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Differential patterns of myosin Va expression during the ontogenesis of the rat hippocampus

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Abstract

Myosin Va is an actin-based, processive molecular motor protein highly enriched in the nervous tissue of vertebrates. It has been associated with processes of cellular motility, which include organelle transport and neurite outgrowth. The *in vivo* expression of myosin Va protein in the developing nervous system of mammals has not yet been reported. We describe here the immunolocalization of myosin Va in the developing rat hippocampus. Coronal sections of the embryonic and postnatal rat hippocampus were probed with an affinity-purified, polyclonal anti-myosin Va antibody. Myosin Va was localized in the cytoplasm of granule cells in the dentate gyrus and of pyramidal cells in Ammon's horn formation. Myosin Va expression changed during development, being higher in differentiating rather than already differentiated granule and pyramidal cells. Some of these cells presented a typical migratory profile, while others resembled neurons that were in the process of differentiation. Myosin Va was also transiently expressed in fibers present in the fimbria. Myosin Va was not detected in germinative matrices of the hippocampus proper or of the dentate gyrus. In conclusion, myosin Va expression in both granule and pyramidal cells showed both position and time dependency during hippocampal development, indicating that this motor protein is under developmental regulation.

Key words: Myosin Va; Growth and development; Central nervous system; Hippocampus; Rat; Immunohistochemistry

Introduction

Brain development requires the coordination of processes that include neuronal proliferation, cell migration and differentiation, synaptogenesis, and programmed cell death. In the cerebrum of most mammals, neuronal proliferation and migration occur mainly during the prenatal period, followed by synaptogenesis and apoptosis. In contrast, granule neurons of the dentate gyrus, olfactory bulb and cerebellum originate also during the postnatal period. In the adult hippocampus, new neurons are generated throughout life, and are added to the granule cell layer of the dentate gyrus. This addition of neurons allows the mature neuronal network of the dentate gyrus to be optimized according to functional demands (1-3), and underscores the importance of the hippocampus in health and disease (1,4).

The embryonic formation of the hippocampus has proved to be an interesting model of ontogenesis because it presents neurogenic patterns that obey temporal gradients of neuronal proliferation and differentiation (5,6). There are three distinct spatiotemporal gradients (5,7-10). First, granule cells of the dentate gyrus located in the superficial portions of the granule cell layer are generated before those located more deeply, according to an outside-in pattern. This gradient differs from that of the cerebral cortex, where newly generated cells have to migrate through the cell layers formed earlier, resulting in an inside-out pattern (11). Second, the supra-pyramidal limb of the granule cell layer is generated before the infra-pyramidal limb. Finally, the cells in the temporal regions of the dentate gyrus tend to be generated earlier than those at the septal pole (5,7), obeying a temporal-septal gradient. Although the cytoarchitecture and development of the hippocampus, including the process of neuronal migration guided by glial cells, are well described (5,8-10), the participating components and molecular mechanisms involved in these processes remain mostly unknown.

The development of the hippocampus requires move-

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ment of cells, organelles and vesicles, as it occurs during the ontogenesis of all brain regions. Thus, it requires molecular motors that can convert chemical energy into force and/or movement. Motor proteins include the microtubule-based kinesins and dyneins, and the actin-based myosins (12). The myosin superfamily is composed of at least eighteen structurally distinct classes of myosins, which are widely expressed in eukaryotes (13). Among them, myosin Va (14) is a two-headed, non-filament forming, processive myosin (15-17). Myosin Va is present at low levels in many vertebrate tissues, but it is most concentrated in nervous tissue (18). It occurs in the nervous tissue of the embryonic chick (19), rat (18), mouse (20), and man (21). Most brain regions of the adult rat express myosin Va (22). Myosin Va is found in neurons and glial cells, and is localized to neurite tips, growth cones, and the perinuclear region (14). Myosin Va binds to synaptic vesicle-associated proteins (23-25), participates in organelle and vesicle movement along axons (26,27), is apparently involved in neuronal growth cone motility (28), and is involved in the localization of the smooth endoplasmic reticulum to the dendritic spines of Purkinje cells (29). The expression of myosin V during the early phases of development of the chick nervous system suggests that myosin Va participates in specific cellular tasks (19). The immunolocalization of myosin Va in mammalian brain from the embryonic to postnatal stage has not yet been described. Since this is a necessary step towards understanding the role of myosin Va in the developing nervous system, we have studied the expression of myosin Va by immunohistochemistry during pre- and postnatal ontogenesis of the rat hippocampus, using an affinity-purified anti-myosin Va antibody.

Material and Methods

Establishment of gestational age

Albino Wistar rats were used and the experiments were approved by and conducted according to guidelines of the Faculty of Medicine of Ribeirão Preto Committee for Animal Care and Use. These guidelines are in accordance with those set by the National Institutes of Health, USA. Rats were housed in plastic cages and maintained in a room under controlled temperature (20 ± 2°C) and illumination (12-h light-dark cycle). Water and laboratory rat chow were given *ad libitum.* Timed pregnancies were obtained by housing 6 females with a male rat overnight. On the following day, the females were checked for vaginal plugs and vaginal smears were obtained. The sperm-positive day was designated as embryonic day 0 (E0). Once paired, presumed pregnant dams were separated from their mates to ensure accurate determination of gestational age.

Tissue processing and microwave antigen retrieval

Pregnant and postnatal rats were deeply anesthetized with Nembutal (45 mg/kg body weight). Brains of E15 to E18 rats were surgically removed from their mothers, and immersion-fixed for 4 h in 50 mM sodium phosphate buffer, pH 7.4, containing 2% (w/v) paraformaldehyde, 75 mM lysine and 10 mM sodium meta-periodate, prepared just before use. Brains of E19 to adult rats were perfusion-fixed through the left heart ventricle in the same fixative for 40 to 60 min, and immersion-fixed for another hour. Tissues were then dehydrated through graded alcohols and xylene, and embedded in paraffin. Five-micrometer sections were cut and mounted on gelatin-chromalumn-coated glass slides. Tissue sections were incubated at 56°C for 2 h to insure adherence of the tissue to the slides. Deparaffinized sections were hydrated and incubated in 50 mM sodium phosphate buffer, pH 7.4, containing 0.3% (v/v) H_2O_2, for 15 min at room temperature to block endogenous peroxidase.

Retrieval of antibody immunoreactivity was done by incubating tissue sections in 10 mM sodium citrate buffer, pH 6.0, for 15 min in a microwave oven (Sharp model RB-4A33, with an internal capacity of 34 L and a working frequency of 2.45 GHz), which was used at the maximum nominal power, 900 W. To assure that the tissue was entirely covered by the fluid, the Coplin jars containing the slides were placed inside larger plastic jars filled with the same solution. After microwave treatment, slides were allowed to cool. Sections were then incubated in 0.1 M Tris-glycine buffer, pH 7.4, for 30 min. To minimize non-specific adsorption of antibodies, tissue sections were incubated for 4 h in 20 mM sodium phosphate buffer, pH 7.4, containing 0.45 M sodium chloride, 0.2% (v/v) Triton X-100, 5% (w/v) non-fat dry milk and 15% (v/v) normal goat serum (blocking buffer) (30).

Immunohistochemistry

Hippocampal sections were incubated overnight with the affinity-purified, rabbit anti-myosin Va antibody 32a (14) or with anti-myosin Va antibody 32a pre-adsorbed against myosin Va (control), both diluted 1:100 (v/v) in blocking buffer, or with rabbit anti-bovine glial fibrillary acidic protein (GFAP; Dako, USA), diluted 1:2000 (v/v) in blocking buffer. After incubation, sections were rinsed with 20 mM sodium phosphate buffer, pH 7.4, containing 0.45 M sodium chloride and 0.2% (v/v) Triton X-100. The detection of anti-myosin Va and anti-GFAP antibodies was carried out using a biotinylated swine anti-rabbit IgG (Dako) diluted 1:200 (v/v), followed by detection of the biotinylated second antibody using the ABC kit (Vectastain Elite Kit, Vector, USA), according to the manufacturer protocol. The peroxidase reaction was developed using 3,3'-diaminobenzidine tetrahydrochloride (Pierce, USA) as the chromogen. The reaction was carried out for 12 min and stopped with distilled water. After dehydration, sections were cleared in xylene and coverslipped with Permount (Thomas, USA). Negative controls included the use of anti-myosin Va antibody preadsorbed with myosin Va protein (30), the substitution of anti-myosin Va and anti-GFAP (Dako) primary antibodies by non-immune serum, and the omission of the primary antibodies. All incubations

were carried out at room temperature. Sections were analyzed using an Olympus BX60 (Japan) light microscope. Micrographs were obtained using Ektachrome 64TPro film (Kodak, USA), an Olympus PM C35DX camera, and an Olympus PM30 exposure control. The film was developed using an AT6-2500 automated processor. The slides were then digitalized using a ProPalette 8000 scanner (Polaroid, Japan). Digital images were processed using the Photoshop software version 7.01 (Adobe, USA) so that contrast was changed by +15 and brightness from 54 to 47% for all images. Micrographs were printed using a Phaser 780 printer (Tectronics, USA).

Results

The hippocampal formation comprises: i) the dentate gyrus, which appears as a "C"-shaped trilaminar cortical structure consisting of the molecular layer, the granular layer that contains mainly granule cells, and the hilus, a polymorphic cell layer that contains several neuronal types; ii) the hippocampus proper, which is subdivided into cornu ammonis (CA) CA1, CA2 and CA3 fields; iii) the subicular complex, and iv) the entorhinal cortex.

The hippocampus of E17 rat embryos contains two well-defined zones, one of differentiating and the other of proliferating cells (Figure 1A). Cells in the fimbria, dentate notch and differentiating zone of the prospective hippocampus exhibited a strong myosin Va staining (Figure 1B). In contrast, proliferating cells in the hippocampal neuroepithelium did not express myosin Va (Figure 1B). Many GFAP-immunoreactive-like fibers were observed, mainly in the most lateral portion of the hippocampus, including the neuroepithelial region and the dentate notch (Figure 1C).

Myosin Va was strongly expressed in large cells with clear nuclei in the differentiating zone of the primitive hippocampus proper (E18) that resembled pyramidal neurons (Figure 2). Some of these cells were spindle-shaped, exhib-

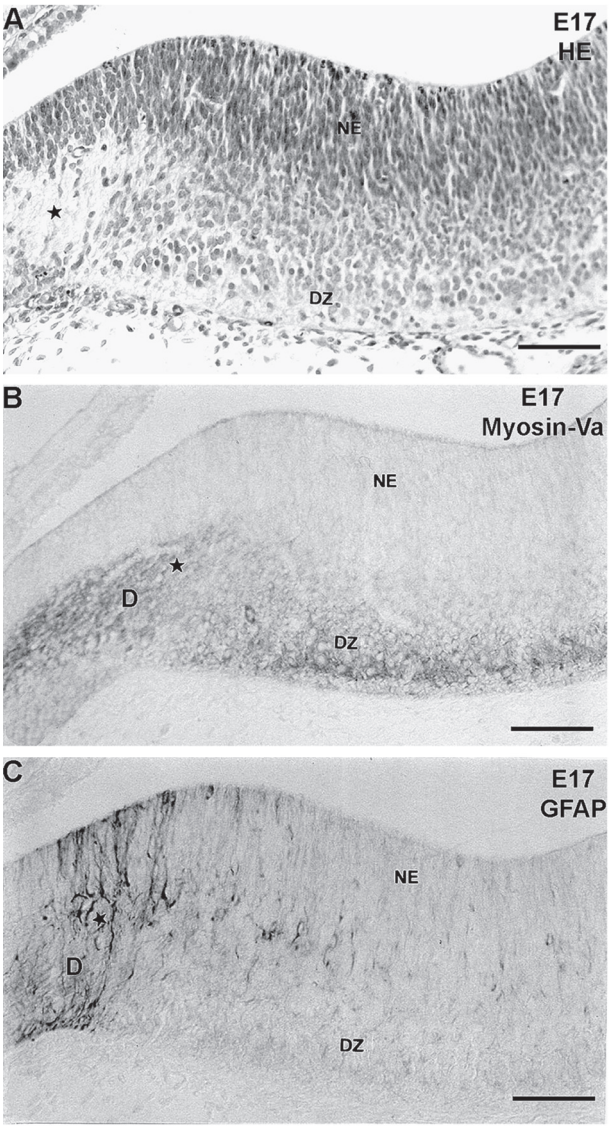

Figure 1. Adjacent coronal sections (5 µm) of a rat hippocampus on embryonic day 17 (E17) stained with hematoxylin and eosin (A); labeled with anti-myosin Va antibody (B); labeled with anti-glial fibrillary acidic protein (GFAP) antibody (C). Note in Panel B that cells in the neuroepithelium (NE) are not expressing myosin Va, in contrast to cells present in the differentiating zone (DZ) and dentate notch (D), which show a strong staining. Note an intense glial fiber staining in the dentate notch (Panel C). The star indicates the same location in all sections. Bar = 50 µm.

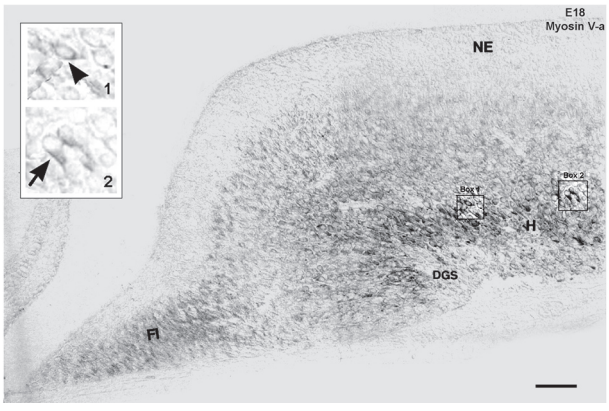

Figure 2. A coronal section (5 µm) of a rat hippocampus on embryonic day 18 (E18) showing the expression of myosin Va in the primitive hippocampus proper (H). Some myosin Va-stained cells show a characteristic migratory profile (inset, arrowhead in box 1), whereas others exhibit the characteristic cell morphology of differentiating pyramidal cells (inset, arrow in box 2). Granule cell progenitors present in the secondary dentate matrix (DGS) express much less myosin Va than cells in the primitive hippocampus proper. NE = neuroepithelium; FI = fimbria. Bar = 50 µm.

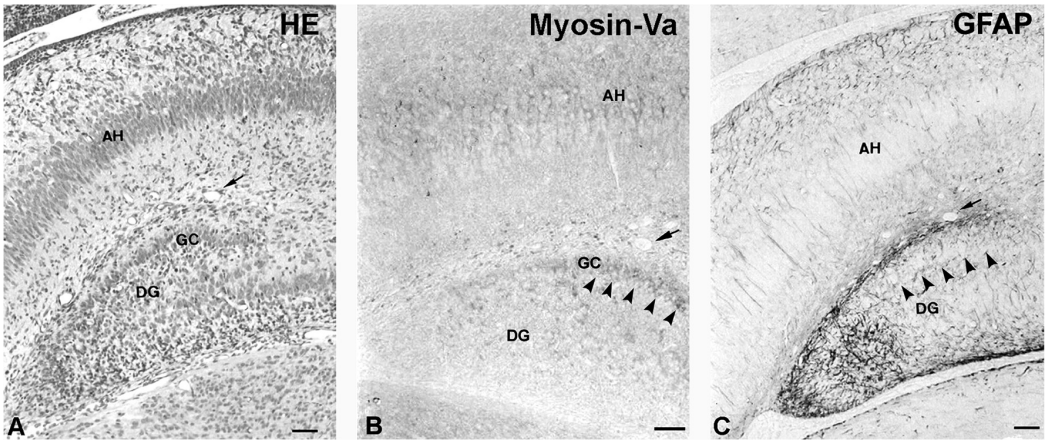

Figure 3. Adjacent coronal sections (5 µm) of a rat hippocampus on prenatal day 21 were stained with hematoxylin-eosin (A), labeled with anti-myosin Va antibody (B), or labeled with anti-glial fibrillary acidic protein (GFAP) antibody (C). Note the expression of myosin Va in granule cells (GC, indicated by arrowheads) in the supra-pyramidal blade. These cells are located above the proliferative region. Note that in the dentate gyrus (DG) and in Ammon's horn (AH), cells that are myosin Va-positive (Panel B) appear to be GFAP-negative (arrowheads indicate GC localization in Panel C). The arrows point to the same blood vessel in A-C. Bar = 50 µm.

iting a migratory profile (arrowhead, inset 1), while others were more triangular-shaped (arrow, inset 2), resembling differentiating pyramidal cells. On the other hand, myosin Va was barely detectable in the secondary dentate matrix of the E18 hippocampus, a region of neuronal proliferation (Figure 2).

By E21 the supra-pyramidal blade of the dentate gyrus was delineated, as shown by hematoxylin-eosin staining (Figure 3A), and this region was myosin Va immunoreactive (Figure 3B), but was GFAP-negative (adjacent section in Figure 3C). At E21, the cytoplasm of the CA1 pyramidal cells was stained for myosin Va (Figures 3B and 4A). Many GFAP-positive fibers were observed in the secondary dentate matrix, in a layer above the supra-pyramidal blade, and as radially oriented fibers passing through Ammon's horn (Figure 3C). At this developmental stage, cells located in the proliferative region that underlies the supra-pyramidal blade, and in the prospective infra-pyramidal blade were myosin Va-negative (Figure 3B).

During the transition from pre- to postnatal (P) hippocampal development, E21 to P2, myosin Va expression increased in the CA3 field, such that at P2 CA3 pyramidal cells were found near the polymorphic layer (Figure 4B), whereas at E21 myosin Va-positive pyramidal cells did not occupy the whole CA3 region (Figure 4A). Anti-myosin Va antibody stained the cytoplasm of many rounded cells with clear nuclei in all CA fields, but staining of pyramidal cell processes was especially observed in the CA2 field (Figure 4B). Fibers in the fimbria at P2 were strongly stained by anti-myosin Va (Figure 4B), whereas there was no fimbria staining at E21 (Figure 4A). At the P2 stage, myosin Va was expressed in the supra-pyramidal blade and in the curving dentate gyrus that projects towards the infra-pyramidal blade (indicated by arrowheads in Figure 5).

At stage P7, both the supra- and infra-pyramidal blades

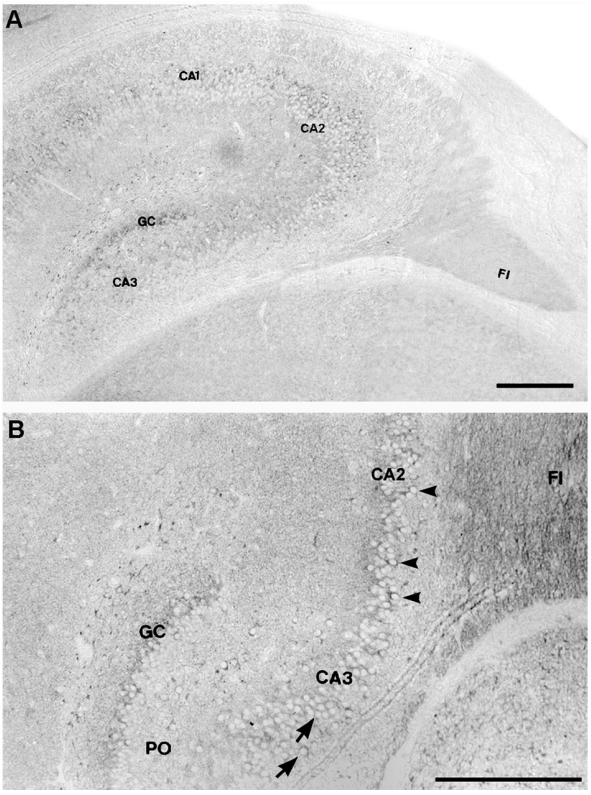

Figure 4. Coronal sections (5 µm) of a rat hippocampus on embryonic day 21 (E21, A) and postnatal day 2 (P2, B) probed with anti-myosin Va antibody. Note in Panel A that pyramidal cells located in the cornu ammonis (CA1, CA2 and CA3) regions are expressing myosin Va. In P2 (Panel B), myosin Va-positive pyramidal cells in the CA3 region (arrows) reached the polymorphic layer (PO). The cytoplasm and processes of pyramidal cells in CA2 were strongly stained by anti-myosin Va (arrowheads). Myosin Va staining of fimbria (FI) fibers was strong at P2, but was not detected at E21. GC = granule cells. Bar = 50 µm.

of the dentate gyrus expressed myosin Va, but the later generated infra-pyramidal blade cells presented more intense staining with the anti-myosin Va antibody than the supra-pyramidal cells (Figure 6A). At P12, cells in the infra- and supra-pyramidal blades exhibited a similar staining intensity (Figure 6B). This pattern of staining by anti-myosin Va antibody was also observed in the adult hippocampus (Figure 6C). During postnatal ontogenesis of the hippocampus proper, the expression of myosin Va by pyramidal cells in the CA3 region increased from E21 (Figure 4A) up to P7 (Figure 7B), and the staining of dendritic processes was more conspicuous by P7. After P7, myosin Va expression in pyramidal cells changed to the adult pattern, characterized by staining of the cytoplasm, of a few dendritic poles and dendrites, and unstained nuclei (Figure 7C).

The fimbria of the mature brain is composed of hippocampal afferent and efferent fibers. It only begins to be a distinctive region in E16 rat embryos (8). From its appearance at E16 and up to the adult, the fimbria expressed myosin Va (Figures 2, 7D-F). At P2, a massive fiber tract immunoreactive to anti-GFAP (Figure 7D) was observed. A similar staining pattern for myosin Va was seen in an adjacent section (Figure 7A). As the fibers became mature and began to be replaced by astrocytes (Figure 7E,F), myosin Va immunoreactivity changed to a weak, finely punctate staining (Figure 7B,C), as can be seen at P7 and in adult sections probed with anti-myosin Va and anti-GFAP.

Discussion

The prenatal pyramidal cells of Ammon's horn and the postnatal granule cells of the dentate gyrus derive from two different germinative matrices, the periventricular neuroepithelium (7,9,10), and the subgranular zone of the dentate gyrus itself (8), respectively. The precursors of the subgranular zone neurons migrate from the ventricular neuroepithelium (8). The present report shows that myosin Va exhibits a gradient of expression that appears to accompany the formation of the hippocampal dentate gyrus and Ammon's horn (5,8-10,31).

Myosin Va was not detected in proliferative zones

The germinal matrix of the hippocampus is a medially situated, convex neuroepithelial lobule of the lateral ventricle that is divisible into three morphogenic components. These components, from dorsal to ventral, are the putative ammonic neuroepithelium, the putative primary dentate neuroepithelium, and the putative fimbrial glioepithelium. The progenitors present in the neuroepithelium proliferate and migrate to specific structures to form the different fields of the

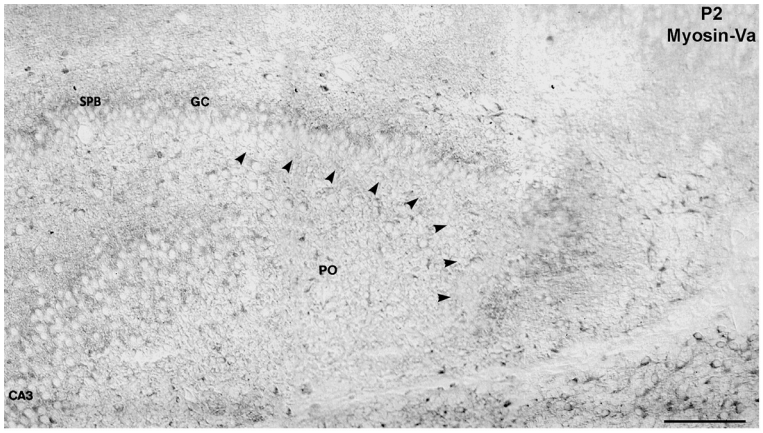

Figure 5. Coronal section (5 µm) of a rat hippocampus on postnatal day 2 (P2) probed with anti-myosin Va antibody. Note that the granule cells that are myosin Va-positive are located in the supra-pyramidal blade (SPB), and start curving down to occupy the infra-pyramidal blade (indicated by arrowheads). GC = granule cell layer; PO = polymorphic cell layer; CA3 = cornu ammonis CA3 region. Bar = 50 µm.

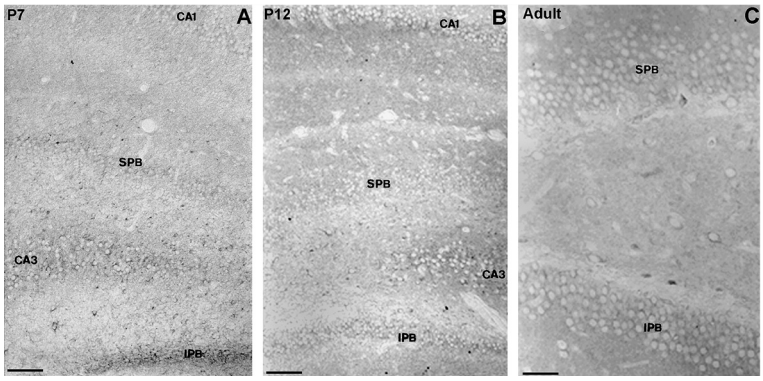

Figure 6. Coronal sections (5 µm) of a rat hippocampus at postnatal days 7 (P7, A) and 12 (P12, B), and at adult stage (C) were probed with anti-myosin Va antibody. Note that the granule cells present in the infra-pyramidal blade (IPB) of a P7-hippocampal section (A) demonstrate a stronger staining than those in the supra-pyramidal blade (SPB). In the rat hippocampus at P12 (B) and in the adult phase (C), note that the intensity of the myosin Va expression in the granule cells present in the SPB and IPB is about the same, and lower than in the IPB at P7. CA1 and CA3 = cornu ammonis CA1 and CA3 regions, respectively. Bar = 50 µm.

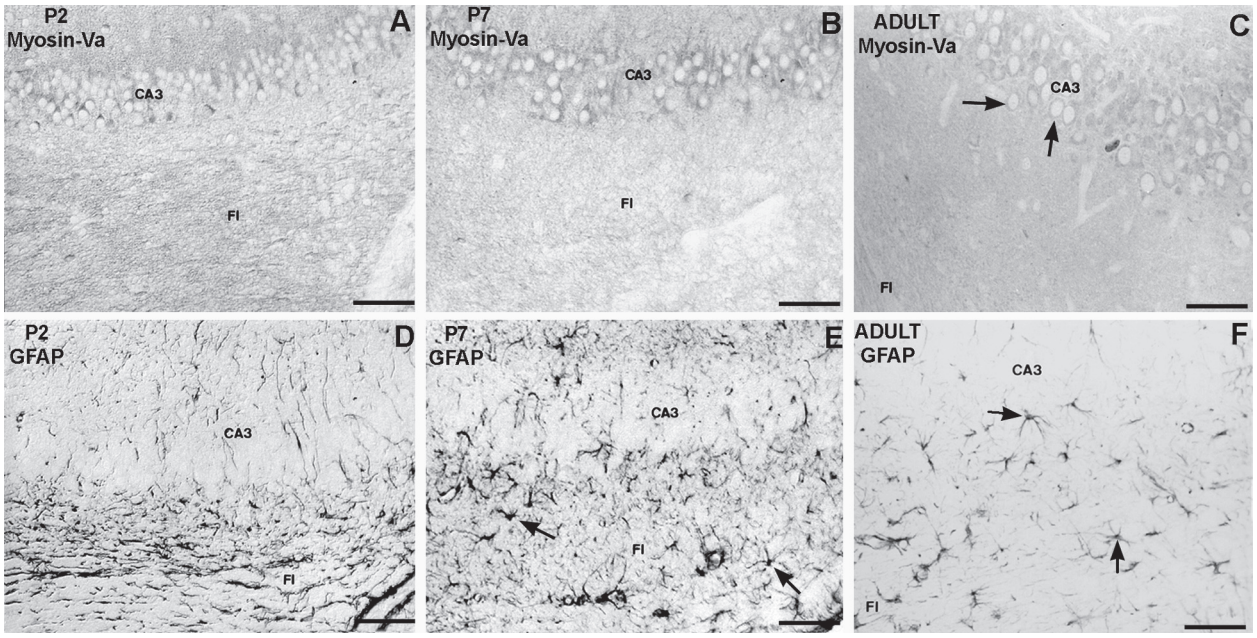

Figure 7. Adjacent coronal sections (5 µm) of a rat hippocampus on postnatal days 2 (P2, A and D), 7 (P7, B and E) and in the adult phase (C and F) probed with anti-myosin Va (A, B and C) or with anti-GFAP (D, E, and F) antibodies. In P2, note that fibers in both sections, A and D, respectively, are strongly labeled and have a parallel orientation in the fimbria. Myosin Va-positive pyramidal cells seen in A, B, and C are GFAP-negative, as shown in D, E, and F. Note that the fiber staining pattern of fimbria (FI) by anti-GFAP has changed from P2 to adult (Panels D-F) such that the predominance of GFAP-positive fibers has been largely and progressively replaced by glial cell bodies and their processes in the adult stage (arrows in E and F). The fimbria, stained with anti-myosin Va, presents a finely punctate and diffuse staining. Arrows in C point to adult neurons that express myosin Va. GFAP = glial fibrillary acidic protein; CA3 = cornu ammonis CA3 region. Bar = 50 µm.

hippocampus. In addition to the duplication and separation of chromosomes, mitosis is characterized by disassembly, partitioning, and reorganization of intracellular membranous organelles. Myosin Va was demonstrated to be located at the centrosome in several cultured mammalian cells at all stages of the cell cycle, suggesting that myosin Va could play a role during cell division (32). However, myosin Va was not detected in hippocampal proliferative matrices, namely the neuroepithelium and the secondary dentate matrix of the dentate gyrus at any age studied here. Although the resolution of the method used here is not appropriate to identify the centrosome and the mitotic spindle, it is clear that the hippocampus germinative matrices do not express myosin Va in the cytoplasm at levels comparable to other developing hippocampal structures.

Differential expression of myosin Va in granule cells of the dentate gyrus during hippocampal development

The expression of myosin Va in granule cells became apparent after they had migrated from the secondary dentate matrix to the supra-pyramidal blade of the dentate gyrus, and began to differentiate. During the formation of the outside-in gradient within the dentate gyrus (10), myosin Va was expressed primarily in the granule cells that had migrated to the external limb of the supra-pyramidal blade of the dentate gyrus. Thus, myosin Va could be detected in cells that apparently have migrated away from the proliferative zone, but not in the proliferative zone itself. The granule cells of the supra-pyramidal blade are generated and start differentiating before those in the infra-pyramidal blade (8-10). At day P2, the expression of myosin Va became evident in differentiated cells in the infra-pyramidal blade, in accordance with the gradient of cell generation and differentiation from the supra- to the infra-pyramidal blade (5,8-10). At age P7, the infra-pyramidal blade of the dentate gyrus showed stronger staining than the supra-pyramidal blade. In the adult, there was no appreciable difference in the weak myosin Va staining between the infra- and supra-pyramidal blades, in agreement with the findings reported by Tilelli et al. (22). Taken together, the data presented here and the above discussion suggest that myosin Va is developmentally regulated during the ontogenesis of the hippocampal granule cells.

Myosin Va is expressed in both undifferentiated and differentiated pyramidal cells

The CA fields of the hippocampus are anatomically

and neurochemically distinct, and appear to make different contributions to the role of the hippocampus in learning and memory (33). The development of normal hippocampal circuitry and functions also depends on the correct division of the hippocampal anlage into its component CA fields. In the first step of this process, distinct populations of hippocampal cells must be specified to follow a particular developmental program, and thereby take on a particular CA field identity.

Throughout hippocampal development, pyramidal cells are generated between E15 and E19 following a subicular-to-dentate morphogenetic gradient (9). Myosin Va expression was observed in differentiating pyramidal cells as early as E17. These cells were localized to the differentiating zone of the future CA region. As the pyramidal cells differentiate they start to spread out first forming the CA3 region and then forming the CA1 region. The myosin Va expression shown here follows the subicular-to-dentate morphogenetic gradient (9), where pyramidal cells populate first the CA3 region and then continue by populating regions CA2 and CA1, respectively. The expression of myosin Va in pyramidal cells of the different hippocampal subfields varies during differentiation, suggesting that myosin Va is under developmental control.

Myosin Va expression in fimbria fibers

The fimbrial glioepithelium is situated between the primary dentate neuroepithelium and the tip of the hippocampal rudiment. The fimbrial glioepithelium is clearly present by day E15, two days before the fimbria becomes a distinct fiber tract. As the fimbria emerges, cells of the putative glial matrix migrate into it (8). At E15 we were not able to detect myosin Va in the fimbrial glioepithelium. However, as soon as the fimbria became a distinct fiber tract, expression of myosin Va was evident, suggesting that this motor protein might be involved in its formation and/or function. As the fimbrial structure becomes more mature it continues to express myosin Va. A comparison of developing hippocampal sections labeled with anti-myosin Va and anti-GFAP antibodies showed that at the beginning of hippocampal development, when the fimbria is mainly constituted of fibers, both proteins presented basically the same staining pattern. As the fibers start to be replaced by mature astrocytes, myosin Va expression loses its fiber-like pattern, and the staining becomes finely punctate and diffuse. This finding suggests that myosin Va might participate in the changes that occur during fimbria development.

Is myosin Va developmentally regulated?

The development and maintenance of neuronal morphology involve a set of cytoskeleton proteins, which include tubulin, microtubule-associated proteins, intermediate filament proteins, and actin. These various filamentous components interact to maintain the elaborate three-dimensional shape of neurons and to play a dynamic role in axonal transport and synaptic plasticity. As such, their expression is critical to the structural and functional changes that occur during neuronal development. In particular, actin is a component of the cytoskeleton that is essential for motility of the growth cones (34). In contrast, the only form of actin detected in migratory neurons is cortical actin, a ring of which is seen in the cell soma (35,36). Migratory neurons (11) characteristically exhibit a microtubule system that forms a “cage” around the nucleus (35), and a basal body (37) that projects microtubules into the highly motile leading process (36,38,39). Vesicles move along this microtubule system. A cessation of vesicle movement accompanies a halt of migration (36).

The association of myosin Va with organelle movement in axons (26) suggests that myosin Va may have a functional role in the transport of materials packed in organelles, and in organelle localization itself. Since these processes take place in differentiating neurons and glial cells, and myosin Va occurs in both cell types (14), it is possible that myosin Va can participate in neural cell differentiation. A second process that requires force generation is cell migration. Neurons navigate through a crowded terrain, and have to squeeze between fibers and cells during migration to attain their final positions (11). Changes that occur in both cell shape and position require force generation.

The results presented here suggest that the expression of myosin Va is developmentally regulated. First, the absence of the expression of myosin Va in proliferative areas suggests that it is not up-regulated during cell division in neural embryonic tissue as seen for cells in culture (32). Second, myosin Va expression by pyramidal and granule cells at specific regions and times that include processes of cell differentiation and migration, suggests that myosin Va can participate in the dynamic cellular rearrangements occurring during morphogenesis. Third, its increased expression in granule and pyramidal cells clearly undergoing differentiation, followed by its decrease after differentiation, suggests a role in cell maturation. In conclusion, the expression of myosin Va was more intense in differentiating than in proliferating and in already differentiated granule and pyramidal cells, and in developing neural tracts, but was not expressed in proliferating cells, suggesting that myosin Va is developmentally regulated and can play a role in hippocampal ontogenesis.

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