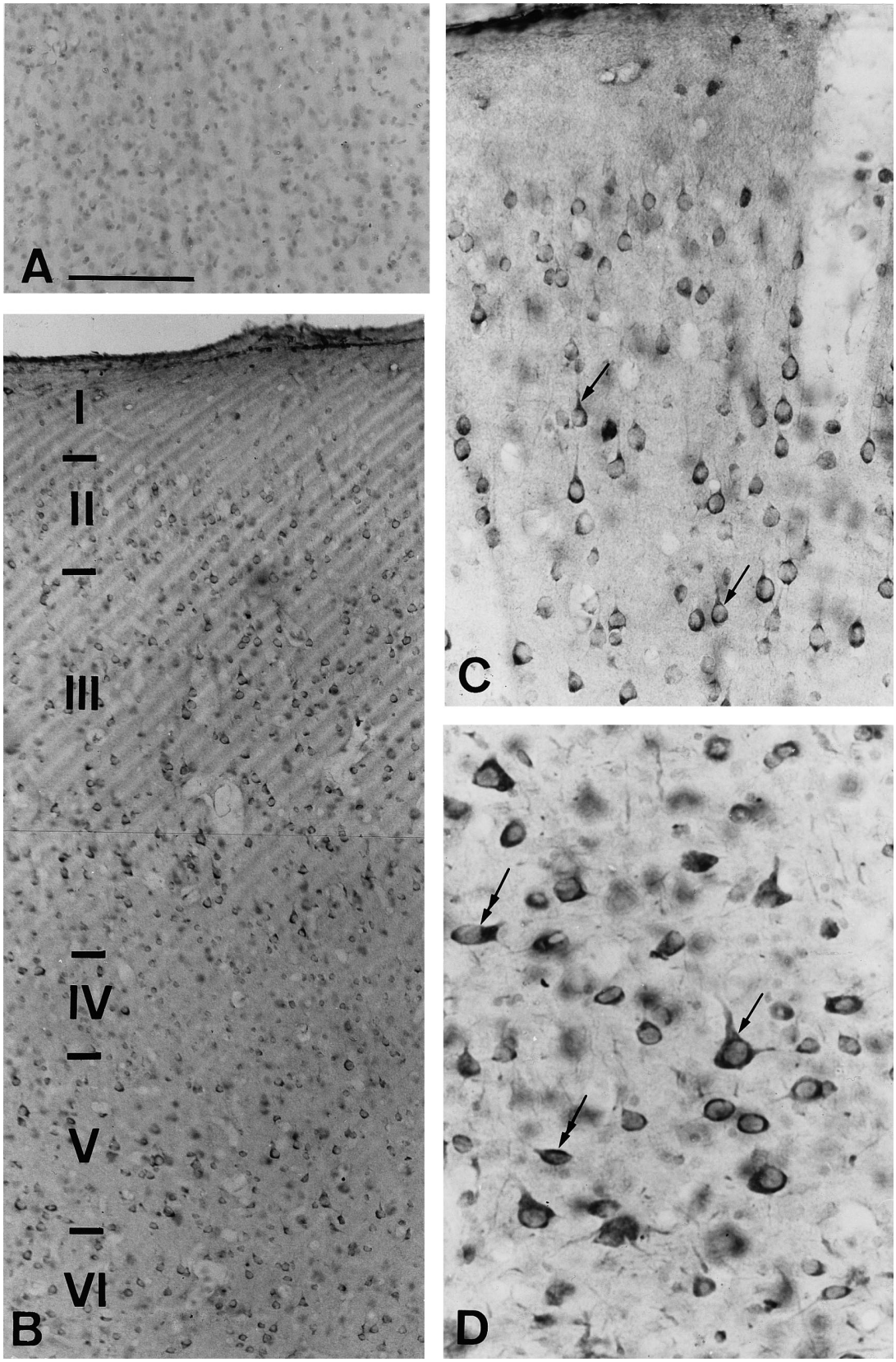

Fig. 1. Light micrographs of the cerebral cortex. (A) Layer III of the middle temporal gyrus, from the same brain as Fig. 1D, Fig. 2A–C. The section was incubated with antibody and immunizing protein (1 μg/ml), and shows no specific staining. The very lightly stained structures are nuclei counterstained with Methyl Green. (B) Montage of the frontal cortex, showing large numbers of densely labeled neurons. These were especially numerous in layers II, III, V and VI, whilst layer IV appeared as a relatively clear band. (C, D) higher magnification of layer III of the middle temporal gyrus (C), layer V of the inferior parietal lobule (D), showing labeled pyramidal neurons (arrows) and non-pyramidal neurons (double arrows). Scale bars: (A) = 160 μm; (B) = 250 μm; (C, D) = 100 μm.

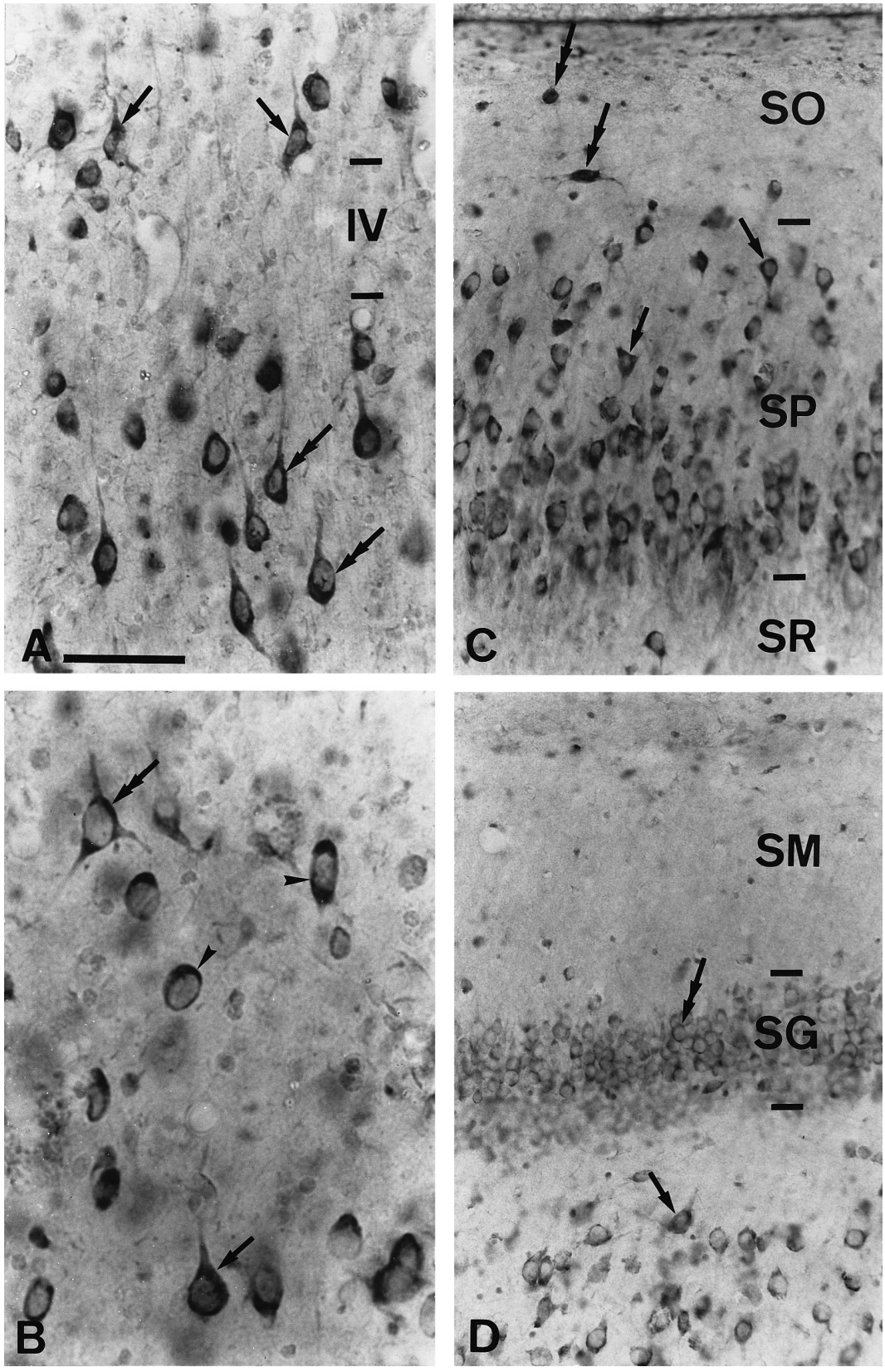

Fig. 2.

pyramidal neurons in CA1-4 (Fig. 2C), and in a small number of non-pyramidal neurons in the stratum oriens and radiatum. The neuropil was also densely labeled, and contained a dense meshwork of immunoreactive processes. Dense staining was also observed in some hilar neurons, whilst light staining was observed in granule neurons in the dentate gyrus (Fig. 2D).

Basal nuclei. The caudate nucleus, nucleus acumbens, and putamen (Fig. 3A, B) were on the whole, lightly stained for CB1. Light staining was observed in small diameter 10–15 μm neurons, although denser staining was observed in scattered, large diameter 20–25 μm neurons, in these nuclei (Fig. 3B).

The globus pallidus was even more lightly stained than the caudate nucleus and putamen, and contained only scattered large diameter (25–50 μm) neurons (Fig. 3C) in both the pars externa (external pallidal segment) and interna (internal pallidal segment). Very few immunoreactive fibers were observed in the neuropil. The substantia nigra pars reticulata, which has similar functional connections as the globus pallidus pars interna,[40] was also lightly labeled (Fig. 3D).

In contrast to the pars reticulata, many densely labeled, large (35–40 μm) neurons with fusiform outlines were observed in the substantia nigra pars compacta (Fig. 3D, E).

Thalamus and hypothalamus. The thalamus was, as a whole, lightly stained for CB1. Many densely labeled neurons were observed in the reticular nucleus, whilst the rest of the thalamus contained moderately labeled neurons (Fig. 4A, B). Many moderately densely labeled neurons were also observed in the hypothalamus (Fig. 4B).

Cerebellum. A population of Purkinje cells in the cerebellar cortex were labeled for CB1 (Fig. 4C). Staining occurred throughout the cerebellum, but not all Purkinje cells were stained. When stained, reaction product was observed in the cell bodies and stem dendrites of Purkinje neurons, and in a meshwork of immunoreactive processes in the molecular layer (Fig. 4C).

Cerebellar granule neurons and the cerebellar white matter were unlabeled, while the deep cerebellar nuclei were very lightly labeled (Fig. 4D).

Brainstem. In contrast to the densely packed, labeled neurons in the substantia nigra pars compacta, only scattered labeled neurons were observed in the rest of the brainstem. The latter included lightly labeled neurons in the red nucleus.

Electron microscopy

Cerebral neocortex. Many labeled pyramidal neurons were observed in the cerebral cortex (Fig. 5A). The cell bodies were regular in outline, and measured 15 μm in diameter. The nucleus was also regular in outline except for the occasional small indentations, and contained evenly dispersed, fine heterochromatin clumps. The cytoplasm contained large numbers of free ribosomes and mitochondria with loosely packed cristae, and a few profiles of rough endoplasmic reticulum (Fig. 5A). The Golgi apparatus was also frequently observed. CB1 immunoreactivity was associated with free ribosomes and ribosomes of the rough endoplasmic reticulum, but was absent from the nucleus, Golgi apparatus, and the interior of mitochondria. This pattern of distribution is similar to that seen for the metabotropic glutamate receptor (mGluR)1A receptor in human cortex, where prominent localization to ribosomes was observed.[24] A large apical dendrite was observed in continuity with the cell body. In addition to pyramidal neurons, labeled neurons with deep folds in the nuclear membrane and dense cytoplasm, and features of non-pyramidal neurons in the monkey cortex[4,34] were also observed (Fig. 5B).

Many labeled dendrites or dendritic spines were observed in the neuropil (Fig. 5C). These formed asymmetrical synaptic contacts with unlabeled axon terminals containing small round vesicles (Fig. 5C). Label was concentrated at the postsynaptic densities, but was also present in the cytosol of the spines. Not all spines that formed asymmetrical synapses were labeled, however, and unlabeled spines were often observed, close to CB1-positive ones.

Myelinated axons, and cells with features of astrocytes,[23] oligodendrocyte-like cells,[26] microglia,[23]

Fig. 2. Light micrographs of limbic structures in the brain, including layers III–V of the entorhinal cortex (A), the lateral nucleus of the amygdala (B), field CA1 of the hippocampus (C), and the dentate gyrus (D). (A) In entorhinal cortex, dense reaction product is visible in the cell bodies and large stem dendrites of pyramidal neurons in layers III (arrows) and V (double arrows), but is absent from neurons in layer IV. (B) In amygdala, label is observed in putative pyramidal or projection neurons with a single large diameter dendrite (arrow) or multiple stem dendrites (double arrow), and putative non-projection neurons the lacked large stem dendrites (arrowheads). (C) In field CA1 of the hippocampus, dense staining is observed in the pyramidal neurons in the stratum pyramidale (arrows) and non-pyramidal neurons in the stratum oriens (double arrows). (D) In dentate gyrus, dense staining is evident in hilar neurons (arrow), while light staining is observed in granule neurons in the stratum granulosum (double arrow). SG, stratum granulosum of the dentate gyrus; SM, stratum moleculare of the dentate gyrus; SO, stratum oriens; SP, stratum pyramidale; SR: stratum radiatum. Scale bars: (A, B) = 40 μm; (C, D) = 160 μm.

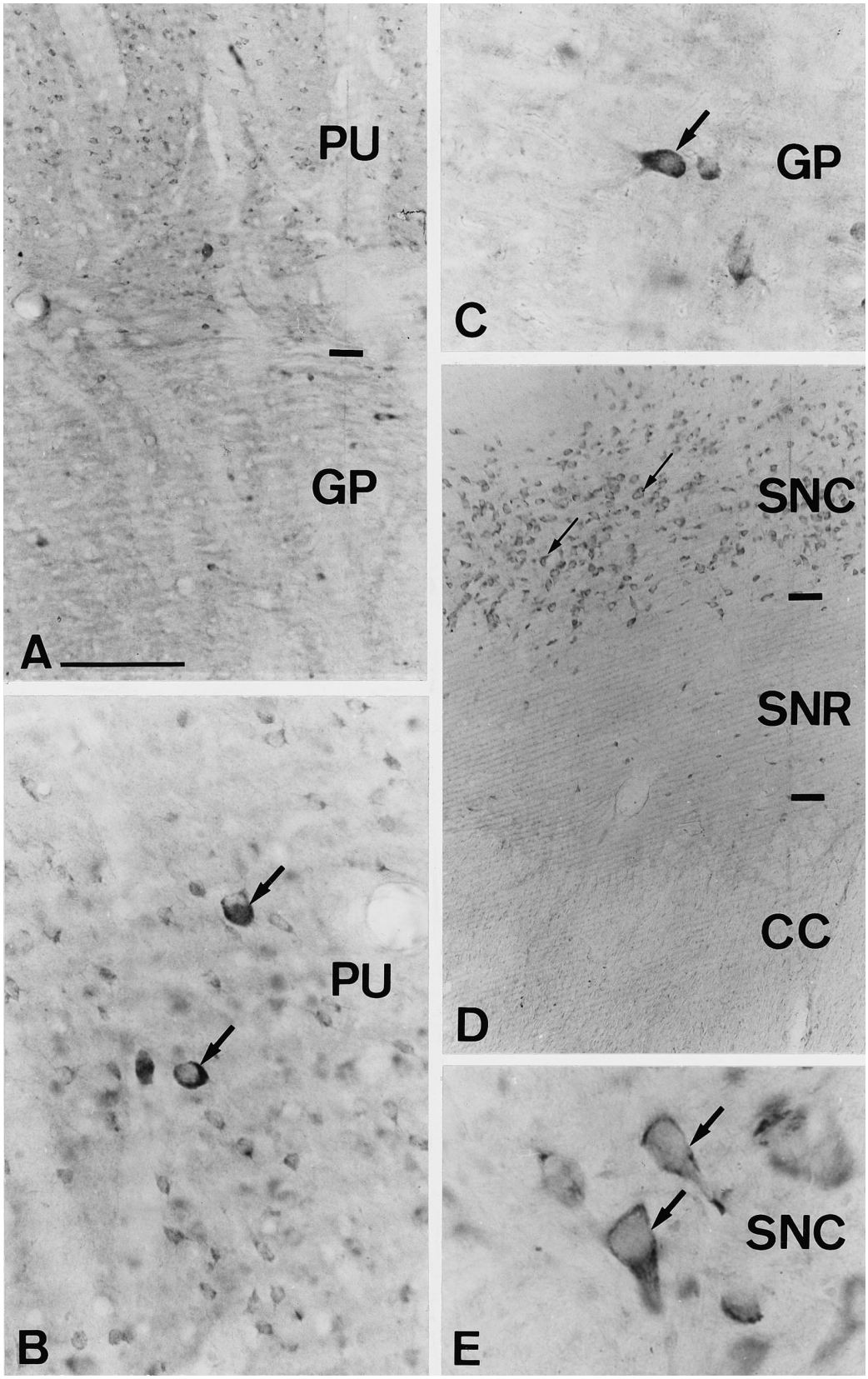

Fig. 3. Light micrographs of the basal nuclei, including the putamen (PU in A, B), globus pallidus (GP in A, C), and the substantia nigra pars compacta (SNC in D, E) and substantia nigra pars reticulata (SNR in D). (A) Low magnification micrograph showing overall light staining in the putamen and globus pallidus. (B) Dense staining is observed in large diameter neurons, whilst light staining is observed in small neurons in the putamen. (C) The globus pallidus is almost unstained, except for occasional, densely labeled neurons (arrow). (D, E) Many densely labeled neurons are observed in the substantia nigra pars compacta (arrows in D, E), while the pars reticulata and the cerebral peduncles (CC) are unstained. Scale bars: (A) = 250 μm; (B, C) = 100 μm; (D) = 630 μm; (E) = 160 μm.

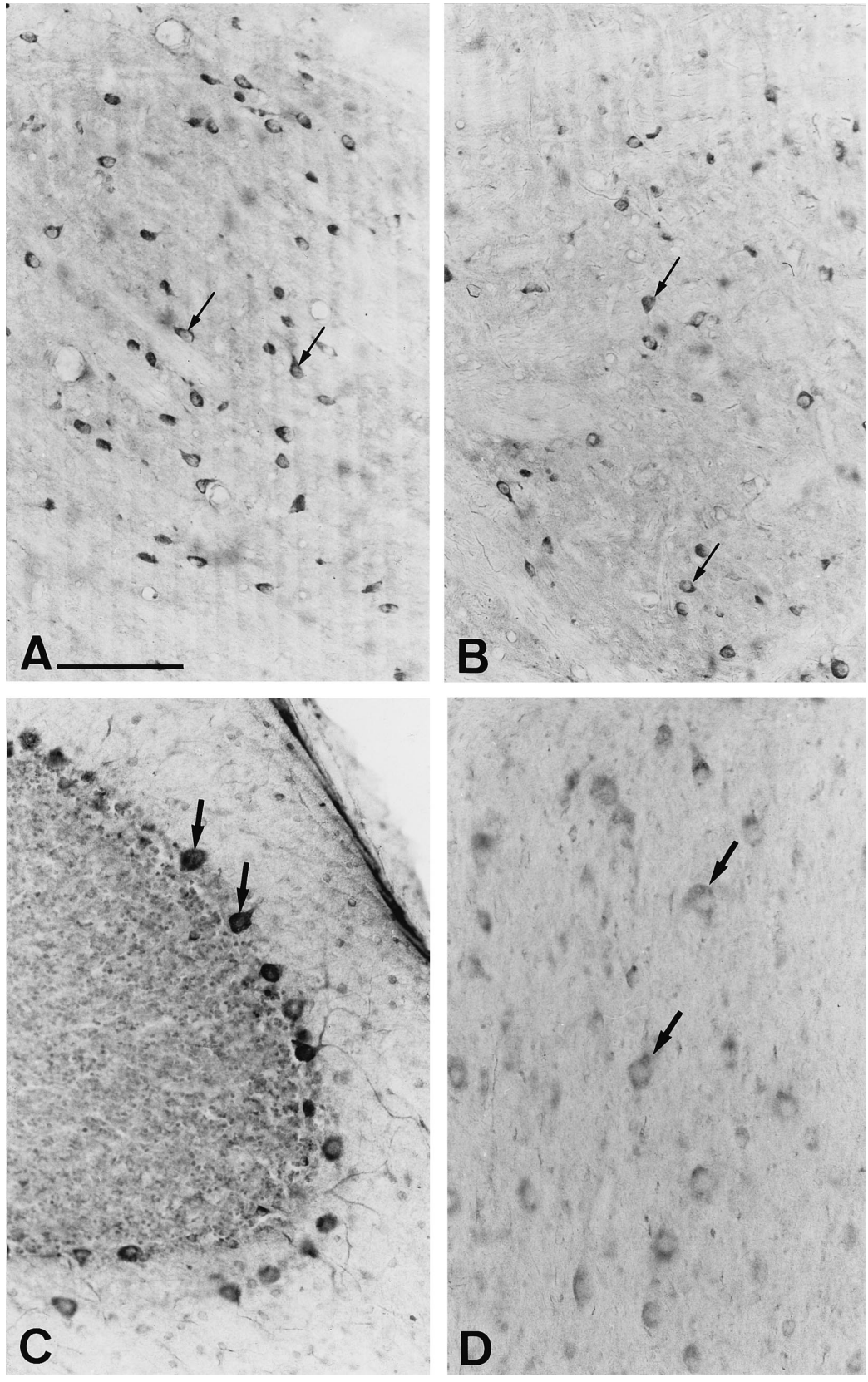

Fig. 4. Light micrographs of the thalamus, cerebellum, and dentate nucleus. (A, B) Lateral (A) and medial (B) groups of nuclei of the thalamus, showing scattered labeled cells (arrows). (C) Cerebellar cortex, showing densely labeled Purkinje (arrows). (D) Dentate nucleus, showing very lightly labeled neurons (arrows). Scale bars = 160 μm.

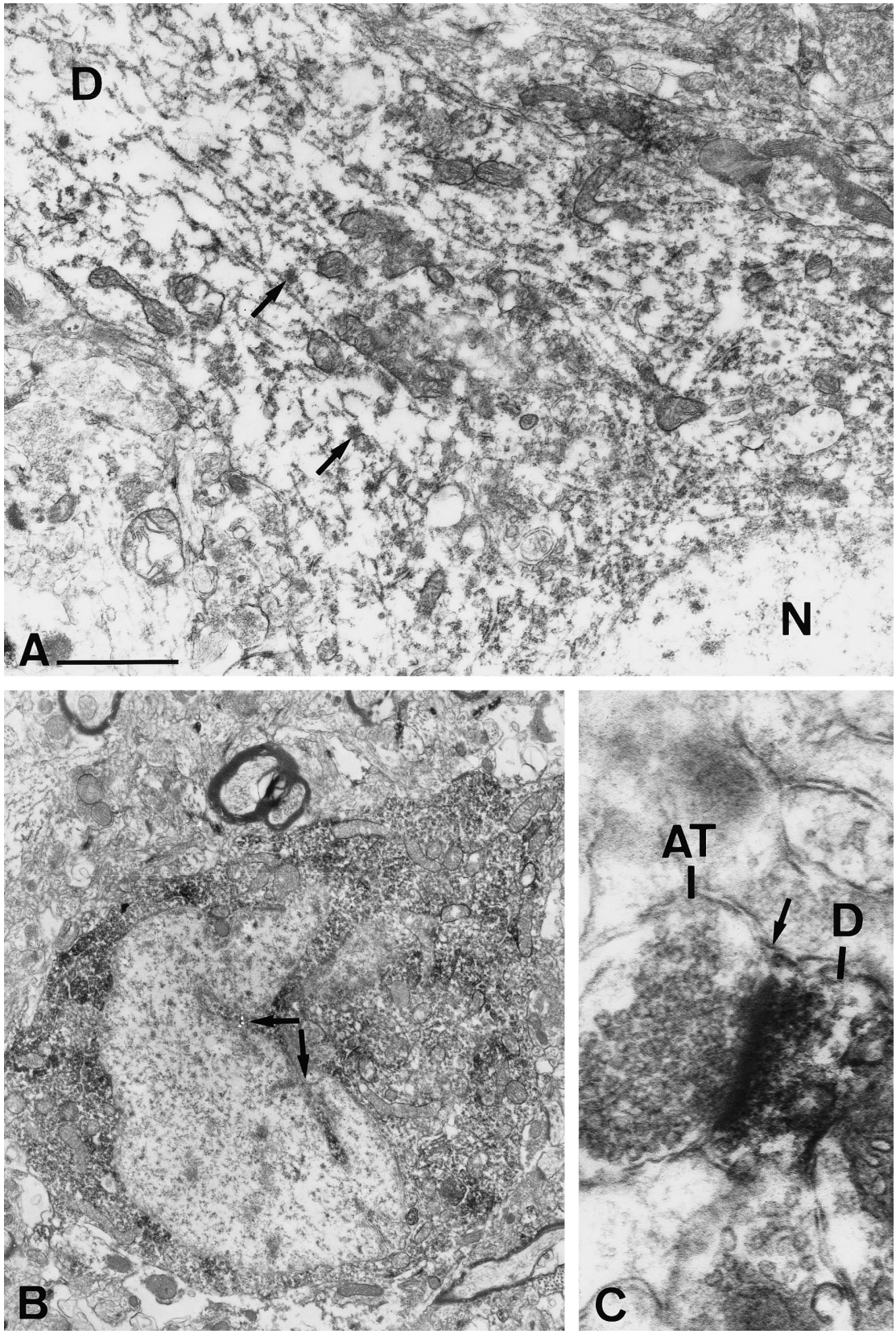

Fig. 5. Electron micrographs of the cerebral cortex. (A) Portion of the nucleus (N) and somatic cytoplasm, and apical dendrite (D) of an immunolabeled pyramidal neuron. Arrows indicate floccules of reaction product in the cytoplasm. (B) Labeled non-pyramidal cell with deep folds (arrows) in the nuclear envelope. (C) Asymmetrical synapse (arrow) between an axon terminal containing small, round vesicles (AT) and a labeled small diameter dendrite/dendritic spine. Scale bars: (A) = 1.2 μm; (B) = 2.3 μm, (C) = 0.3 μm.

and mural cells in the walls of blood vessels[41] of the human cortex were unlabeled.

Amygdala. Labeled large (16–22 μm) cell bodies with features of pyramidal or projection neurons in the amygdala[20] were commonly observed (Fig. 6A). The nucleus was regular in outline except for occasional small indentations, and contained evenly dispersed, fine heterochromatin clumps. The cytoplasm contained large numbers of free ribosomes, mitochondria with loosely packed cristae, and profiles of rough endoplasmic reticulum (Fig. 6A). Large stem dendrites were often observed, in continuity with the cell bodies. In addition to the large pyramidal or projection neurons, labeled smaller (12–14 μm) neurons with deep folds in the nuclear membrane and dense cytoplasm, features of GABAergic neurons in the monkey amygdala[21,30] were also observed.

Many densely labeled dendrites were observed in the neuropil. These formed asymmetrical synaptic contacts with unlabeled axon terminals (Fig. 6B). In addition to dendrites, occasional labeled axon terminals were also observed. These formed asymmetrical synaptic contacts with unlabeled dendrites (Fig. 6C).

Hippocampal formation. CA fields Labeled pyramidal neurons were easily identified in the CA fields, due to the presence of a prominent apical dendrite. These had similar ultrastructural features to pyramidal neurons in the cerebral neocortex.

Many lightly labeled large diameter dendrites or dendritic shafts, but densely labeled dendrites or dendritic spines were observed in the neuropil (Fig. 7A). The dendritic spines often formed asymmetrical synaptic contacts with unlabeled axon terminals containing small round vesicles (Fig. 7A).

Molecular layer of the dentate gyrus Occasional labeled dendrites and axon terminals were observed in the molecular layer of the dentate gyrus. The immunolabeled dendrites formed asymmetrical synapses with unlabeled axon terminals, while the labeled axon terminals formed asymmetrical synapses with unlabeled dendrites (Fig. 7C).

Substantia nigra pars compacta. The substantia nigra pars compacta contained many densely labeled neurons with large numbers of cytoplasmic organelles (Fig. 8A), including granules with the classical triphasic substructure of neuromelanin.[22] These granules were surrounded by an outer limiting membrane, and contained fine granular material with indistinct linear arrays, lipid-like globules, and very dense, coarsely granular material (Fig. 8B). Neurons in the pars compacta that contained neuromelanin granules were identified as dopaminergic neurons. Immunolabeled dendrites were observed to form asymmetrical synapses with unlabeled axon terminals, whilst labeled axon terminals were occasionally observed to form asymmetrical synapses with unlabeled dendrites.

Molecular layer of the cerebellar cortex. Many small diameter dendrites or dendritic spines were labeled in the molecular layer of the cerebellar cortex. These formed asymmetrical synaptic contacts with unlabeled axon terminals containing small round vesicles (Fig. 8C).

DISCUSSION

The present study aimed to elucidate the distribution of cannabinoid receptors in the primate brain and their ultrastructural localization. Dense staining was observed in putatively glutamatergic pyramidal or projection neurons of the cerebral neocortex, entorhinal cortex, hippocampus, and amygdala, but was also observed in non-pyramidal, putatively GABAergic neurons of the amygdala, GABAergic Purkinje neurons in the cerebellum, and dopaminergic neurons of the substantia nigra pars compacta. This pattern of staining is in general agreement with results of CB1 localization in rat brain, where high levels of CB1 mRNA and densely labeled cell bodies or processes were also observed in the rat cerebral cortex, amygdala, hippocampus, and cerebellar cortex, whilst only relatively low levels of CB1 mRNA and protein were observed in the thalamus and brainstem.

Despite these similarities, obvious differences exist, in the density of staining of various populations of neurons. Dense staining, for instance, was observed in pyramidal neuronal cell bodies in the monkey cerebral cortex and hippocampus, whereas in the rat, these cell bodies were moderately densely labeled in the cortex, or unlabeled in the hippocampus.[36] Staining was also observed in Purkinje neurons of the cerebellar cortex in the monkey, where it was absent in the rat.[36] It is possible that these differences are due to higher levels of expression of CB1 in monkey pyramidal and Purkinje neurons, by expression of splice variants in the two species, or methodological differences between the two studies. Examples of potentially important methodological differences include the different fixation protocols and antibody staining conditions used in the two studies.

The most obvious difference between primate and rat CB1 was in the staining of the globus pallidus. The primate globus pallidus contained very few immunoreactive neuronal cell bodies and processes except for some scattered, large diameter 25–50 μm neurons. Very few immunoreactive fibers were observed in the monkey substantia nigra pars reticulata, which has similar functional connections as the globus pallidus pars interna.[40] In contrast, the rat globus pallidus and substantia nigra pars reticulata contained a dense plexus of immunoreactive

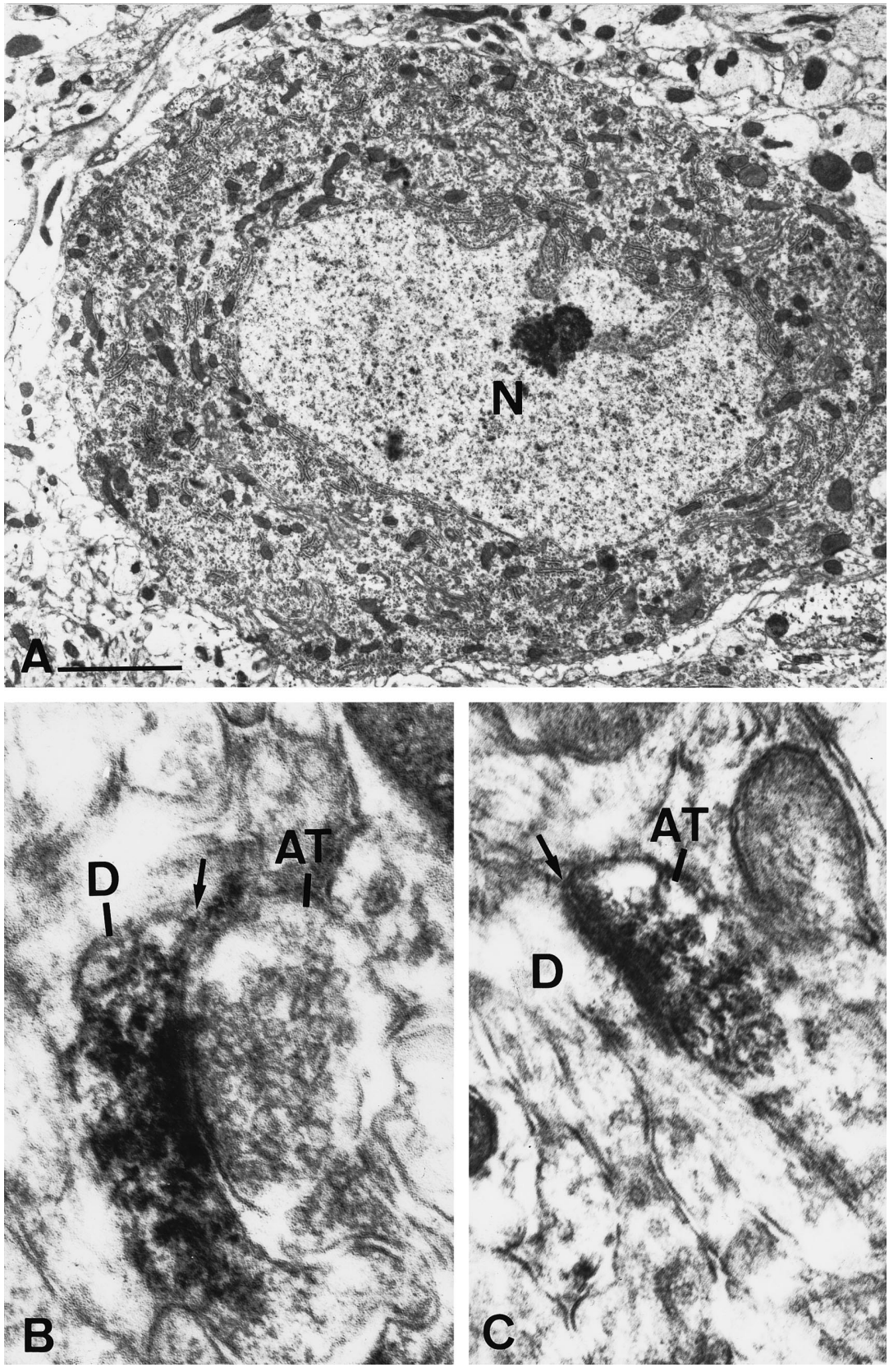

Fig. 6. Electron micrographs of the amygdala. (A) Portion of the nucleus (N) and cytoplasm of a large diameter neuron in the corticomedial nucleus of the amygdala. This neuron has features of pyramidal or projection neurons in the amygdala,[20] including a nucleus with fairly dispersed heterochromatin clumps and large numbers of organelles in the cytoplasm. (B) Asymmetrical synapse (arrow) between an unlabeled axon terminal containing small vesicles (AT) and a labeled dendrite/dendritic spine (D) in the lateral nucleus. (C) Asymmetrical synapse (arrow) between a labeled axon terminal (AT) and an unlabeled dendrite (D) in the lateral nucleus. Scale bars: (A) = 3 μm; (B) = 0.2 μm; (C) = 0.25 μm.

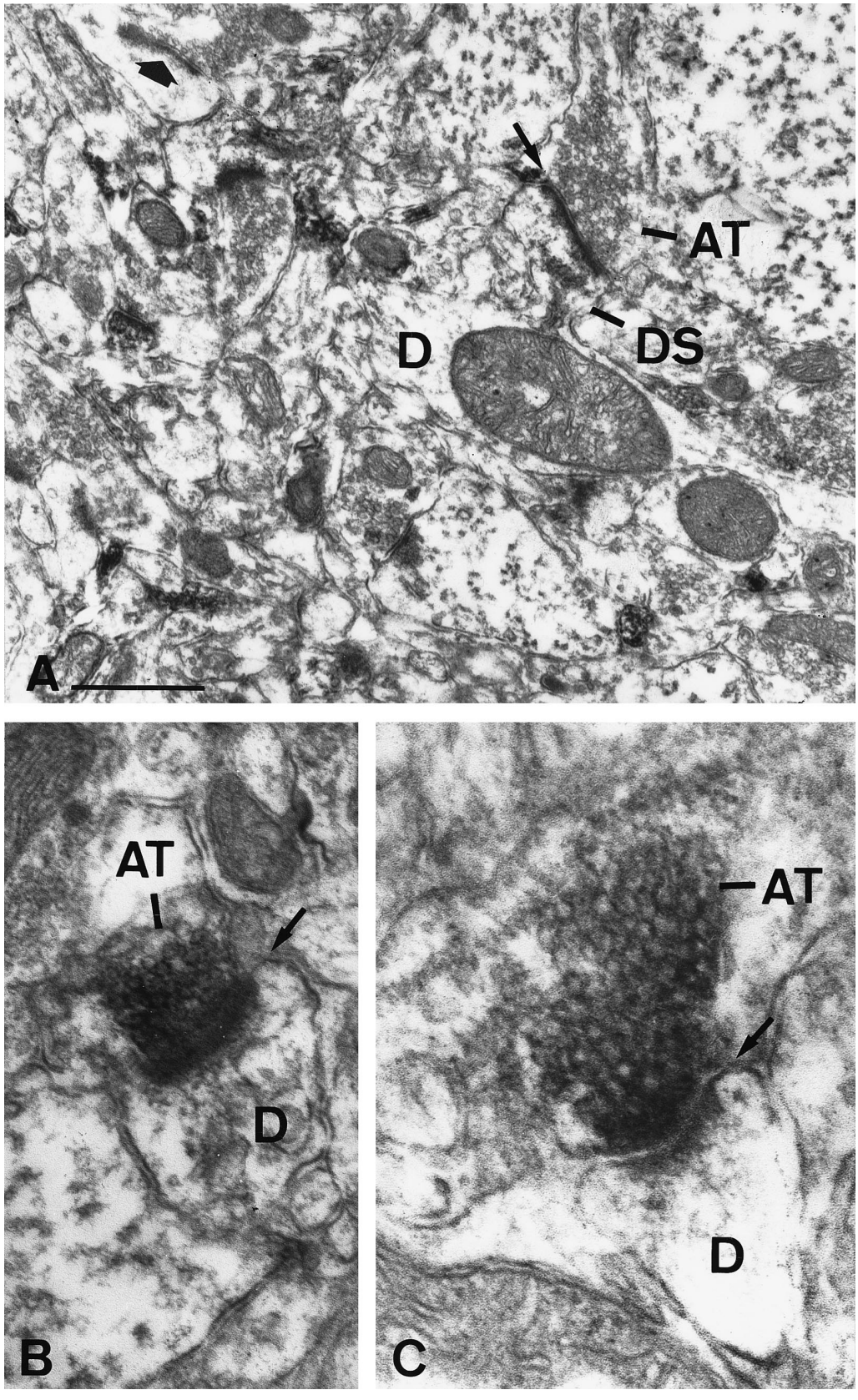

Fig. 7. Electron micrographs of the neuropil of field CA1 (A, B) and the stratum moleculare granulosum (C) of the hippocampal formation. (A) Synapse (arrow) between an unlabeled axon terminal containing round vesicles (AT) and a labeled dendritic spine (DS). There is accumulation of reaction product on the postsynaptic density, and the dendritic spine is more densely labeled than the parent dendrite (D) Unlabeled synapses (arrowhead) were also evident. (B, C) Asymmetrical synapses (arrows) between labeled axon terminals (AT) and unlabeled dendrites (D). Scale bars: (A, C) = 0.2 μm; (B) = 0.3 μm.

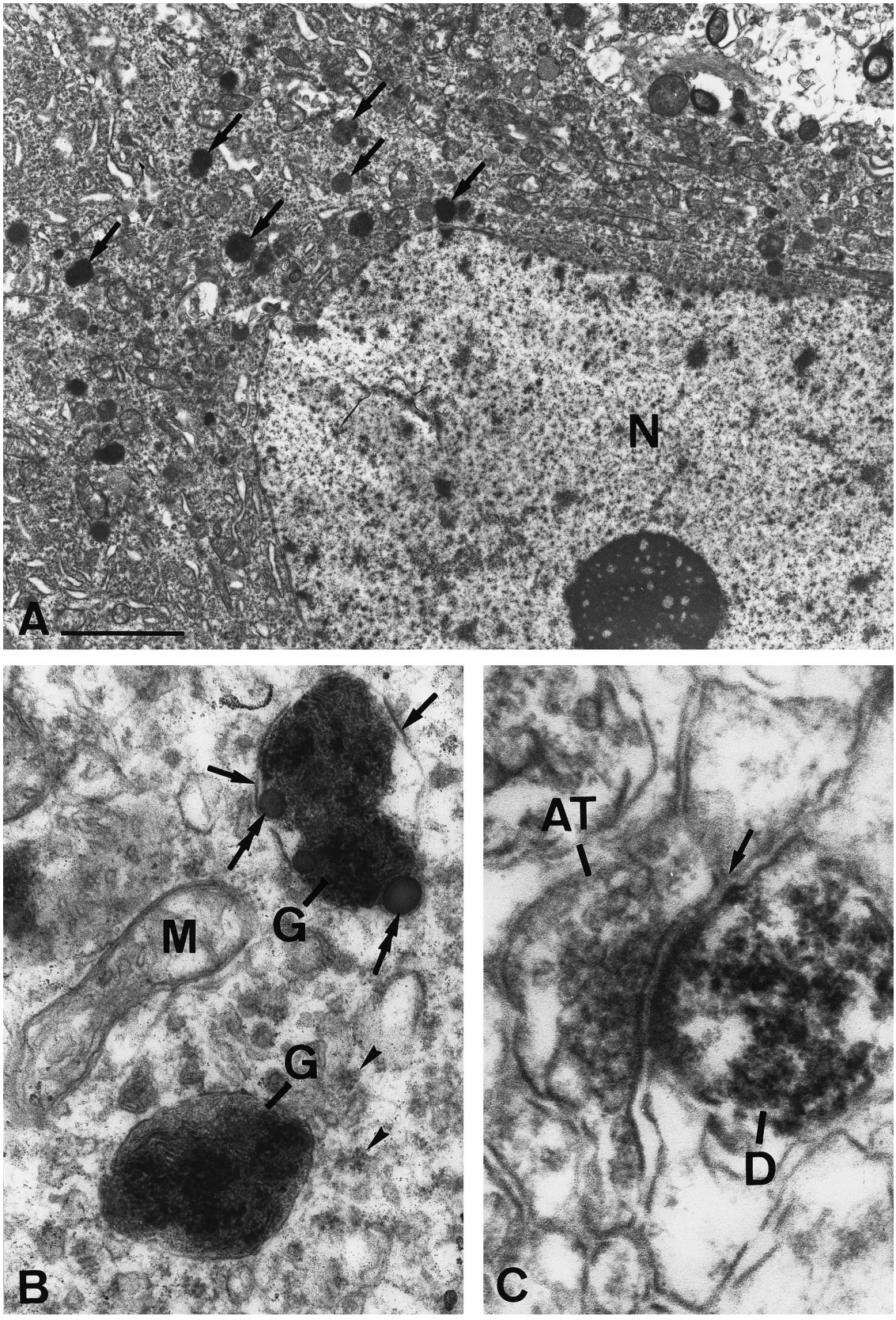

Fig. 8.

fibers.[36] Non-specific staining by our antibody is unlikely, since control sections showed an absence of staining and staining was abolished by preabsorption of the antiserum by the immunizing protein (Fig. 1A). A possible explanation for some of these differences is that a splice variant of CB1 (for example, CB1A[33]) may be expressed in these regions. This hypothesis can be tested once the appropriate antibodies are raised. Another possibility is that the antibody used in this study does not penetrate as well into the white matter tracts as does the small lipophilic CP55,940. A third, less likely possibility is that CP55,940 binds to a receptor in addition to the CB1 receptor.

Physiological processes in which cannabis, and endogenous cannabinoid receptor agonists including anandamide may serve as chemical mediators include cognition, memory, mood, perception, movement co-ordination, sleep, thermoregulation, appetite and immune response (for review, see Refs 28 and 29). Our observation of densely labeled pyramidal or projection neurons CB1 in the hippocampus, amygdala and cerebral cortex suggest that localization of the receptor to these neurons could be the basis for the effects of cannabinoids on cognition, memory, mood and perception. In the cerebral cortex, the CB1-containing spines are very likely to be those of pyramidal neurons, since very little staining was observed in layer IV where most of the other densely spiny cell type in the cortex, the spiny stellate neurons are located.[13] Similarly, densely stained spiny dendrites in CA1 are almost certainly those of pyramidal neurons, since the other type of densely spiny neurons in the hippocampus, the calretinin-positive spiny non-pyramidal neurons, have been observed in CA3 but not CA1 in the rat hippocampus,[6] but are apparently absent from the monkey hippocampus.[31]

The densely labeled neurons in the substantia nigra pars compacta, and Purkinje cells of the cerebellum suggest that these may be the substrates for the effects of cannabinoids on movement co-ordination. Lightly labeled neurons in various portions of the hypothalamus may be the basis for the effects of cannabinoids on sleep, thermoregulation and appetite.

In addition to a postsynaptic location, CB1 immunoreactivity was present in some axon terminals, especially in cortex and CA1 (Fig. 5C; Fig. 7C, D). This suggests cannabinoid receptors may have a major role in inhibiting presynaptic calcium channels, reducing release of glutamate from axon terminals in these regions.[32] However, the dendritic localization of the CB1 receptor in these and other regions, suggests its role is broader than merely mediating presynaptic inhibition.

CONCLUSION

Cannabinoids have been postulated to play a role as endogenous neuroprotective agents (for review, see Ref. 28). Thus the high levels of cannabinoid receptors in the various components of the limbic system, including the entorhinal cortex, hippocampus and amygdala, as well as the substantia nigra pars is of particular interest. Most of the CB1 immunoreactivity found in these regions is on postsynaptic structures, including small diameter dendrites or dendritic spines. This places the receptor in a position to interact with multiple messenger systems to decrease excitability and calcium influx. Examples of systems potentially activated by CB1 receptors include inwardly rectifying potassium channels[15] and N- and P/Q-type voltage-dependent calcium channels,[37] which are present in distal dendrites of neurons from field CA3 of the hippocampus.[38,39] Thus during periods of ischemia or hypoxia, cannabinoid receptor activation could serve to limit the extent of depolarization and calcium influx, decreasing excitotoxic injury. Thus CB1 receptor activation by lipid mediators produced during periods of ischemia,[7] may serve a neuroprotective role in selected brain regions.

Acknowledgements—This work was supported by a grant from the National University of Singapore (RP950365), grants from the NIH (DA00286, DA08534, DA11322). We thank Ms Leong Lo Ngah, Ms Ng Leh See, and Mr Sean Brown for excellent technical assistance.

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Fig. 8. Electron micrographs of the substantia nigra pars compacta (A, B) and the cerebellar cortex (C). (A) Portion of the nucleus (N) and cytoplasm of a large diameter neuron. This has features of dopaminergic neurons in the substantia nigra pars compacta, including a nucleus with fairly evenly dispersed heterochromatin, and cytoplasm that contains large numbers of organelles, including neuromelanin granules (arrows). (B) Higher magnification of a labeled neuron, showing neuromelanin granules (G). These are surrounded by an outer limiting membrane (arrows) and contained fine granular material with indistinct linear arrays, and lipid-like globules (double arrows). Arrowheads indicate floccules of reaction product in the cytoplasm. M, unlabeled mitochondrion. (C) Asymmetrical synapse (arrow) between an unlabeled axon terminal (AT) containing small round vesicles and a labeled small diameter dendrite or dendritic spine (D). Scale bars: (A) = 2.6 μm; (B) = 0.4 μm; (C) = 0.17 μm.

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(*Accepted* 11 *January* 1999)