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The spatial and temporal pattern of fatty acid amide hydrolase expression in rat hippocampus during postnatal development

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Abstract

GABAergic synaptic transmission is efficiently controlled by endogenous cannabinoids in cortical structures. Fatty acid amide hydrolase (FAAH) is one of the metabolizing enzymes of endocannabinoids in the brain. In this study we investigated the cellular and subcellular distribution of FAAH at various timepoints during the first postnatal weeks, when GABA is still depolarizing, and plays a crucial role in network events. FAAH immunoreactivity is strong in the CA3 region already at postnatal day 0 (P0), but in CA1 only after P8. During this period, FAAH levels in hilar mossy cells decrease and in granule cells slowly increase. Pyramidal cells express FAAH first in the soma and proximal dendrites, and gradually in more distal segments, reaching adult levels in the most distal dendrites only at P22. Transient expression of FAAH was found in a small number of stratum radiatum cells that may be interneurons and in ependymal cells at the border of the alveus and corpus callosum between P2 and P8. At the ultrastructural level, FAAH distribution at all ages was very similar to the adult pattern, i.e. it was largely associated with the membrane of cytoplasmic vesicles, mitochondria and endoplasmic reticulum. During postnatal development of the hippocampus, the spatio-temporal expression of FAAH correlates well with the general pattern of neuronal maturation, but not with the arrival of afferent pathways, which suggests that FAAH – and its major endocannabinoid substrate, anandamide – is unlikely to be involved in the presynaptic control of neurotransmission. Instead, FAAH may subserve general roles as the inactivating enzyme for many fatty acid amides, in addition to anandamide.

Introduction

Both exogenous and endogenous cannabinoids are known to reduce GABA release via cannabinoid type 1 receptors (CB_1), and high concentrations of cannabinoid agonists reduce glutamate release as well via an as yet unidentified cannabinoid-sensitive receptor (for review see Alger, 2002; Wilson & Nicoll, 2002; Freund *et al*., 2003). Strong depolarization, a transient elevation of calcium concentration or stimulation of phospholipase C-coupled metabotropic receptors in the postsynaptic neuron was shown to induce the down-regulation of afferent GABAergic synaptic currents for over 10 s. This phenomenon, termed depolarization-induced suppression of inhibition (Llano *et al*., 1991; Pitler & Alger, 1992), was shown to be mediated by endocannabinoids (Kreitzer & Regehr, 2001; Ohno-Shosaku *et al*., 2001; Wilson & Nicoll, 2001; Wilson *et al*., 2001), which can be synthesized and released by the postsynaptic cell, and act on CB_1 receptors selectively located on axons of the cholecystokinin (CCK)-containing subset of GABAergic interneurons (Katona *et al*., 1999). The question arises as to whether endocannabinoid signalling operates in the same way during the first 1–2 postnatal weeks, when GABA is still depolarizing, and plays a major role in the generation of synchronous network events called giant depolarizing potentials that are required for the maturation of hippocampal networks (Ben-Ari *et al*., 1989, 1997; Cherubini *et al*., 1991; Ben-Ari, 2002). EEG sharp waves in the adult hippocampus are known to be terminated by a synchrony-dependent recruitment of GABAergic inhibition (Buzsaki, 1986). However, how can giant depolarizing potentials be terminated, and epileptic activity prevented, in the immature hippocampus, if recruitment of GABAergic interneurons enhance rather than inhibit neuronal activity? Retrograde signalling via endocannabinoids appears to be an ideal solution, because they are synthesized and released in an activity-dependent manner, and can reduce both GABAergic and glutamatergic currents in adult as well as in immature hippocampus (Hájos *et al*., 2000, 2001; Gozlan *et al*., 2003). Thus, studying the development of crucial components of the endocannabinoid signalling system may provide an answer to this question. We have demonstrated previously that CB_1 receptors are present in the membrane of axon terminals of CCK-containing interneurons already in newborn rats (Morozov & Freund, 2003a). Furthermore, other components of the G-protein-coupled signalling system are functional at this time point, as was shown for GABA-B receptors (McLean *et al*., 1996). Here we studied the distribution of one of the enzymes that are able to degrade endocannabinoids, fatty acid amide hydrolase (FAAH; Schmid *et al*., 1985; Deutsch & Chin, 1993; Cravatt *et al*., 1996), to elucidate further the sites of potential involvement of endocannabinoid signalling in the development of hippocampal networks.

FAAH, an enzyme that inactivates endocannabinoids and other fatty acid amides (Natarajan *et al*., 1984; Schmid *et al*., 1985; Deutsch & Chin, 1993; Cravatt *et al*., 1996, 2001), is expressed by hippocampal

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principal neurons and is located exclusively in their soma-dendritic compartment in adult rats (Egertova *et al*., 1998; Tsou *et al*., 1998; Gulyás *et al*., 2004). FAAH and CB_1 receptors appear to be co-distributed in several areas of the rodent brain, and are located in complementary cell compartments, i.e. FAAH-immunoreactive dendrites and cell bodies are innervated by CB_1-containing axons (Egertova *et al*., 1998, 2003). The apparent postsynaptic location of FAAH was claimed to indicate that the enzyme might be involved in the regulation of the concentration or the releasable pool of endocannabinoids in postsynaptic neurons, which are the sources of the retrograde signal molecules, and could thereby influence the magnitude of depolarization-induced suppression of inhibition. FAAH mRNA is detectable in the rat brain on the 14th embryonic day, and is increased gradually until the 10th postnatal day (P10; Thomas *et al*., 1997). The cellular and subcellular distribution of FAAH protein in the developing postnatal rat hippocampus are still unknown.

The aim of the present study was to investigate the spatial and temporal pattern of FAAH expression in the hippocampus at several postnatal ages from birth until P22 when adult-like levels are reached. This is compared with the regional/laminar distribution and development of CB_1 receptor-containing afferent inputs.

Materials and methods

Newborn (P0, $n = 2$), 2- (P2, $n = 2$), 4- (P4, $n = 3$), 8- (P8, $n = 3$), 12- (P12, $n = 2$) and 22- (P22, $n = 2$) day-old male Wistar rats were used for the present study. All experimental procedures were performed according to the Institute of Experimental Medicine Ethical Codex and the Hungarian Act of Animal Care and Experimentation (1998, XXVIII, Section 243/1998), which is in accordance with the NIH Guide to the Care and Use of Laboratory Animals. Every effort was made to minimize the number of animals used. Transcardial perfusion was performed under deep anaesthesia with chlornembutal (0.03 mL/10 g). Two different fixatives were used to optimize conditions for immunostaining as well as for ultrastructural preservation. One each of 4-, 8-, 12- and 22-day-old animals were perfused with a mild fixative allowing deep penetration of antibodies and intensive immunostaining. This fixative contained 2% paraformaldehyde and 0.1% glutaraldehyde and was used at a graded pH (from 6.5 to 8.5) according to the procedure described in detail previously (Sloviter *et al*., 2001). Other animals were perfused with a fixative containing 4% paraformaldehyde, 0.2% picric acid and 0.1% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4.

After fixation, the brain was removed from the skull, and coronal 60-μm-thick sections were cut from the block containing the dorsal hippocampus, using a vibratome. After extensive washes with phosphate buffer, the sections were freeze-thawed over liquid nitrogen, blocked in 5% bovine serum albumin and incubated in the solution of primary antibodies (rabbit anti-FAAH) for 48 h at 4 °C. Two different anti-FAAH antisera were used: one was raised against FAAH–GST fusion protein (dilution 1 : 5000 or 1 : 3000), and another against FAAH lacking its transmembrane domain (truncated and his-tagged recombinant; ΔTM–FAAH; dilution 1 : 10 000 or 1 : 5000; Egertova *et al*., 1998; Patricelli *et al*., 1998; Cravatt *et al*., 2001; Gulyás *et al*., 2004).

The sections were then processed further either for immunoperoxidase reaction or for the immunogold–silver procedure. For immunoperoxidase visualization, the sections were immersed in a solution of biotinylated antirabbit IgG made in goat (1 : 300) followed by avidin-biotinylated-horseradish peroxidase complex (Elite ABC, both from Vector Laboratories, Burlingame, CA, USA). 3,3′-Diaminobenzidine-4HCl was used as a chromogen. For the immunogold–silver procedure, goat antirabbit IgG conjugated with 1-nm gold particles (Aurion, Wageningen, The Netherlands) was used as a secondary antiserum. Silver intensification of gold was performed with Aurion R-Gent SE-LM according to the manufacturer's instructions. Thereafter, all the sections were postfixed with OsO_4, dehydrated and embedded in Durcupan (ACM; Fluka, Buchs, Switzerland) on microscope slides and coverslipped. Selected sections were photographed with an Axioplan 2 microscope (Karl Zeiss, Germany). For electron microscopic investigations, selected areas were re-embedded into Durcupan blocks and cut using a Reichert ultramicrotome into 60-nm-thick sections. These sections were then stained with lead citrate and evaluated in a Hitachi 7100 electron microscope.

The possible change of FAAH distribution between different intracellular compartments in the hippocampal neurons was investigated in two animals each at P0, P4, P8 and P12. The number of immunogold/silver particles was counted using low-power (6000×) electron micrographs taken from the CA1 and CA3 stratum radiatum and the dentate hilus. The percentage of particles attached to the outer surface of mitochondria, or to the inner surface of cell membrane, as well as those located in the cytoplasm were counted and calculated for each subfield of each animal. The data were expressed as means ± standard deviation.

The specificity of each FAAH antiserum was confirmed by the lack of immunostaining in FAAH-knockout mice, as shown in a parallel study (Gulyás *et al*., 2004). Immunolabelling of FAAH with two different antisera used in the present study revealed identical patterns, which provides additional evidence for their specificity. Specificity of the method was tested by replacing the primary antibody with normal rabbit serum (1 : 200). No specific staining was observed in these sections.

Results

At all ages studied, the most intensive FAAH immunolabelling was demonstrated in principal neurons, i.e. in pyramidal, granule or mossy cells. Interneurons could be identified as FAAH-negative elements embedded in the strongly stained neuropil. This is in line with previous publications demonstrating the absence of FAAH from hippocampal interneurons in adult rodents (Egertova *et al*., 1998, 2003; Tsou *et al*., 1998; Gulyás *et al*., 2004). Thus, the sections below only deal with the pattern and time course of development of FAAH expression in principal neurons of the hippocampus, with the exception of sparse transient expression in ependymal cells and unidentified neurons in stratum radiatum and alveus.

Time course of FAAH development

At the first postnatal days (P0–P2), FAAH immunolabelling was robust in somata and proximal dendrites of CA3 pyramidal cells as well as hilar mossy cells. In the CA1 and subicular pyramidal cells, FAAH immunoreactivity was very low during this period (Figs 1A, and 2, A_1 and B_1). There is a pronounced difference in FAAH content between the CA1 and CA3 regions until P4. Between the P4 and the P8, a gradual increase in FAAH immunoreactivity takes place in the subiculum and CA1 strata pyramidale and radiatum, whereas stratum lacunosum-moleculare remains negative. CA3 stratum lacunosum-moleculare and dentate granule cell bodies (beginning with their outer rows towards the hilar border) also show an increase in immunoreactivity. From the P8 onwards, the neuropil in the stratum lucidum shows a faint immunoreactivity for FAAH due to the appearance of numerous mossy fibres, all of which appear to lack FAAH (Figs 1B and C, and 2, $A_{2,3}$ and B_2).

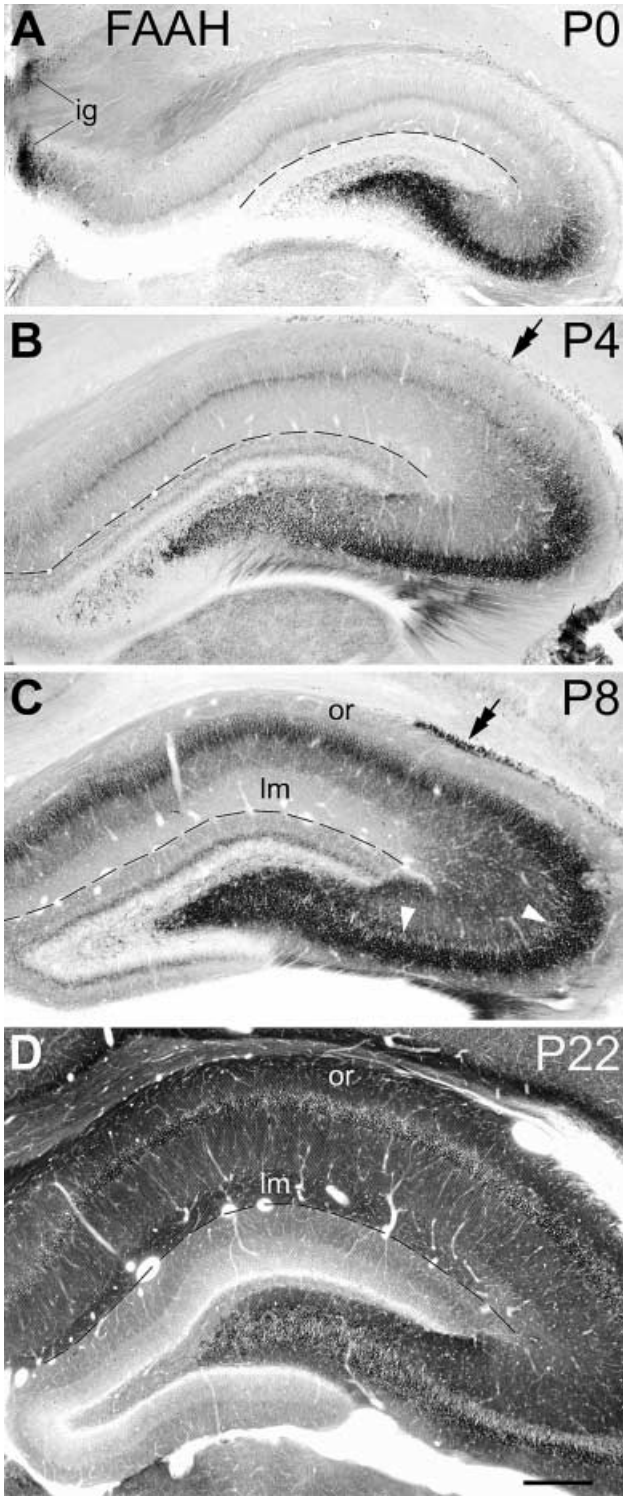

FIG. 1. Time course of development of FAAH immunoreactivity in postnatal rat hippocampus. (A and B) On the day of birth until the 4th postnatal day, FAAH is strongly expressed in the hilar mossy and CA3 pyramidal cells. CA1 shows low FAAH content (see also Fig. 2, $A_{1,2}$). (C) At P8, all the subfields of the hippocampus proper became immunolabelled. A low density remains in the strata oriens (or) and lacunosum-moleculare (lm), which nevertheless become very heavy labelled zones at P22 (see D). The hippocampal fissure is indicated with dashed lines. FAAH-containing ependymal cells that line the lateral ventricle from the surface of hippocampus are indicated with a double arrow (see also Fig. 3, C_{1-3}). The stratum lucidum is indicated with white arrowheads. Abbreviation: ig, indusium griseum. Scale bar on D (valid for all), 250 μm.

At P12–P22, immunoreactivity for FAAH gradually becomes stronger throughout all hippocampal subfields and layers. CA1 stratum lacunosum-moleculare becomes immunoreactive later than the adjacent strata radiatum and moleculare but, at P22, it appears at least as darkly stained as the bordering stratum radiatum. In the dentate gyrus, all of the granule cells are FAAH-immunoreactive by the 22nd day, although their labelling intensity always remains below the level seen in pyramidal cells. The cells that remain immunonegative during the entire postnatal period are found in the inner row of the cells in the stratum granulosum, which mostly correspond to interneurons (Figs 1D, and 2, A_4 and $2B_{3,4}$; see Freund & Buzsaki, 1996). The adult-like pattern of FAAH expression was reached generally by P12, when robust immunolabelling appears in the cells scattered in the alveus (see below). This cell population cannot be found before P8, but they are present in the adult rat hippocampus.

Somata and dendrites of hilar mossy cells are strongly FAAH-immunoreactive from the day of birth until P8. During this period, they are stained as intensely as adjacent pyramidal cells, and sharply contrast with the immunonegative neuropil of the hilus. On the 12th postnatal day, staining of mossy cells becomes noticeably weaker than that of nearby pyramidal neurons in CA3c, and at P22 only weakly labelled cell bodies remain in the hilus. Their dendrites can no longer be followed very clearly, but the hilar neuropil is more densely labelled (Fig. 2B). Neurons in the indusium griseum display a similar pattern and time course of decreasing immunoreactivity (data not shown).

Transient FAAH-immunoreactive cells and processes in the hippocampal formation

At the first postnatal days, FAAH-containing cells appear in the dendritic layers of the hippocampus proper (Fig. 3A and B). All of these cells had horizontal or multipolar dendritic orientation. A total of 61 such cells were found in the CA1–3 stratum radiatum during light microscopic analysis of 35 sections (on average about two cells per section). These cells demonstrated robust immunolabelling even in the CA1 subfield of newborn animals where pyramidal neurons had low FAAH content. On the 4th postnatal day, FAAH-containing cell bodies in the stratum radiatum were rather rare (eight cells per 13 sections). At P8, only two such cells were found in 12 sections, and none on the 12th postnatal day. At P22, FAAH-immunoreactive cell bodies were numerous in this layer, and most had a vertical dendritic orientation characteristic of misplaced pyramidal cells. In the stratum oriens, numerous FAAH-containing cell bodies were detectable at all ages studied. We did not analyse this cell population quantitatively because it was not always easy to separate them from misplaced pyramidal cells and FAAH-containing cells in the alveus (see below).

At P0–P2, weak FAAH immunolabelling was found in ependymal cells that line the lateral ventricle from the surface of the hippocampus and corpus callosum (Figs 1B and 3, C_1). On P4–P8, the labelling of these cells became robust (Figs 1C and 3, C_2) but, later, from P12 onwards, their immunoreactivity gradually decreased in comparison with the adjacent alveus and stratum oriens. Finally, at P22, these cells could barely be found. By this time point, numerous FAAH-containing cells appeared in the white matter of the alveus and corpus callosum (Fig. 3, C_3). It is not clear whether this was due to migration of ependimal cells into the tissue or whether another cell population became FAAH-immunoreactive. Robust FAAH immunoreactivity of ependymal cells was demonstrated also in adult mouse brain (Egertova *et al*., 2003).

At the first postnatal days, thin FAAH-immunoreactive processes were running horizontally within CA1 stratum lacunosum-moleculare. They were moderately varicose and branching very rarely (Fig. 3D).

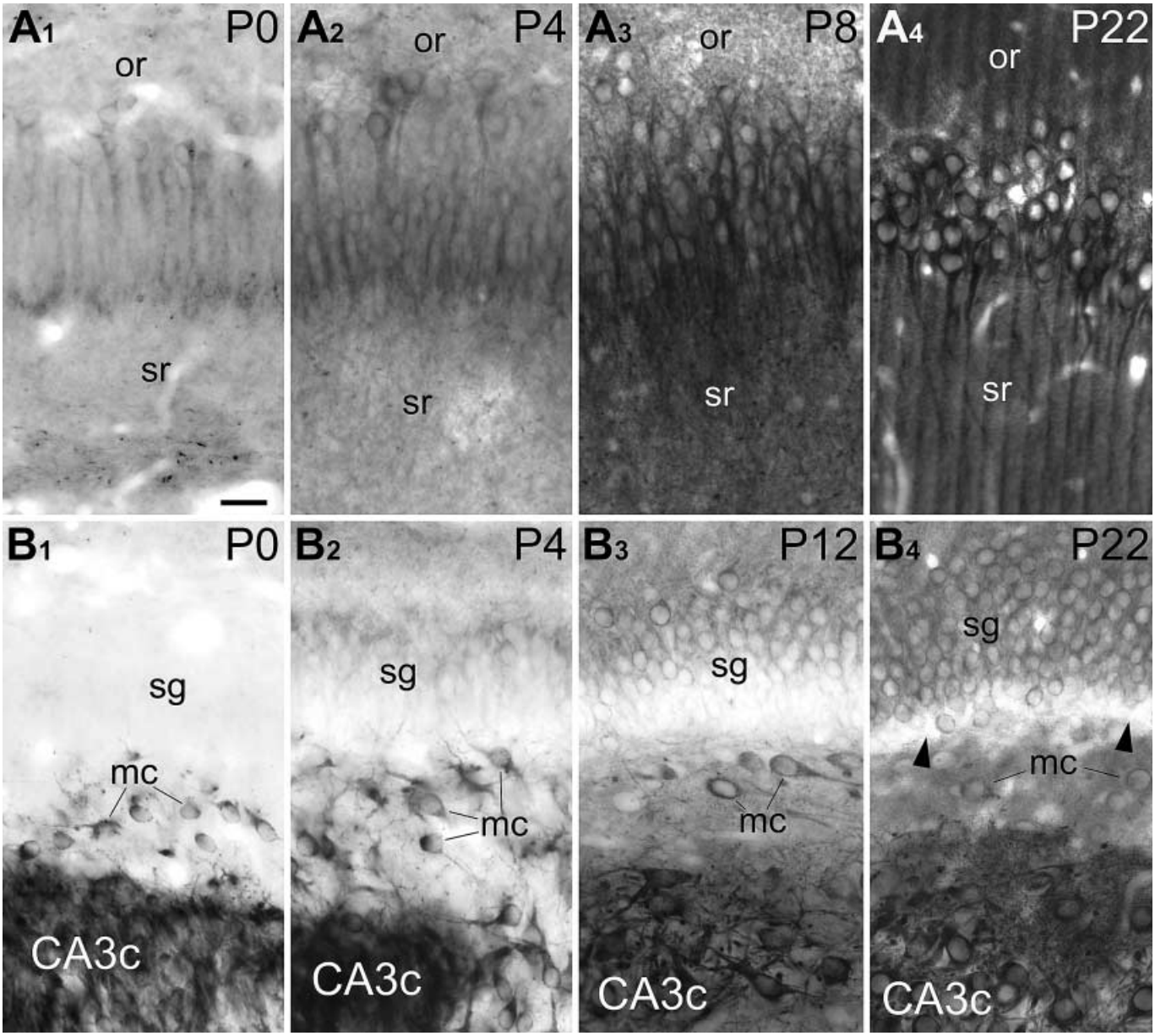

FIG. 2. Time course of postnatal development of FAAH immunoreactivity in the CA1 subfield (A) and in the suprapyramidale blade of the dentate gyrus (B) as visualized by immunoperoxidase reaction. (A_1) In newborn rats, CA1 pyramidal neurons express very low levels of FAAH. (A_2) In 4-day-old animals, pyramidal cell bodies and short fragments of their proximal apical dendrites are weakly immunostained. (A_3) The staining of CA1 pyramidal cell bodies and proximal apical dendrites is robust on the 8th postnatal day. (A_4) At P22, basal dendrites in the stratum oriens (or) are strongly immunoreactive as well. (B_1) On the day of birth, FAAH content is high only in the hilar mossy (mc) and CA3c pyramidal cells. (B_2) On the 4th postnatal day, FAAH appears also in the granule cell bodies in their outer rows. (B_3) By P12, about half of the granule cell bodies become immunolabelled. At this time point, hilar mossy cells (mc) are stained less intensely than adjacent pyramidal cells and, by P22, they lose even more immunoreactivity (B_4). Most of the granule cell bodies are immunoreactive at this age, although not as strongly as adjacent pyramidal cells. Only the inner cell row in the stratum granulosum (arrowheads) remains largely immunonegative. This row contains numerous interneurons, which do not express FAAH. Note the intense immunoreactivity of the hilar neuropil at P12–P22 but not at P0–P4. Abbreviations: sg, stratum granulosum of the suprapyramidale blade of dentate gyrus; sr, stratum radiatum. Scale bar in A_1 (valid for all), 25 μm.

Synapses were not formed by these varicosities, as seen under the electron microscope, but they probably represent axons because they contain clusters of synaptic-like vesicles (data not shown). These processes disappeared from P4 onward.

Soma-dendritic location of FAAH

Electron microscopy following pre-embedding immunoperoxidase or immunogold labelling for FAAH revealed a similar antigen distribution, at each examined time point, in the cytoplasm of different cell types, including pyramidal, granule and mossy cells, as well as transient cells in CA1 stratum radiatum (see above). Immunoreaction end-product could be seen in contact with cisternae of endoplasmic reticulum in the cell bodies and most proximal dendrites (Fig. 4A and B). Nuclei of all examined FAAH-containing cells were immunonegative. In the dendrites, FAAH immunoreactivity was found in the following characteristic locations: (i) in the cytoplasm often associated with small vesicles, (ii) attached to the outer surface of mitochondria or (iii) attached to the

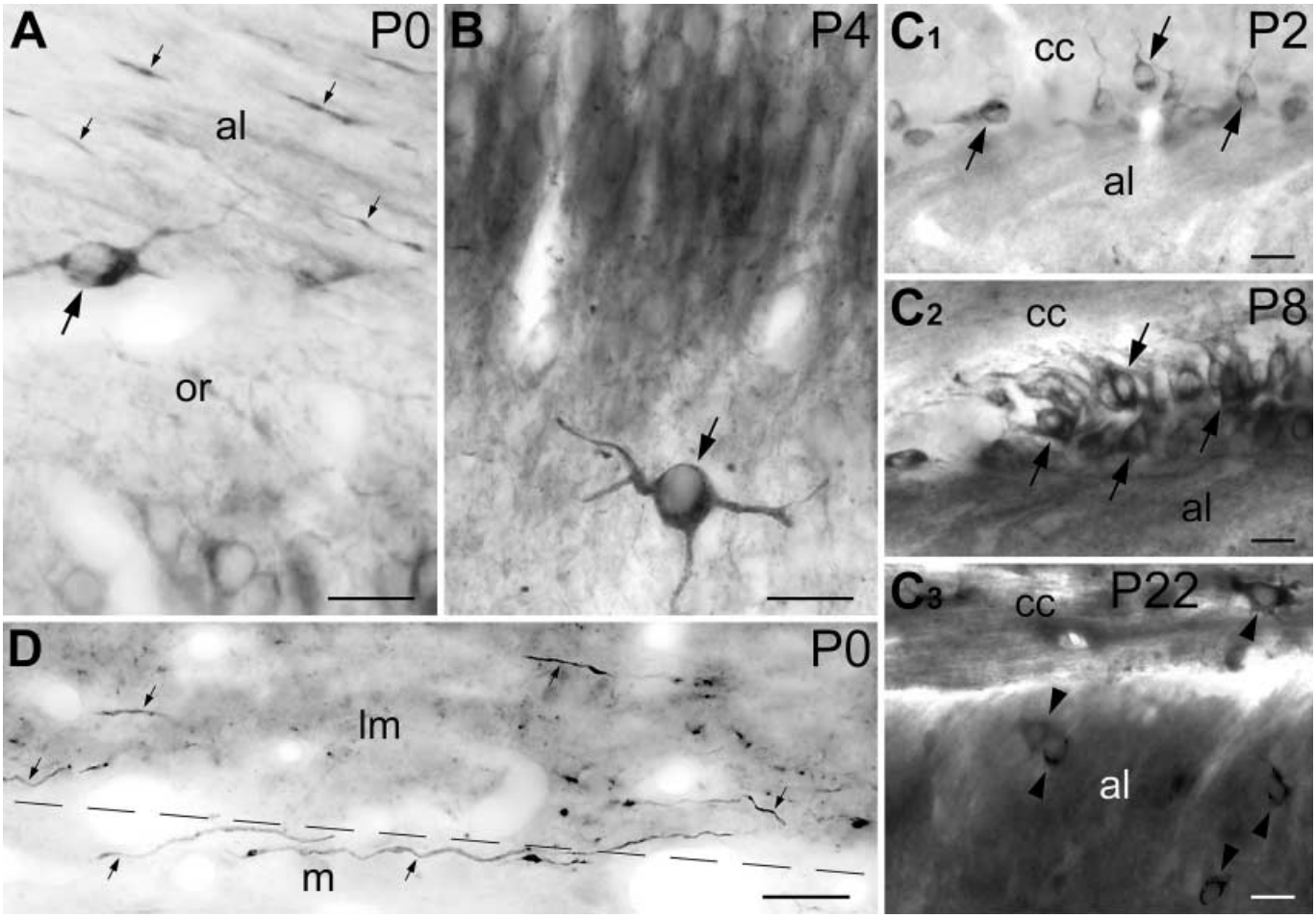

FIG. 3. Cells or processes that transiently express FAAH. (A) An FAAH-containing cell (arrow) with horizontal dendrites is visible at the oriens/alveus border in the CA3 region of a newborn rat. Numerous thin FAAH-containing processes (small arrows) with unidentified origin are present in the alveus. (B) An FAAH-containing cell (arrow) in the CA1 stratum radiatum of a 4-day-old rat. This cell is shown at the electron microscopic level in Fig. 4. (C) FAAH-containing ependymal cells (arrows) that line the lateral ventricle from the surface of the hippocampus and corpus callosum (cc) adjacent to the CA2 subfield. These cells are weakly FAAH-immunoreactive at P2 (C_1), intensely stained at P8 (C_2) and can no longer be found on the 22nd postnatal day (C_3). However, at P22, numerous FAAH-containing cells (arrowheads) can be found in the alveus, stratum oriens and the corpus callosum. (D) FAAH-containing varicose processes (small arrows) in the strata moleculare (m) and lacunosum-moleculare (lm) of a newborn rat. These processes are no longer visible after P4. The hippocampal fissure is indicated with a dashed line. Scale bars, 20 μm.

inner surface of the cell membrane (Fig. 4C). An accumulation of FAAH immunoreactivity near synaptic contacts was not observed.

A quantitative survey of FAAH distribution in association with mitochondria, cell membrane and small cytoplasmic vesicles did not reveal a consistent age-related difference in the CA1 and CA3 stratum radiatum and in the hilus. Therefore, data from P0, P4, P8 and P12 animals were pooled. The vast majority of the silver particles (over 80%) was found in the cytoplasm attached to small vesicles. Particles attached to the cell membrane were rare at all ages studied (below 5%). By contrast, FAAH immunoreactivity linked to mitochondrial membrane was more frequent; of 4902 silver particles detected in a random sample, 484 (10%) were in direct contact with the outer surface of mitochondria. This distribution is remarkably similar to that observed in the adult hippocampus, cerebellum or amygdala (Gulyás *et al*., 2004).

Discussion

The main findings of the present study are as follows. (i) FAAH immunoreactivity reaches adult-like expression levels first in CA3 (already at P0), and only much later (after P8) in CA1. During this period, FAAH levels in hilar mossy cells decrease and in granule cells slowly increase. (ii) FAAH expression develops in the proximal-to-distal direction in pyramidal cells, reaching adult levels in the most distal dendrites only at P22. (iii) At least two cell populations – stratum radiatum neurons and ependymal cells at the border of the alveus and corpus callosum – showed a transient expression of FAAH during hippocampal development. (iv) At the ultrastructural level, FAAH distribution at all ages was very similar to the adult pattern, i.e. it was largely associated with the limiting membrane of cytoplasmic organelles, including transport vesicles, mitochondria and endoplasmic reticulum.

FAAH expression correlates with the spatial and temporal pattern of hippocampal cell maturation, but not with the arrival of afferent inputs

FAAH immunoreactivity was robust in mossy cells and CA3c pyramidal cells already on the day of birth. It became clearly detectable in the CA1 region only several days later. At the cellular level, the development of FAAH expression followed a proximal-to-distal

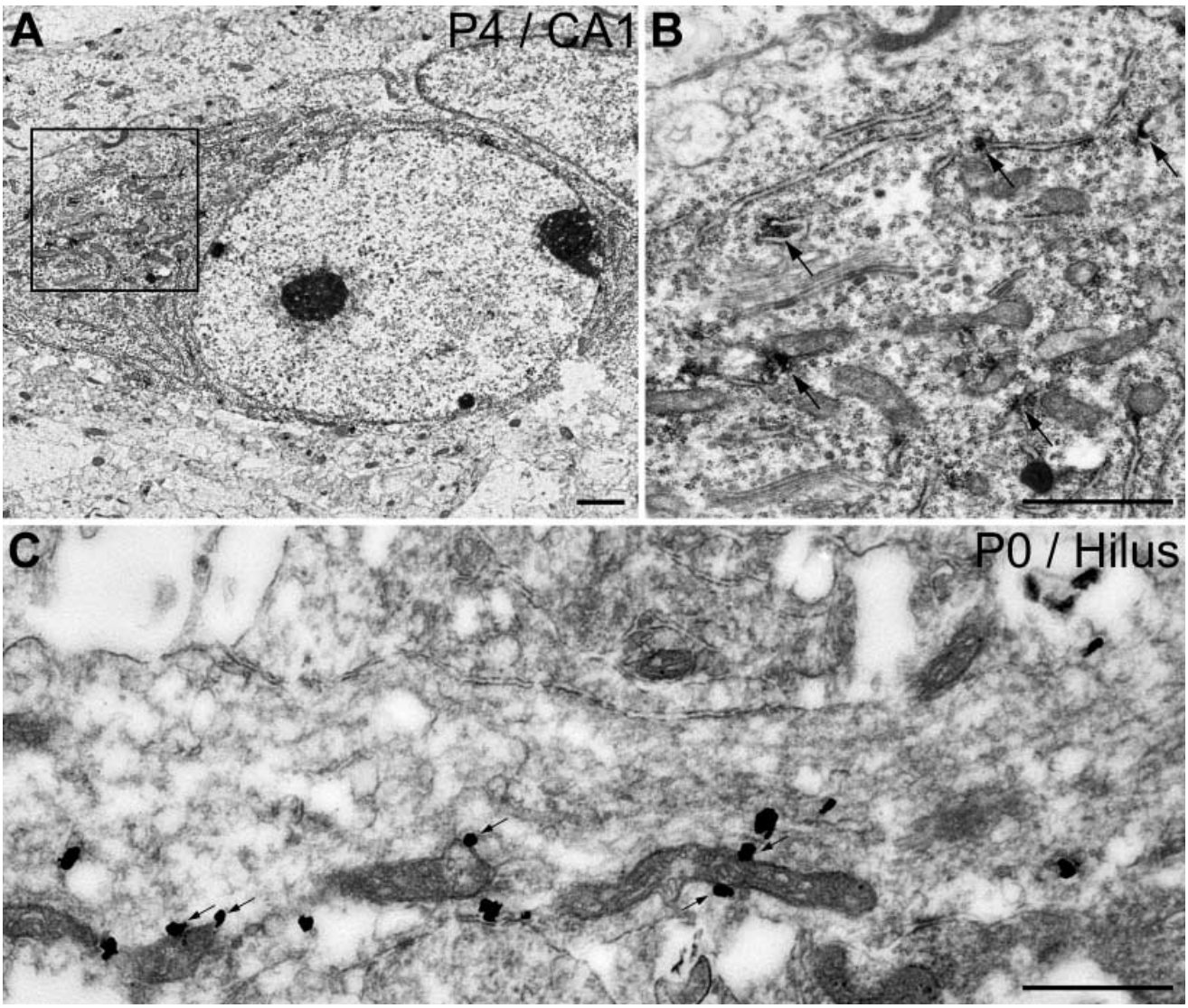

FIG. 4. (A) Low-power electron micrograph of an FAAH-containing cell body in the CA1 stratum radiatum at P4. A light micrograph of the framed area is shown in B at higher magnification. The immunoperoxidase reaction end product is indicated by arrows. (C) Short segment of a mossy cell dendrite in the hilus of a newborn rat. FAAH is visualized by pre-embedding immunogold reaction and silver intensification. Electron-dense gold/silver particles attached to the outer membrane of mitochondria are indicated with small arrows. Scale bars, 1 μm.

direction, i.e. pyramidal cell bodies and thick proximal dendrites in stratum radiatum were stained first, followed by staining of the entire stratum radiatum, and finally the strata lacunosum-moleculare and oriens. In the dentate gyrus, on the day of birth, only mossy cells expressed FAAH, whereas granule cells became FAAH-immunoreactive only after the first postnatal week. Immunolabelling appeared first in the outer rows of stratum granulosum and gradually shifted towards the inner layers. The general developmental sequence of FAAH expression summarized above corresponds well to the spatial and temporal pattern of hippocampal cell maturation. Indeed, dendritic arborization of hippocampal pyramidal neurons develops earlier in the CA3 than in the CA1 subfield (Lopez-Gallardo & Prada, 2001). Neurogenesis and maturation of the granule cells takes place later than that of the pyramidal neurons, mostly in the postnatal period, and their outer rows mature earlier than the inner rows (Altman & Das, 1965; Zimmer & Haug, 1978; Bayer, 1980; Gaarskjaer, 1985). By contrast, the major extrinsinc input of the hippocampus arrives from the perforant path already at P2, and starts to invade even the immature dentate molecular layer at P3 (Super & Soriano, 1994). It terminates on the most distal branches of pyramidal and granule cell apical dendrites. GABAergic input to the perisomatic region of granule cells also develops quite early, before P5 (Seress *et al*., 1989; Morozov & Freund, 2003b). Thus, neither the proximal-to-distal nor the regional/laminar distribution pattern of FAAH expression correlates with the arrival of afferent inputs at any postnatal age studied. However, there is a clear correlation with the general pattern of cell maturation. This suggests that FAAH and its substrates (including the endocannabinoid anandamide) are unlikely to be involved in the control of afferent synaptic input, but may have a more general role in fatty acid amide metabolism.

This suggestion is supported by other lines of evidence. In CA1 stratum lacunosum-moleculare, FAAH expression is very low until the 8th postnatal day (present study). However, CB_1-positive axons were shown to innervate dendrites in CA1 stratum lacunosum-moleculare as well as in the outer molecular layer of the dentate gyrus already at the first postnatal week (Morozov & Freund, 2003a). These axons form

symmetric synapses, suggesting that they are GABAergic (although, unlike perisomatic CB_1-containing axons, they do not contain CCK). Thus, in these layers there is a mismatch of CB_1-positive afferent input and postsynaptic endocannabinoid metabolism.

According to Golgi-electron microscopy, hilar mossy cells in the rat hippocampus are immature at birth, and their dendritic trees reach an adult-like appearance by postnatal day 21 (Ribak *et al*., 1985). The present data demonstrate that mossy cells are strongly FAAH-immunoreactive already on the day of birth, and their immunoreactivity starts to decrease approximately from the 8th postnatal day, reaching an adult-like low level on the 22nd day (Gulyás *et al*., 2004). These time points (i.e. P8 and P22) coincide with (i) the appearance of the first CB_1/CCK-containing axons around the somata of hilar mossy cells (P8), and (ii) reaching the final density of perisomatic innervation (P20), respectively, as shown in our earlier studies (Morozov & Freund, 2003a, >b). This represents another example of mismatch, i.e. mossy cells are strongly FAAH-immunoreactive but lack CB_1-positive afferent input during the first postnatal week, whereas during the late postnatal period, mossy cells have a robust innervation from CB_1/CCK-containing basket axons, but have a decreased level of the enzyme metabolizing one of the endogenous CB_1 ligands, anandamide.

Differences between the rat and mouse hippocampus in FAAH expression pattern but not in CB_1 receptor distribution

In contrast to previous publications (e.g. Tsou *et al*., 1998) claiming that FAAH is absent from dentate granule cells in the rat, here we demonstrated the presence of relatively low levels of FAAH in granule cells, which follows the same spatiotemporal pattern of expression as normal neuronal maturation (Fig. 2B). Our findings are in line with the demonstration of FAAH mRNA in these cells (Thomas *et al*., 1997). Egertova *et al*. (2003) demonstrated recently in adult mouse that granule cells of the dentate gyrus, hilar mossy cells and neurons of the indusium griseum are immunoreactive for FAAH, at least as much as adjacent pyramidal neurons. This is in contrast with the present findings in rat, where these cell populations express considerably lower levels of FAAH than pyramidal cells. Another difference between rat and mouse is the density of FAAH immunoreactivity in the stratum lacunosum-moleculare; it is very low in the mouse (Egertova *et al*., 2003), whereas in the rat, it reaches the density seen in the adjacent strata radiatum and moleculare. Thus, there are considerable differences in FAAH distribution between the rat and mouse throughout hippocampal formation, in spite of the fact that the distribution of CB_1-containing axons is almost identical in the two species (Marsicano & Lutz, 1999; Tsou *et al*., 1999; Egertova & Elphick, 2000; Egertova *et al*., 2003).

In summary, there is no obvious correlation between the laminar/regional distribution of FAAH-positive dendrites and the arrival of CB_1-containing afferent inputs either during postnatal development of the rat hippocampus at any time point or in the adult hippocampus of the mouse or rat. Because CB_1-expressing axons mark the sites where endocannabinoid-mediated retrograde synaptic signalling takes place – at least regarding the GABAergic afferents – we hypothesize that FAAH, and its endocannabinoid substrate anandamide, is not involved in depolarization-induced suppression of inhibition. The other endocannabinoid, 2-arachidonoylglycerol (selectively metabolized by monoglyceride lipase; Dinh *et al*., 2002), is a likely candidate for this function. Indeed, electrical stimulation in hippocampus was shown to increase 2- arachidonoylglycerol, but not anandamide levels (Stella *et al*., 1997). FAAH may rather play a role in the metabolism of other fatty acid amides (Natarajan *et al*., 1984; Deutsch & Chin, 1993; Cravatt *et al*., 1996); this activity appears to develop in parallel with the metabolic maturation of neurons.

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Abbreviations

CB_1, cannabinoid type 1 receptor; CCK, cholecystokinin; FAAH, fatty acid amide hydrolase; P, postnatal day.

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