Chapter 2

A-kinase anchoring protein 150 in the mouse brain is concentrated in areas involved in learning and memory

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Abstract

A-kinase anchoring proteins (AKAPs) form large macromolecular signaling complexes that specifically target cAMP-dependent protein kinase (PKA) to unique subcellular compartments and thus, provide high specificity to PKA signaling. For example, the AKAP79/150 family tethers PKA, PKC and PP2B to neuronal membranes and postsynaptic densities and plays an important role in synaptic function. Several studies suggested that AKAP79/150 anchored PKA contributes to mechanisms associated with synaptic plasticity and memory processes, but the precise role of AKAPs in these processes is still unknown. In this study we established the mouse brain distribution of AKAP150 using two well-characterized AKAP150 antibodies. Using Western blotting and immunohistochemistry we showed that AKAP150 is widely distributed throughout the mouse brain. The highest AKAP150 expression levels were observed in striatum, cerebral cortex and several other forebrain regions (e.g. olfactory tubercle), relatively high expression was found in hippocampus and olfactory bulb and low/no expression in cerebellum, hypothalamus, thalamus and brain stem. Although there were some minor differences in mouse AKAP150 brain distribution compared to the distribution in rat brain, our data suggested that rodents have a characteristic AKAP150 brain distribution pattern.

In general we observed that AKAP150 is strongly expressed in mouse brain regions involved in learning and memory. These data support its suggested role in synaptic plasticity and memory processes.

Keywords: AKAP150; localization; immunohistochemistry; cAMP-dependent protein kinase
Introduction

cAMP-dependent protein kinase (PKA) is involved in several intracellular signaling cascades and it regulates multiple cellular functions (Scott, 1991; Skalhegg & Tasken, 2000). A potential mechanism to explain how such a multifunctional and broad substrate kinase mediates precise signaling events, is colocalization of its substrate to specific subcellular compartments. Compartmentalization arises in part from the association of the enzyme with so-called A-kinase anchoring proteins (AKAPs) (Glantz et al., 1993; Lohmann, et al., 1984). AKAPs represent a group of more than 70 identified functionally related proteins (Wong & Scott, 2004). Although they share little primary structure similarities, they all have the ability to bind PKA, and therefore to regulate specific cAMP signaling pathways by sequestering PKA to a specific subcellular location. This compartmentalization of individual AKAP-PKA complexes occurs through unique targeting domains that are present on each anchoring protein.

To date, AKAPs have been identified in a wide range of species, tissues and cellular compartments (Angelo & Rubin, 2000; Jackson & Berg, 2002, Sarkar et al., 1984; Wong & Scott, 2004). In the mammalian brain, several AKAPs have been characterized. One of these AKAPs is AKAP79/150. This family of proteins consists of three orthologues: bovine AKAP75, murine AKAP150 and human AKAP79. Initially AKAP75 was identified as a contaminant of PKA regulatory subunit II (RII) purified cytosolic brain preparations (Bregman et al., 1991; Sarkar et al., 1984). In addition, Bregman and colleagues retrieved AKAP150 by screening a rat cDNA library using radiolabeled RIIβ as functional probe (Bregman et al., 1989). Finally AKAP79 was identified as a constituent of postsynaptic densities (PSD) in human cerebral cortex (Carr et al., 1992).

Interestingly, AKAPs function as multi-assembly scaffold molecules interacting with other signaling enzymes. AKAP79/150 has the ability to bind protein phosphatase 2B/calcineurin (PP2B/CaN) (Dell’Acqua et al., 2002) and protein kinase C (PKC) (Faux et al., 1999) besides PKA. By tethering both kinases and phosphatases AKAP79/150 provides a unique platform for integrating opposite signaling events to the same subcellular site.

It has been suggested that in excitatory synapses at the PSD, AKAP79/150 targets its anchored proteins and forms a multi protein complex with AMPA and NMDA receptors.
(AMPAR and NMDAR), synaptic adhesion molecules, and cytoskeleton proteins. These proteins play an important role in synaptic function (Colledge et al., 2000; Kennedy, 1997; Malenka & Bear, 2004; Yamauchi, 2002; Ziff, 1997).

The first evidence that anchoring of PKA is crucial for the regulation of synaptic function was reported by Rosenmund et al. (1994). In their study, blocking the PKA anchoring to AKAPs prevented the PKA-mediated regulation of AMPA/kainate currents in cultured hippocampal neurons. Moreover, recent findings strongly indicate that anchored PKA is crucial for maintaining AMPA currents during glutamate stimulation (Hoshi et al., 2005).

Interestingly, disruption of AKAP-PKA anchoring leads to CaN-dependent, long-term depression (LTD)-like down-regulation of AMPAR currents, implicating an important role for AKAP79/150 in AMPAR regulation (Tavalin et al., 2002). In general, the AKAP79/150 scaffold molecule has emerged as an important element in regulating AMPAR phosphorylation in long-term potentiation (LTP) and LTD at the PSD (Dell’Acqua et al., 2006; Genin et al., 2003; Snyder et al., 2005). Since strengthening or weakening of synaptic transmission is widely considered to be the cellular mechanism that underlies learning and memory, a role of AKAP79/150 in learning and memory can be expected.

To date, only a few immunohistochemical localization studies illustrate the distribution of AKAP79/150 in different brain compartments in various species. In human brain high levels of AKAP79/150 were reported at the PSD of the forebrain (Carr et al., 1992). A more detailed analysis of AKAP150 protein distribution in the rat brain showed that AKAP150 is widely distributed throughout the brain and is expressed in many classes of neurons that constitute the rat CNS (Glantz et al., 1992). A more recent study focused on the distribution of AKAP150 at rat CA1 pyramidal cell asymmetric and symmetric PSD and its colocalization with several markers of excitatory and inhibitory receptors. In this study, the interaction of AKAP150 with components of the excitatory PSD was confirmed, whereas AKAP150 immunoreactivity (IR) was not associated with inhibitory synapses (Lilly et al., 2005).

The distribution of AKAP150 protein in the mouse brain has not been established yet. To elucidate the specific role of AKAP150 in learning and memory processes it may be important to use genetically modified mice in future research. Therefore, besides characterizing AKAP150 expression throughout the whole mouse brain, we specifically
focused on AKAP150 expression in areas known to be involved in learning and memory processes. We established the distribution of AKAP150 protein in the mouse brain using immunohistochemistry and Western blot techniques.

**Material and Methods**

**Animals and housing conditions**
The present study is based on brain tissue from 9-week-old male C57Bl/6J inbred mice (n=8) obtained from Harlan, Horst, The Netherlands. Animals were group-housed in standard macrolon cages placed in an environmentally controlled room for temperature (22 ± 1 °C) and humidity (45 ± 10%) under a 12:12h light/dark cycle with lights on at 7.00 a.m. Animals had free access to water and standard food pellets. Collection of brain tissue was performed after at least one week of accommodation under the above conditions. All procedures concerning animal care and treatment were in accordance with the regulation of the ethical committee for the use of experimental animals of the University of Groningen, The Netherlands (License: DEC 4278A).

**Western blotting**
CO₂/O₂ anesthetized animals were quickly decapitated and brain tissue was removed on ice. The following brain regions were excised, immediately frozen in liquid nitrogen and stored at -80 °C before further processing: hippocampus, striatum, olfactory bulb, cortex, cerebellum, hypothalamus and brain stem.

Brain tissue was mechanically homogenized in 10 volumes of homogenization buffer [50 mM HEPES (pH 7.4), 150 mM NaCl, 0.2 % NP-40, 4 mM EGTA, 10 mM EDTA, 15 mM sodium pyrophosphate, 100 mM β-glycerophosphate, 50 mM sodium fluoride, 5 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM PMSF, and Complete Mini Protease Inhibitor Cocktail (Roche)]. The homogenate was centrifuged at 20,000 x g for 10 min at 4 °C, and the resulting supernatant was assayed for protein concentration using the Bradford method.
Protein samples (20 µg per sample) were separated on a 10 % SDS polyacrylamide gel and transferred to PVDF membranes (Millipore, USA). The blots were blocked for 1 h in blocking buffer (0.2 % I-Block (Tropix), 0.1 % Tween 20) and then incubated overnight at 4 °C either with goat anti-AKAP150 N-19 (1:500, sc-6446 Santa Cruz, CA, USA) or goat anti-AKAP150 C-20 (1:2,500, sc-6445 Santa Cruz, CA, USA). Mouse anti-actin antibody (1:40,000; MP Biomedicals, Irvine, CA, USA) served as control for protein loading. The blots were incubated with alkaline phosphatase-conjugated secondary antibodies [AP conjugated donkey anti-goat IgG (1:10,000)] (sc-2022 Santa Cruz, CA, USA) and AP-conjugated goat anti-mouse (1:10,000) (AC32ML, Tropix). Western blots were developed using the chemiluminescence method (Nitroblock and CDP-Star, Tropix). The immunoblots were digitized and quantified using a Leica DFC 320 image analysis system (Leica, Cambridge, UK).

**Immunohistochemistry**

Animals were anesthetized by intraperitoneal injection with sodium pentobarbital 6% solution and sacrificed by transcardial perfusion with saline solution containing heparin, followed by 4 % paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB). After perfusion, the brains were kept at 4 °C in 0.01 M phosphate buffered saline (PBS) containing 0.1% sodium azide for 72 h. All brains were dehydrated for 48 h in a 30 % buffered sucrose solution. After dehydration, 20 µm coronal or sagittal sections were cut on a cryostat microtome. Sections were stored at 4 °C in 0.01 M PBS containing 0.1 % sodium azide. The avidin/biotin immunoperoxidase staining method was used to visualize mouse AKAP150 IR. Coronal and sagittal sections covering the whole mouse brain were rinsed in 0.01 M PBS. Sections were preincubated with 0.3 % H₂O₂ to reduce endogenous peroxidase activity and afterwards rinsed in PBS. Non-specific binding sites were blocked by preincubating the sections with 5 % normal rabbit serum in 0.01 M PBS for 30 min. Subsequently sections were incubated either with goat anti-AKAP150 N-19 (1:500) or goat anti-AKAP150 C-20 (1:500) in 0.01 M PBS containing 5 % normal rabbit serum and 0.3% Triton X-100 for 2 h at room temperature (RT) and left overnight at 4 °C. Afterwards the sections were rinsed (3 x 15 min in PBS) and incubated at RT for 2 h with biotin-conjugated rabbit anti-goat antibody (1:400) (Jackson Inc.) in 0.01 M PBS containing 1 %
normal rabbit serum and 3 % Triton X-100. Another rinsing step with 0.01 M PBS at 4 °C was followed by incubation with avidin complex containing biotinylated horseradish peroxidase (1:400) (Vectastain ABC Kit, Burlingame, CA, USA) for 2 h at RT. Finally, staining was visualized with 0.03 % diaminobenzidine (DAB) chromogen substrate and 0.001 % H$_2$O$_2$. The incubation reactions were stopped by rinsing the sections with 0.01 M PBS.

Immunostained sections were analyzed with an Olympus BH2 microscope (Olympus, Japan). Two independent investigators established semi-quantification of mouse AKAP150 IR. For each brain region, material from 6 animals was examined to establish AKAP150 immunostaining. The scoring system used for establishing the relative AKAP IR was classified as follows: absent (-), low (+-), moderate (+), high (++) and very high (+++) In addition, for a subjective quantification of AKAP150 protein distribution in the mouse brain, relative densitometry measures for some major brain regions and nuclei have been determined (Table 2) (Quantimet 550 IW, Cambridge, UK). Photographs were taken with a DM1000/DFC280 Leica image analysis system (Leica, Cambridge, UK).

**Antibody specificity**
Both AKAP150 antibodies are affinity purified goat polyclonal antibodies raised against a peptide mapping at the carboxyl terminus part (C-20) or N-terminus (N-19) of AKAP150 of rat origin. They showed, in parallel staining experiments, high specific AKAP150 IR in the mouse brain and displayed a similar pattern of staining. Because polyclonal antibodies were used, different affinities and avidities of the antibodies could influence the staining intensity. Therefore Table 1 reflects only relative amounts of AKAP150 IR, rather than a quantitative comparison between the two AKAP150 antibodies. The specificity of both antibodies in the mouse brain was assessed by parallel staining performed without primary antibodies and with secondary antibodies alone and showed no staining signals (data not shown). In addition, incubation of the conjugate with AKAP150 antibody/blocking peptide (1:10; Santa Cruz, CA, USA) was carried out at 4 °C overnight, followed by 25 min centrifugation (12,000 rpm), before application to the tissue sections. Following preabsorption, no AKAP150 staining was observed (Fig. 4B). Furthermore, preincubation
steps were performed to increase the specificity of AKAP150 antibodies in the mouse brain.

Results

Western blot analysis of AKAP150 expression levels in various brain compartments

Western blot analysis with two AKAP150 antibodies (a N-terminal or C-terminal antibody) in protein extracts from different mouse brain regions revealed the highest expression level of AKAP150 protein in cortex and striatum (Fig. 1). High expression levels were also detected in the hippocampus and olfactory bulb, while the cerebellum and the hypothalamus revealed low levels of AKAP150 expression. The lowest expression level of AKAP150 was found in the brain stem (Fig. 1).

Fig. 1 Western blot analysis of AKAP150 expression levels in mouse brain. (A) Bar graph representing AKAP150 expression level in different compartments of mouse brain. Values represent mean percentages of integrated optical density (I.O.D). ± S.E.M. Cortex was set to 100% (B) Representative Western blot with protein extracts from different brain regions. Actin served as control for protein load.

The results of the Western blot expression levels corresponded with the distribution pattern of this protein in the immunohistochemical analysis of the mouse brain (Fig. 2; Tables 1 and 2).

General overview of AKAP150 IR in mouse brain

AKAP150 IR was widely distributed throughout the brain. Highest IR was found in striatum and olfactory tubercle, but in cortex, hippocampus and amygdala AKAP150 expression was also very abundant (Fig. 2).
In several brain regions, AKAP150 IR was limited to specific cell layers (e.g. the Purkinje cell layer of the cerebellum) or nuclei (e.g. reticular thalamic nucleus, ethmoid nucleus) (Table 1). Some brain regions did not show any IR for AKAP150 protein (e.g. numerous nuclei in midbrain and hindbrain) (Fig. 2; Table 1).

**Detailed description of AKAP150 expression in mouse brain**

**Olfactory system**

AKAP150 IR varied from moderate to relatively high in the olfactory bulb and its various layers. Superficial layers (glomerular layer and external plexiform layer) as well as deep layers (internal plexiform layer and granule layer) of the olfactory bulb showed moderate expression of AKAP150 (Table 1). Only the mitral cell layer of the olfactory bulb was highly immunoreactive for AKAP150 protein (Table 1). The olfactory tubercle showed a different staining pattern than the rest of the olfactory system. Here we observed a very
high AKAP150 IR pattern. The excessive staining made it difficult to distinguish subcellular compartments within the olfactory tubercle (Figs. 2A and B).

**Cerebral cortex**

In mouse cerebral cortex, AKAP150 protein was found to be expressed throughout all cortical layers (Figs. 2 and 3A). Very high AKAP150 IR levels were observed in cortical layers I (molecular layer), II (external granular layer) and IV (internal granular layer). Cortical layers II and IV were highly immunoreactive for AKAP150 protein in perikarya of granule cells (Figs. 3B and C).

![Fig. 3 Distribution of AKAP150 protein in the cerebral cortex. (A) Overview of AKAP150 protein distribution in cerebral cortex somatosensory 1, trunk region. Boxed areas represent the localization of panel B-F. (B) Dense staining is observed in cortical layers I-III. (C) Perikarya staining in cortical layer IV. (D) AKAP150 staining in barrel cortex. (E) Both fibers and cell bodies are immunopositive for AKAP150 in cortical layer V. Note the weaker staining and less perikarya IR for AKAP150 in this layer as compared with cortical layer IV. (F) AKAP150 IR in cortical layer VI.](image)

In layer IV of the somatosensory region of the mouse cerebral cortex, cortical barrels displayed a dense and characteristic AKAP150 IR pattern (Fig. 3D). Cortical layer III (external pyramidal layer) showed pronounced AKAP150 IR (Figs. 3A and B) whereas...
cortical layer V (internal pyramidal layer), which represents a principal output system of the neocortex, revealed only moderate immunostaining for AKAP150 (Fig. 3E). The deepest cortical layer, layer VI (polymorphic layer), was strongly AKAP150 IR. (Fig. 3F). The highest IR of AKAP150 protein in mouse cerebral cortex was found in cerebral cortex layer I (molecular layer) and in the stellate cells (interneurons) of both external and internal granular layers (cortex layers II and IV). In addition, the entorhinal cortex, the major source of afferents to the hippocampal formation, was intensely stained (Table 1).

**Hippocampal formation**

Although there were some differences within this laminar structure, overall the hippocampal formation displayed high IR for AKAP150 protein (Fig. 4A; Tables 1 and 2).

![Image](image-url)

Fig. 4 Immunostaining pattern for AKAP150 in the hippocampal formation. (A) AKAP150 IR in stratum oriens (SO), stratum radiatum (SR), stratum lacunsum moleculare (SLM), stratum pyramidale (Py) of CA1-3 and stratum lucidum (SLu) of CA3 and in molecular layer (ML), dentate granular cell layer (GCL) and hilus (hil) of the dentate gyrus. (B) AKAP150 IR after preabsorption of AKAP150 antiserum with the antigenic peptide. (C) Higher magnification of boxed area in panel A, showing immunopositive perikarya of pyramidal neurons (Py) in CA1, (D) Higher magnification of boxed area in panel A, showing stained pyramidal cell bodies (Py) in CA3. (E) Higher magnification of boxed area in panel A, showing immunopositive granule cell perikarya in the dentate gyrus (GCL).
Especially in the CA1-3 region of the hippocampus, AKAP150 showed a very high expression in basal dendrites (stratum oriens) and apical dendrites (stratum radiatum) of pyramidal neurons (Fig. 4A). A somewhat lower IR was observed in both stratum lacunsum moleculare and stratum lucidum (Fig. 4A). A characteristic staining pattern was observed in the pyramidal cell body layer. In CA1 the pyramidal cell bodies were moderately stained (Figs. 4A and C) whereas in CA3 pyramidal neurons perikarya were rather densely stained (Figs. 4A and D).

The subiculum also showed high AKAP150 IR (Table 1). In the dentate gyrus, AKAP150 IR was characterized by very high staining in the granule cell dendrites of the medial and lateral blade of the molecular layer (Fig. 4A). However, the dentate granule cells showed only moderate staining, whereas the hilus was slightly more immunoreactive (Figs. 4A and E). Interestingly, scattered non-principal cells in the dentate gyrus were also strongly stained for AKAP150 protein (Fig. 4E).

**Amygdala**

In the amygdala, AKAP150 IR patterns revealed a high level of this protein in the various subcompartments. The distribution of AKAP 150 was clear and strong throughout all amygdaloid nuclei with only slight differences in the density of staining (Table 1). Uniform but strong staining was detected in cortical amygdaloid nuclei, medial amygdaloid nucleus, basomedial amygdaloid nucleus, amygdalohippocampal area and amygdalopiriform transition area (Table 1, Fig. 5A).

![Fig. 5 Distribution of AKAP150 in the amygdala.](image)

*Fig. 5 Distribution of AKAP150 in the amygdala. (A) AKAP150 IR in the lateral amygdaloid nucleus, dorsolateral part (LaDL), lateral amygdaloid nucleus, ventrolateral part (LaVL) and the anterior part of the basolateral amygdaloid nucleus (BLA). (B) Higher magnification of boxed area in panel A, showing AKAP150 IR in the central amygdala. The capsular part (CeC), lateral division (CeL) and the medial posteroverentral part of the central amygdala (CeMPV) show staining in both cell bodies and fibers (see magnification in panel).*
The most prominent staining in the amygdala for AKAP150 protein was detected in the central amygdaloid nucleus, lateral amygdaloid nucleus and basolateral amygdaloid nucleus (anterior, posterior and ventral) (Fig. 5A). The basolateral complex revealed a strong IR whereas the central amygdala was characterized by a dense staining in which both neuronal perikarya and fibers were detected (Figs. 5A and B; Table 1).

**Septal nuclei and striatopallidal system**
In these brain regions, well-defined AKAP150 IR was observed. In the septum, the lateral nucleus was strongly stained, whereas the medial nucleus showed only faint AKAP150 staining (Fig. 2B; Tables 1 and 2).

The caudate putamen was the most strongly stained structure in the entire mouse brain. The very dense AKAP150 IR pattern made it difficult to distinguish the subcellular distribution of AKAP150, although some perikarya were observed. Clastrum and ventral pallidum exhibited moderate to high AKAP150IR, while the globus pallidus showed almost no staining (Fig. 2; Table 1).

**Epithalamus and thalamus**
In the epithalamus AKAP150 protein showed a moderate expression. Both the medial and lateral habenula displayed a diffuse IR with faint perikarya staining (Table 1).

At the level of the thalamus distinct patterns of expression were detectable although the overall distribution of AKAP150 protein in this brain region was rather low (Fig. 2; Table 1). Detailed analysis of the stained thalamus revealed moderate staining in reticular thalamic nucleus, subgeniculate nucleus and ethmoid nucleus, where scattered fibers (varicose fibers) were specifically stained. Low levels of AKAP150, described as faint staining, were found in geniculate nucleus, anterior nucleus, lateral nucleus, ventral nucleus and zona incerta (Fig. 2). The remainder of the thalamus showed no AKAP150 IR (Table 1).

**Hypothalamus**
In general, the expression level of AKAP150 in the hypothalamus was moderate to low. Accordingly, the supraoptic nucleus, paraventricular nucleus and suprachiasmatic nucleus
showed a very faint and diffuse pattern of staining (Fig. 2; Table 1). Among the regions with the highest IR, the median eminence, arcuate nucleus and mammillary nuclei displayed a homogeneous staining (Table 1).

**Midbrain**

AKAP150 protein showed a moderate to low expression in the midbrain region (Table 1). Most of the staining was observed in the superior colliculus, substantia nigra pars reticulata and pars compacta and periaqueductal gray (Fig. 2E). The IR in ventral tegmental area showed only moderate levels of AKAP150 (Fig. 2E). Low levels of AKAP150 IR were detectable in inferior colliculus and interpeduncular nuclei (Table 1).

**Cerebellum**

In the cerebellum, AKAP150 IR showed a moderate to low expression. Most of the staining was seen in the molecular layer of the cerebellar cortex in what appeared to be dendrites of the Purkinje neurons (Fig. 6B). The Purkinje perikarya were not well stained although punctuate staining of AKAP150 IR was detectable in the Purkinje layer throughout the whole cerebellar cortex (Fig. 6A). In the granule cell layer, the staining was considered faint, corresponding to a low expression of AKAP150 protein (Fig. 6A).

![Fig. 6 AKAP150 expression in the cerebellum. (A) AKAP150 IR in the cerebellar molecular layer (Mol). (B) Higher magnification of boxed area in panel A, showing staining of Purkinje neurons (arrow, also in panel A) staining in both cell body and dendrites.](image)

**Pons and medulla oblongata**

In the brainstem, the overall expression level of AKAP150 was low. Only the inferior olivary complex and nucleus of the solitary tract showed moderate immunostaining (Table 1). The remainder of this brain structure showed low or no AKAP150 IR.
Table 1 - Expression of AKAP150 protein in various compartments of the mouse central nervous system.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>C-term</th>
<th>N-term</th>
</tr>
</thead>
</table>

**Telencephalon**

*Olfactory system*

- Glomerular layer: ++
- External plexiform layer: +
- Mitral cell layer: ++
- Internal plexiform layer: +
- Granule layer: +
- Anterior olfactory nucleus: +

*Olfactory tubercle*

- ++

**Cerebral cortex**

- Layer I: +++
- Layer II: ++
- Layer III: ++
- Layer IV: +++
- Layer V: +
- Layer VI: ++

**Hippocampal formation**

- Dentate gyrus: +
- Granule cell layer: +
- Molecular layer: +++
- Hilus: +++
- CA1 region: +++
- Stratum radiatum: +++
- Stratum oriens: +++
- Stratum lacunsum: ++
- Molecular: Stratum pyramidale: +
- Stratum radiatum: +
- Stratum oriens: +++
- Stratum pyramidale: +++
- Stratum lucidum: +
- Subiculum: ++
- Entorhinal cortex: ++

**Amygdala**

- Central amygdaloid nucleus: +++
- Medial amygdaloid nucleus: ++
- Lateral amygdaloid nucleus: ++
- Basolateral amygdaloid nucleus: +++
- Basomedial amygdaloid nucleus: ++
- Cortical amygdaloid nuclei: ++
- Amygdalohippocampal area: ++
- Amygdalopiriform transition area: ++
- Intercalated nuclei: +++

**Septal and basal magnocellular nuclei**

- Bed nucleus stria terminalis: ++
- Nucleus accumbens core: ++
- Nucleus accumbens shell: ++
- Substantia innominata: +

**Septum lateral nucleus**

- ++

**Septum medial nucleus**

- +

**Diagonal band of Broca**

- +

**Striatopallidal system**

- Caudate putamen: +++
- Globus pallidus: +
- Ventral pallidum: +
- Claustrum: +
- Islands of Calleja: +++

**Diencephalon**

*Thalamus*

- Anterior nuclei: -
- Lateral nuclei: -
- Ventral nuclei: -
- Mediodorsal nucleus: -
- Central nuclei: -
- Paracentral nuclei: -
- Parafascicular thalamic nuclei: -
- Paraventricular thalamic nuclei: -
- Zona incerta: +

**Epithalamus**

- Habenula medial: +
- Habenula lateral: +

**Hypothalamus**

- Median eminence: +
- Arcuate nucleus: +
- Supraoptic nucleus: +
- Paraventricular nucleus: +
- Periventricular nucleus: +
- Suprachiasmatic nucleus: +
- Mammillary nuclei: +
- Fornix: -

**Mesencephalon**

*Midbrain*

- Superior colliculus: +
- Inferior colliculus: +
- Interpeduncular nuclei: +

**Substantia nigra**

- Pars reticulata: +
- Pars compacta: +
- Periaqueductal gray: +

**Rhomencephalon**

*Cerebellum*

- Molecular layer: +
### Chapter 2

#### Table 1 - Continuation

<table>
<thead>
<tr>
<th>Brain region</th>
<th>C-term</th>
<th>N-term</th>
</tr>
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<tbody>
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<td>Rhombencephalon</td>
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<td>Cerebellum</td>
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<tr>
<td>Purkinje cell layer</td>
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<td>+</td>
</tr>
<tr>
<td>Granule cell layer</td>
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<td>+</td>
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<tr>
<td>Ambigiuus nucleus</td>
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<td>-</td>
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<tr>
<td>Nucleus of the solitary tract</td>
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<td>+</td>
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<tr>
<td>Inferior olive</td>
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Scoring was classified as absent (-), low (+/-), moderate (+), high (++), very high (+++).

#### Table 2 - Mean optical density of AKAP150 protein in various compartments of the mouse central nervous system.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>O.D. ±SEM</th>
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<tr>
<td>Olfactory system</td>
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<td>Olfactory tubercle</td>
<td>0.52 ± 0.006</td>
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<tr>
<td>Cerebral cortex</td>
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<td>Layer I</td>
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<td>Layer II</td>
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<tr>
<td>Layer III</td>
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<tr>
<td>Layer IV</td>
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<td>Layer V</td>
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<tr>
<td>Layer VI</td>
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<td>Barrel cortex</td>
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<td>Hippocampal formation</td>
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<td>Dentate gyrus</td>
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<tr>
<td>Granule cell layer</td>
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<tr>
<td>Molecular layer</td>
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<tr>
<td>CA1 region</td>
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<tr>
<td>Stratum radiatum</td>
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<tr>
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<td>Stratum lacunum molecularare</td>
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<td>Stratum pyramidalde</td>
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<td>CA3 region</td>
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<tr>
<td>Stratum pyramidale</td>
<td>0.32 ± 0.024</td>
</tr>
<tr>
<td>Stratum lucidum</td>
<td>0.10 ± 0.015</td>
</tr>
<tr>
<td>Amygdala</td>
<td></td>
</tr>
<tr>
<td>Central amygdala</td>
<td>0.33 ± 0.011</td>
</tr>
<tr>
<td>Basolateral amygdala</td>
<td>0.29 ± 0.004</td>
</tr>
<tr>
<td>Lateral amygdala</td>
<td>0.33 ± 0.015</td>
</tr>
<tr>
<td>Septal and basal magnocellular nuclei</td>
<td></td>
</tr>
<tr>
<td>Septum lateral nucleus</td>
<td>0.32 ± 0.012</td>
</tr>
<tr>
<td>Septum medial nucleus</td>
<td>0.14 ± 0.009</td>
</tr>
<tr>
<td>Striatopallidal system</td>
<td></td>
</tr>
<tr>
<td>Caudate putamen</td>
<td>0.46 ± 0.015</td>
</tr>
<tr>
<td>Diencephalon</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
</tr>
<tr>
<td>Lateral nuclei</td>
<td>0.16 ± 0.003</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td></td>
</tr>
<tr>
<td>Dorsomedial hypothalamus</td>
<td>0.09 ± 0.011</td>
</tr>
<tr>
<td>Rhombencephalon</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
</tr>
<tr>
<td>Molecular layer</td>
<td>0.17 ± 0.002</td>
</tr>
<tr>
<td>Granule cell layer</td>
<td>0.13 ± 0.005</td>
</tr>
</tbody>
</table>

Values represent mean of optical density and ± S.E.M. of each brain compartment (n=5). O.D.= optical density.

### Discussion

#### General overview of AKAP150 distribution in the mouse brain

Our results showed that AKAP150 is widely distributed throughout the mouse brain. However, clear differences between brain compartments were observed. Both Western
AKAP150 in the mouse brain

blotting and immunohistochemistry showed the highest expression levels of AKAP150 in striatum and cerebral cortex. In addition, relatively high AKAP150 expression was found in hippocampus and olfactory bulb and low/no expression in cerebellum, hypothalamus, thalamus and brain stem. Although the overall expression of AKAP150 in thalamus, hypothalamus, midbrain and hindbrain was limited, a few nuclei in these regions showed a moderate to high AKAP150 IR (e.g. reticular thalamic nucleus, ethmoid nucleus, mammillary nuclei). Although it remains difficult to speculate on the specific function of a brain region based solely on AKAP150 expression, a higher expression in these specific nuclei may suggest a more prominent role of AKAP150 in these nuclei. E.g. the thalamic reticular nucleus is a sheet of GABAergic neurons. It was recently shown that AKAP150 facilitates the phosphorylation of GABA(A) receptors by PKA which may have profound local effects on neuronal excitation (Brandon et al., 2003).

It is interesting to note that in cholinergic areas such as the nucleus basalis, the medial septum and the diagonal band of Broca, which are known to be involved in the modulation of learning and memory, rather low levels of AKAP150 expression were observed. AKAP150 expression was absent in the large brain fiber systems (e.g. fornix and corpus callosum). With a few exceptions, our mouse AKAP150 data showed a distribution pattern similar to that previously described for the rat brain (Glantz et al., 1992). Like in the mouse brain, in the rat brain AKAP150 is abundantly expressed in olfactory bulb, cerebral cortex, hippocampus and cerebellar Purkinje neurons whereas in thalamus, hypothalamus, midbrain and hindbrain, AKAP150 is quantitatively characterized as modest or extremely low (Glantz et al., 1992 and Lilly et al., 2005).

AKAP150 IR in the cortex, hippocampus and amygdala

In the mouse brain AKAP150 showed high levels of IR in the cortex. Dense staining was observed throughout all cortical layers, except for moderate staining in immunoreactive layer V. The same pattern of expression was observed in the rat neocortex (Glantz et al., 1992). Interestingly, in the mouse barrel cortex (cortical layer IV), AKAP150 showed a strong and characteristic staining which was not reported for the rat brain. The barrel cortex and its afferent pathway from the facial vibrissae in rodents is often used as a model for studying neuronal plasticity (Fox et al., 1996). Although the function of AKAP150 in the
barrel cortex is not known, we can speculate on its involvement in mechanisms of neuronal plasticity in this brain region.

AKAP150 was expressed in all mouse hippocampal subfields but mainly in apical and basal dendrites of pyramidal neurons and dendrites of granule cells in the dentate gyrus. In general these findings correspond to the rat hippocampal AKAP150 distribution as reported previously (Glantz et al., 1992; Lilly et al., 2005). However, we also observed several differences between mouse and rat AKAP150 expression in this region. Overall, CA1 and dentate gyrus showed stronger IR than CA3 in the rat hippocampus (Lilly et al., 2005), whereas the mouse hippocampus has a more homogeneous staining. On a more cellular level the pyramidal neurons of the mouse hippocampus showed a somewhat different staining in comparison to the rat AKAP150 IR. Mouse pyramidal neurons in the CA3 showed very high AKAP150 IR in their cell bodies, whereas cell bodies in the CA1 exhibited a much weaker staining. This specific staining for pyramidal neurons was not reported previously for the rat brain (Glantz et al., 1992; Lilly et al., 2005). Interestingly, detailed observation of the mouse hippocampus indicated that interneurons might also contain AKAP150. For example, AKAP150-positive cells that resemble interneurons (based on shape, size and localization) were seen in the CA1-3 hippocampal area and in the dentate gyrus, which were not previously reported in the rat hippocampus (Glantz et al., 1992; Lilly et al., 2005). However, in the hippocampus from post-mortem human brains of healthy individuals and lobectomy samples from patients with intractable epilepsy, AKAP79 positive cells resembling interneurons were also reported in the CA1 area and in the hilus (Sik et al., 2000). Double staining experiments for AKAP79 and calretinin and parvalbumin as interneuron markers demonstrated numerous double stained dendrites and somata at the electron microscopic level of the human hippocampus (Sik et al., 2000).

Together these findings and our data suggest that AKAP79/150 may have a specific function in these interneurons. Nevertheless, the nature of the non-principal cells, which showed high expression levels of AKAP150 in the mouse brain, was not identified. Since we did not use any cellular markers for interneurons, the staining could also reflect e.g. excitatory mossy neurons.

Substantial levels of AKAP150 were demonstrated both in mouse and rat amygdala (Glantz et al., 1992). The highest expression levels were found in central and basolateral amygdala.
where dense staining marked both perikarya and fibers. This pattern of expression was also reported previously for AKAP79 in human foetal amygdala where it was enriched in dendrites and various neuronal cell types (Ulfig & Setzer, 1999).

**AKAP150 expression parallels the expression of PKA-RIIβ and AMPA receptor subunits**

The AKAP79/150 family has a high affinity for the RIIβ-PKA subunit (Bregman et. al., 1989). RIIβ is the predominant isoform and principal mediator of cAMP action in mammalian central nervous system (Sarkar et. al., 1984). Ventra and colleagues showed that in the rat neocortex and corpus striatum RIIβ mRNA levels paralleled the presence of AKAP150 protein. Conversely, in brain areas showing low RIIβ levels such as cerebellum, hypothalamus and cerebellum, the anchoring protein was absent (Ventra et al., 1996). In the adult mouse brain, high RIIβ mRNA levels were shown in the neocortex, caudate-putamen, hippocampus and reticular thalamic nuclei and a reduced level in the thalamus, midbrain and hindbrain (Cadd & McKnight, 1989). This RIIβ mRNA pattern in the mouse brain corresponds to our AKAP150 expression pattern confirming a parallel presence of both proteins in specific mouse brain regions.

AKAP79/150 has been shown to regulate hippocampal AMPA receptor phosphorylation and function (Colledge et al., 2000; Tavalin et al., 2002). Interestingly, in the murine hippocampus high expression levels of both GluR1 and GluR2 AMPA receptor subunits were reported in CA1-3 stratum oriens, radiatum and lacunosum moleculare and in the stratum moleculare of the dentate gyrus (Yoneyama et al., 2004). This also parallels the AKAP150 expression pattern we observed in this brain structure.

**Concluding remarks**

Overall, our results showed that AKAP150 protein is strongly expressed in numerous mouse brain compartments, and particularly in those areas that were reported to be involved in learning and memory processes. This supports the general notion that AKAP150 might be involved in learning and memory processes. AKAP79/150 bound PKA was found to play an important role in the regulation of AMPA receptor surface expression and synaptic plasticity (Rosenmund et al., 1994). Changes in synaptic plasticity are suggested to be the
possible mechanism underlying learning and memory processes, therefore a role of AKAP79/150 in learning and memory can be expected. Initial evidence for a role of AKAP150 in learning and memory came from a study by Moita and colleagues, who reported that blocking PKA binding on AKAPs in the rat lateral amygdala leads to an impairment of memory consolidation of auditory fear conditioning (Moita et al., 2002). In addition, we could recently show that AKAP150 is upregulated in the mouse hippocampus after exposing mice to a novel context and after associative learning (Nijholt et al., 2007).

In summary, we presented a detailed description of AKAP150 in discrete brain regions of the mouse. Although we observed minor differences between AKAP150 staining in the mouse and rat, our data suggested a characteristic pattern of AKAP150 distribution in these two rodent species.

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