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Original Research Article

Expression of dendrin, neurofilament and glial fibrillary acidic protein in the brain of the dogfish *Scyliorhinus canicula* L.

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Abstract

Background and purpose: *Dendrin is a brain and renal protein that is supposed to be involved in cytoskeletal modifications at the synapse and a part of the slit diaphragm and podocytes. Here, we aimed to investigate dendrin expression in dogfish brain since this newly discovered protein was never reported in fish. We compared the expression of dendrin to those of glial (GFAP) and neuronal (NF) proteins, which have already been described in the dogfish brain.*

Materials and Methods: *Histological and immunofluorescent techniques were performed on tissue samples. The obtained data were statistically analyzed.*

Results: *Our results have shown that dendrin is expressed in all observed parts of the dogfish brain. In the forebrain, both observed parts (telencephalon and olfactory lobes) expressed dendrin. Regarding the percentage area of dendrin expression, it is expressed more in olfactory lobes than in the telencephalon. Compared with GFAP and NF expression, the expression of dendrin significantly differs in both parts of the forebrain. The highest dendrin expression was noticed in the midbrain. In dogfish midbrain, the difference in expression of dendrin in comparison to those of GFAP and NF was even more significant. The percentage area of dendrin expression in the hindbrain (cerebellum and medulla oblongata) was smaller than those in the forebrain and midbrain, contrary the percentage area of intermediate filaments GFAP and NF were significantly higher.*

Conclusion: *These results are the first report on dendrin expression in the dogfish brain opening the path for future studies on its role and function.*

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INTRODUCTION

Dendrin is a hydrophilic and proline-rich protein known as a brain and renal protein because of its specific expression in these two organs. It was originally found in the rat brain and reported as a brain-specific protein *(1)*; and very soon positioned exclusively in forebrain structures and hippocampus *(2)*. Neuner-Jehle et al. *(1)* reported two variants (81 kDa and 89 kDa) of dendrin in the rat brain on both immunoblots and brain tissue sections using affinity-purified polyclonal rabbit anti-dendrin antibodies. Both proteins were found in the rat forebrain areas, and it was noticed that mRNA and protein concentrations of dendrin are differentially downregulated by extending the period of wakefulness *(1)*. Dendrin is encoded by a somatodendritic mRNA and

localized in dendritic spines *(1,2)*. A recent study (3) has shown, for the first time, the localization of dendrin in rats' dorsal horn neurons, where its expression in the nociceptive areas of the spinal cord suggests its possible role in pain perception. As a brain protein, dendrin has been associated with the actin cytoskeleton, interacting with α-actinin in postsynaptic dendritic spines and thus defined as an important component of cytoskeletal modifications at the synapse (4,5). This could suggest that dendrin has an important role in synaptic plasticity, and as a postsynaptic component spatially synthesized, dendrin has a significant impact in modulating synaptic cytoskeleton (6). Kremerskothen et al. (6) have shown that dendrin interacts with two postsynaptic proteins, α-actinin and scaffolding protein MAGI/S-SCAM, suggesting how dendrin links postsynaptic lattice and microfilaments of the spine. Another scaffold protein named KIBRA is also highly expressed in the brain; it regulates memory and learning processes, and specifically binds with dendrin. Inhibition of this binding can disrupt spatial learning and memory and consequently induce severe disorders such as Tourette syndrome and Alzheimer's disease (7). An increased rate of dendrin mRNA was noticed in the prefrontal cortex of the adolescent rat brain during acute nicotine uptake (5). The brain response to nicotine uptake includes plasticity-related changes at the synapse since dendrin, a postsynaptic protein, is an important component of cytoskeletal modifications at the synapse *(5,6)*. Previous studies *(8–11)* have shown that drugs' influence on brain physiology is also age dependent. Adolescents and adults show similar ways of early response genes activation after drug exposure, but the distribution and intensity of induction are different *(8–11)*. The study on dendrin expression in the rat's brain provided by Schochet et al. *(5)* has shown that dendrin expression is higher in the adolescent brain compared to adults. Furthermore, they emphasized that nicotine-induced increases in dendrin found in adolescent rats were not found in adult rats. On the contrary, cocaine uptake and exposure to environmental changes have not expressed dendrin increases in both adolescent and adult rats *(5)*. Interestingly, some other markers of synaptic plasticity, such as Arc and Homer 1A, were strongly induced in the rat's brain when exposed to a novel environment *(12)*. After first being discovered in the brain *(1)*, dendrin was also described in the renal tissues *(4,13–18)*. It was noticed that dendrin has a significant role in glomerular physiology and pathology *(14)*. In kidneys, dendrin is localized at the slit diaphragm in podocytes, and it was found that in pathological conditions, dendrin translocates to the nucleus of injured podocytes, causing podocyte apoptosis *(13,15)*. Moreover, blocking dendrin function could delay the onset of proteinuria and podocyte loss *(16)*. Since dendrin is a newly found protein, the data on how it functions in the neurons are still sparse. Kremerskothen et al. *(6)* suggested that dendrin influences retrograde signalling from the synapse to the nucleus. A recent study *(3)* on the increase of dendrin expression induced by stress suggested that the difference in its expression could be sex-specific and explain the sex-related pain hypersensitivity.

Neurofilaments (NF) and glial fibrillary acidic protein (GFAP) are cytoskeletal structural components that belong to the class of intermediate filaments. Intermediate filaments are cytoskeletal components that, due to their average diameter of 10 nm, differ from microfilaments and microtubules. NFs are type IV intermediate filaments found in high concentrations along the axons of vertebrate neurons *(19)*. GFAP is a type III intermediate filament protein expressed by numerous glial cells of the central nervous system *(19,20)*. NFs are considered among the most prominent and highly conserved structures in the nervous system *(21)*. NFs are known to be needed for the proper neuronal function and to support and control axon diameter and axonal transport. Due to their extremely rich phosphorylation potential, NFs are probably involved also in regulatory mechanisms *(22)*. GFAP is one of the first detected markers of radial glial cells (RDCs) in early, intermediate, and late embryos of dogfish *Scyliorhinus canicula* L. *(23,24)*. RDCs are supposed to be the predominant glial cells in fish *(24)*. Although the expression and function of NFs and GFAP were previously investigated in vertebrates and fish *(21,23–25)*, findings on dendrin expression in nonmammalian vertebrates are still missing. According to present data, there are no records of dendrin presence in the fish brain. The teleost fish had a very important role during vertebrates' evolution. The fish brain is a complex macroscopic structure *(26)* and has some specific features compared to mammals. Different fish species show different anatomical and morphological characteristics of the brain. However, generally, five brain parts similar to those in mammals can be usually observed: telencephalon (forebrain), diencephalon, mesencephalon (midbrain), metencephalon (hindbrain), and myelencephalon (brain stem) *(27,28)*. Compared to limited adult neurogenesis in mammals, teleost has a great potential to generate new neurons in the adult central nervous system *(29,30)*. The ability to regenerate brain tissue, replace damaged neurons, and produce new ones in adulthood is observed in fish and amphibians *(29–31)*. Several neuronal stem cell sites, especially in the cerebrum and optic tectum, have been found as the most proliferating zones in teleost fish *(28)*. The telencephalon in cartilaginous fish and all other jawed vertebrates is formed by evagination instead of being everted as in teleost fish, and it is especially large compared to other parts of the brain *(27)*. The disproportionate growth of this part of the brain and delayed neurogenesis in the dogfish *S. canicula*, characterized as precocial fish, could be related to delayed neuron migration and integration in the olfactory bulbs *(32)*. Many similarities between the shark and developing mammalian telencephalon have been established, especially in its dorsal part and interneurons' migratory ways from subpallium to pallium *(33)*. The olfactory bulbs are well-developed structures in all cartilaginous fish, and

their different shape among species is used as a species-determination tool. The sensory ciliated neurons, which project to the main olfactory bulb in tetrapod vertebrates, are missing in sharks. However, crypt sensory neurons and microvillous olfactory receptor neurons are present in the olfactory epithelium of the catshark *S. canicula (24,34)*. The adult neurogenesis in sharks, which implies juveniles and adults, is characterized by high proliferation and could be the reason why the telencephalon is disproportionally large *(35)*. Unlike the cartilaginous fish, in the teleost fish, the mesencephalon is the largest part of the brain *(28,36)*. On its dorsal side is an optic tectum that evaginates into two bilateral optic lobes *(36)*. The optic tectum of the cartilaginous fish is usually considered more homologous with mammals' cerebral cortex because of the multi-layered arrangement of the nerve cells than to those of teleost fish. The most variable parts, both in terms of shape and size, in the chondrichthyan brain are the telencephalon and the cerebellum *(27)*. Among the vertebrates, even the cerebellum differs considerably in size; the layers of the grey matter are well conserved *(37)*. The cell surface of the Purkinje cells in the dogfish cerebellar cortex possesses numerous dendritic spines in secondary and tertiary branches *(38)*. Alvarez-Otero et al. *(39)* found somatic spines on the basal surface of the Purkinje cells, which are very similar to the climbing fibers of teleost which seem to correspond to those found in the developing mammalian cerebellum *(40)*. In all vertebrates, the centers of origin and termination of all cranial nerves are in the brain stem, while in cartilaginous fishes, the brain stem is divided into columns of nerve fibers *(27)*. These columns are longitudinal ridges and grooves of the internal floor and sides of the medulla oblongata, the most posterior part of the brain, which narrows into the spinal cord. Depending on the transmitted information, they are visceral and somatic sensory or motor columns. The most afferent cerebellar fibers originate in the brain stem, and almost all efferent terminates in the brain stem, implying a strong connection between the cerebellum and the brain stem *(27)*.

Regarding these similarities with mammals' brain structure and specificities of the fish brain itself, as well as the fact that dendrin has never been previously reported in the fish brain, we report in the present study the distribution of dendrin in comparison to those of NF and GFAP in the brain of dogfish *S. canicula* L.

MATERIALS AND METHODS

Experimental animals and tissue collection

Ten young dogfish (*S. canicula*) specimens, fresh-caught in the Adriatic Sea near the city of Zadar in June 2021., were used to perform this study. The brain tissue samples of each animal were sectioned at the forebrain (two olfactory lobes and telencephalon), midbrain (two optic lobes), and hindbrain (cerebellum and medulla oblongata) and prepared for histochemical and immunofluorescent analysis.

Histological analysis

The tissue samples were fixed in 10% formalin, dehydrated in an ascending series of ethanol, cleared in xylene, and then embedded in paraffin wax (Paraffin embedding station HistoCore Arcadia H, Leica Biosystems, Germany). 5 µm thick, paraffin sections were cut on a rotary microtome (HistoCore BIOCUT, Leica Biosystems, Germany) and mounted on glass slides. Deparaffinization of the sections were made in xylenes (2x) and rehydration in descending concentrations of ethanol and water. Basic histochemical analysis *(38)* with hematoxylin-eosin staining (H&E) (Hematoxylin M, BioGnost, Croatia; Eosin Y 1% aqueous, BioGnost, Croatia) was used as an orientation technique, in order to define the area of interest in the dogfish brain for further immunohistochemical analysis.

Immunohistochemistry

For the immunofluorescent analysis, the paraffin sections were labelled with primary rabbit polyclonal Anti-Dendrin Antibody (AB15299-I, Sigma-Aldrich) in dilution of 1:200; primary mouse monoclonal Anti-Neurofilament 200kDa Antibody, clone N52 (MAB5266, Sigma-Aldrich) in dilution 1:200 and primary mouse monoclonal Anti-GFAP Antibody (2E1) (sc-33673, Santa Cruz Biotechnology Inc.) in dilution 1:50. After deparaffinization and rehydration, tissue sections were heated in citrate buffer (pH 6.0) for 10 min in a water steamer and afterward, cooled down to room temperature. A blocking buffer (ab 64226, Abcam, Cambridge, UK) was applied for 30 minutes to exclude unspecific staining. Sections were incubated with the primary antibody in a humid chamber overnight. Secondary detection of dendrin was performed using Alexa Fluor® 488-conjugated AffiniPure Donkey Anti-Rabbit IgG (H+L) (711-545-152, Jackson Immuno Research Laboratories Inc.) in dilution of 1:300. Secondary antibody Alexa Fluor® 488 AffiniPure Donkey Anti-Mouse IgG (H+L) was used for detection of NFs and GFAP in dilution 1:300. Tissue sections were incubated with secondary antibody for 60 min in the dark at room temperature. After washing with PBS, the nuclei were stained with DAPI (4', 6-Diamidino-2-Phenylindole, Dihydrochloride) for 1 min. After the final rinsing in the PBS, the sections were mounted in Aqua-Poly/Mount (Polysciences, Europe, Germany). Negative controls included omitting primary antibodies from the staining procedure, which resulted in no tissue staining.

Antibody Characterization

The Anti-Dendrin Antibody (AB15299-I, Sigma-Aldrich) recognizes Dendrin in humans, mice, and rats. This highly specific rabbit polyclonal antibody targets an

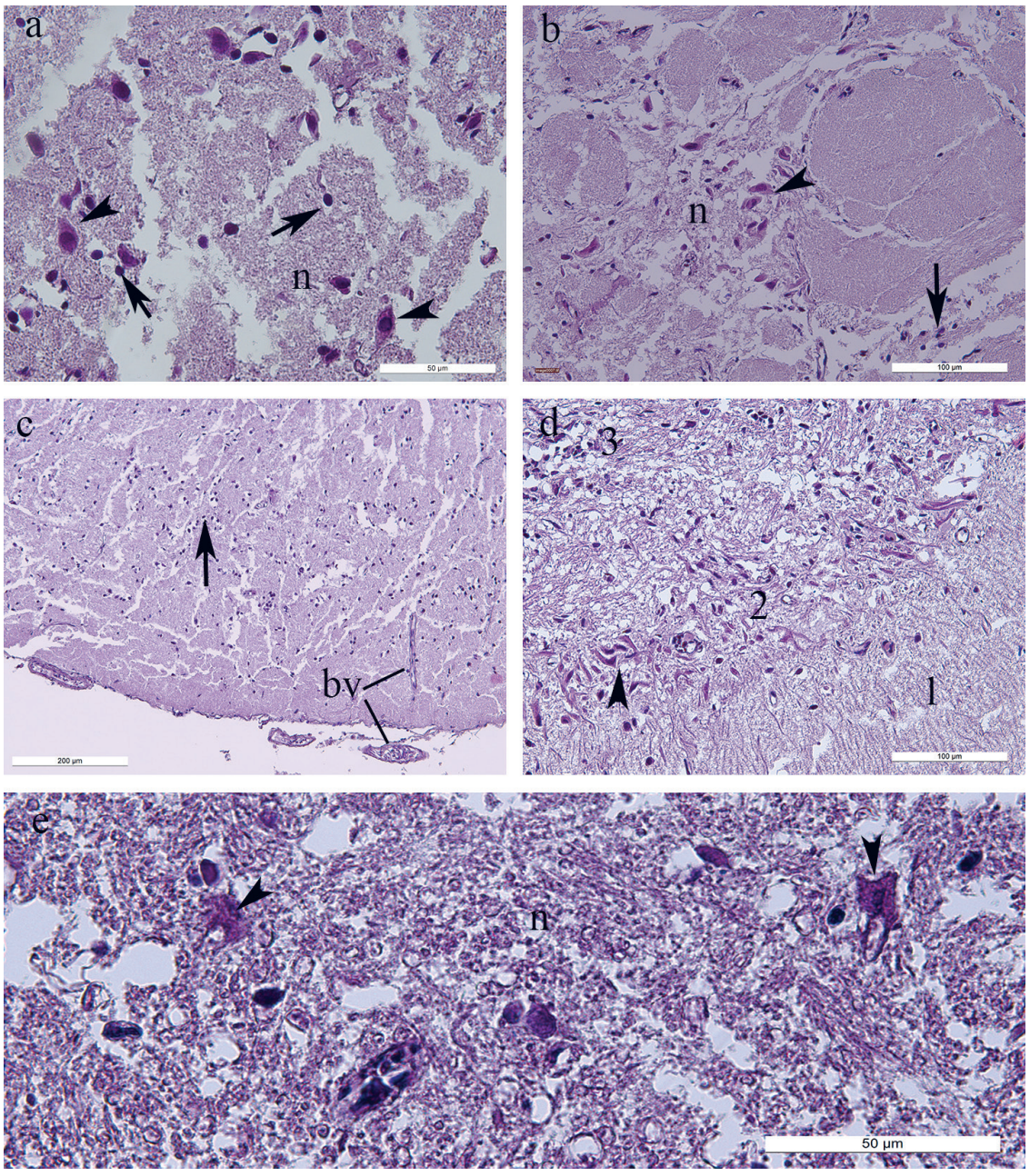

Figure 1. *Morphology of the different brain regions of the dogfish S. canicula. a – telencephalon; neuron (arrowhead); glial cell (arrow); n (neuropil). b – olfactory lobes; neuron (arrowhead); glial cell (arrow); n (neuropil). c – midbrain, glial cells (arrow); bv (blood vessels). d – cerebellum; 1 (outer molecular layer); 2 (middle Purkinje cells layer); 3 (inner granular layer). Note Purkinje cells in the middle layer (arrow). e – brain stem (medulla oblongata); neurons (arrowhead); n (neuropil). H&E.*

epitope of 17 amino acids from the N-terminal region (manufacturer's datasheet).

The Anti-Neurofilament 200 kDa Antibody, clone N52 (MAB5266, Sigma-Aldrich) reacts with the phosphorylated and dephosphorylated H-chain of neurofilament 200kDa (NF-H) in normal tissues/extracts. This primary mouse monoclonal antibody can be used to detect cells of neuronal origin by immunohistochemistry and Western blot (manufacturer's datasheet).

Anti-GFAP Antibody (2E1) (sc-33673, Santa Cruz Biotechnology Inc) is raised against spinal cord homogenate of bovine origin. This mouse monoclonal IgG2b κ GFAP antibody is recommended for the detection of GFAP of the mouse, rat, and human origin (manufacturer's datasheet).

Alexa Fluor® 488-conjugated AffiniPure Donkey Anti-Rabbit IgG (H+L) (711-545-152, Jackson Immuno Research Laboratories Inc.) reacts with whole molecule rab-

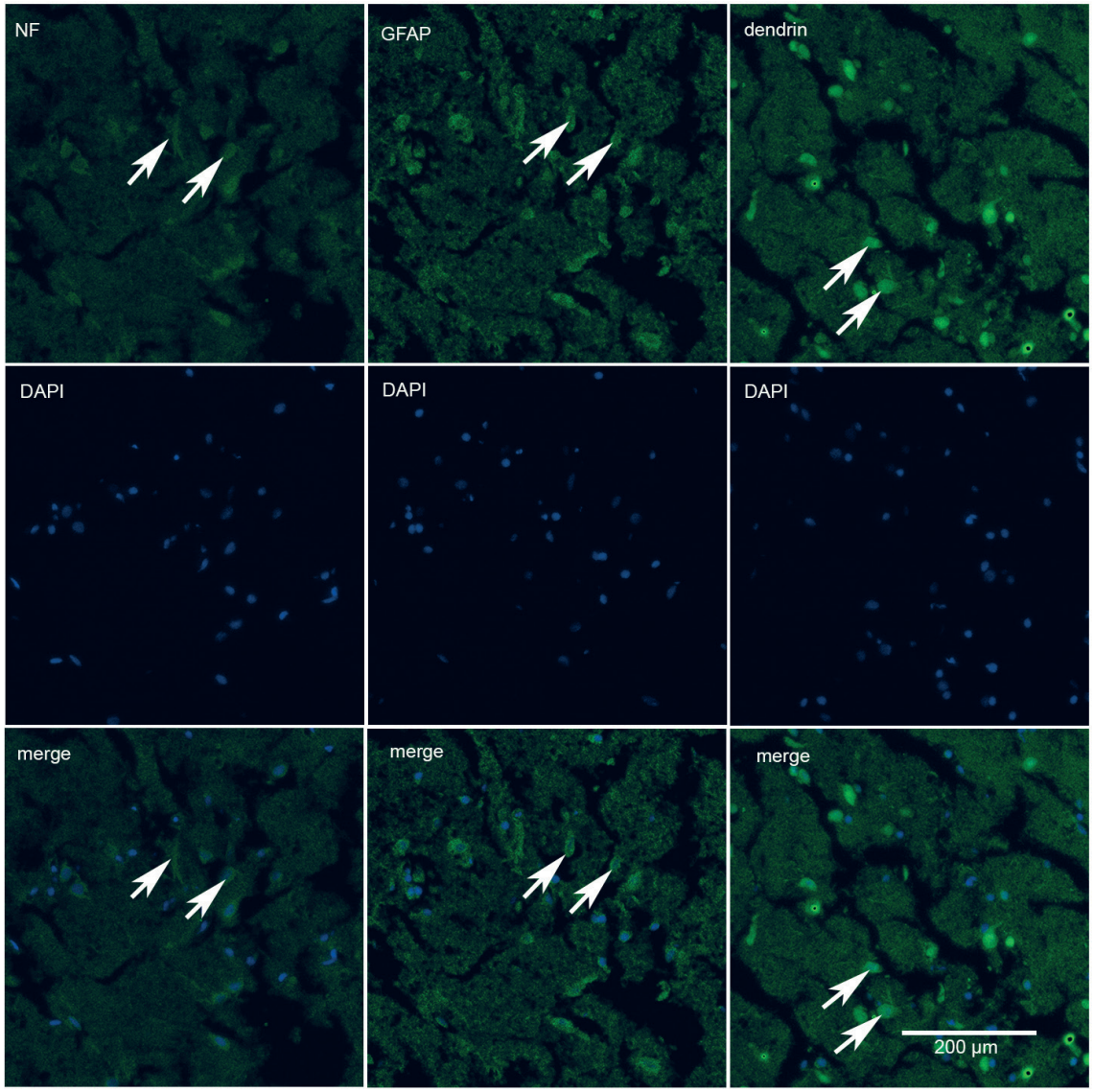

Figure 2. *Expression of NF, GFAP and dendrin in the telencephalon of the dogfish. Arrows represent localization of NF, GFAP and dendrin.*

bit IgG and with the light chains of other rabbit immunoglobulins. No antibody was detected against non-immunoglobulin serum proteins. The whole IgG form of antibodies is suitable for the majority of immunodetection procedures and is the most cost-effective (manufacturer's datasheet).

Data acquisition and statistical analysis

The sections stained with H&E were observed using a Leica DM 3000 LED microscope (Leica Microsystems, Germany) equipped with a digital camera Leica DMC4500 (Leica Microsystems, Germany). These photomicrographs served as an orientation in the brain tissues for further analysis. The microscope software platform was Leica Application Suite (LAS) 4.9 (Leica Microsystems, Germany).

For the immunofluorescent analysis, the slides were observed using an epifluorescence microscope Olympus BX51 (Olympus Corporation, Tokyo, Japan). Figures were captured with a digital camera Nikon DS-Ri1 (Nikon Corporation, Tokyo, Japan) and prepared using Adobe Photoshop (Adobe, San Jose, CA, USA).

For quantitative analysis of dendrin, NFs and GFAP expression, Image J software (National Institutes of Health, Bethesda, MD, USA) was used. Ten photomicrographs (20x objective magnification) were transferred to 8-bit and thresholded. Image J software was used to detect the percentage of dendrin, NFs and GFAP immunoreactive areas and fluorescence intensity.

For statistical analysis a paired t-test was used to examine the differences in the expression of dendrin, NFs and GFAP proteins in the dogfish brain regions. The data

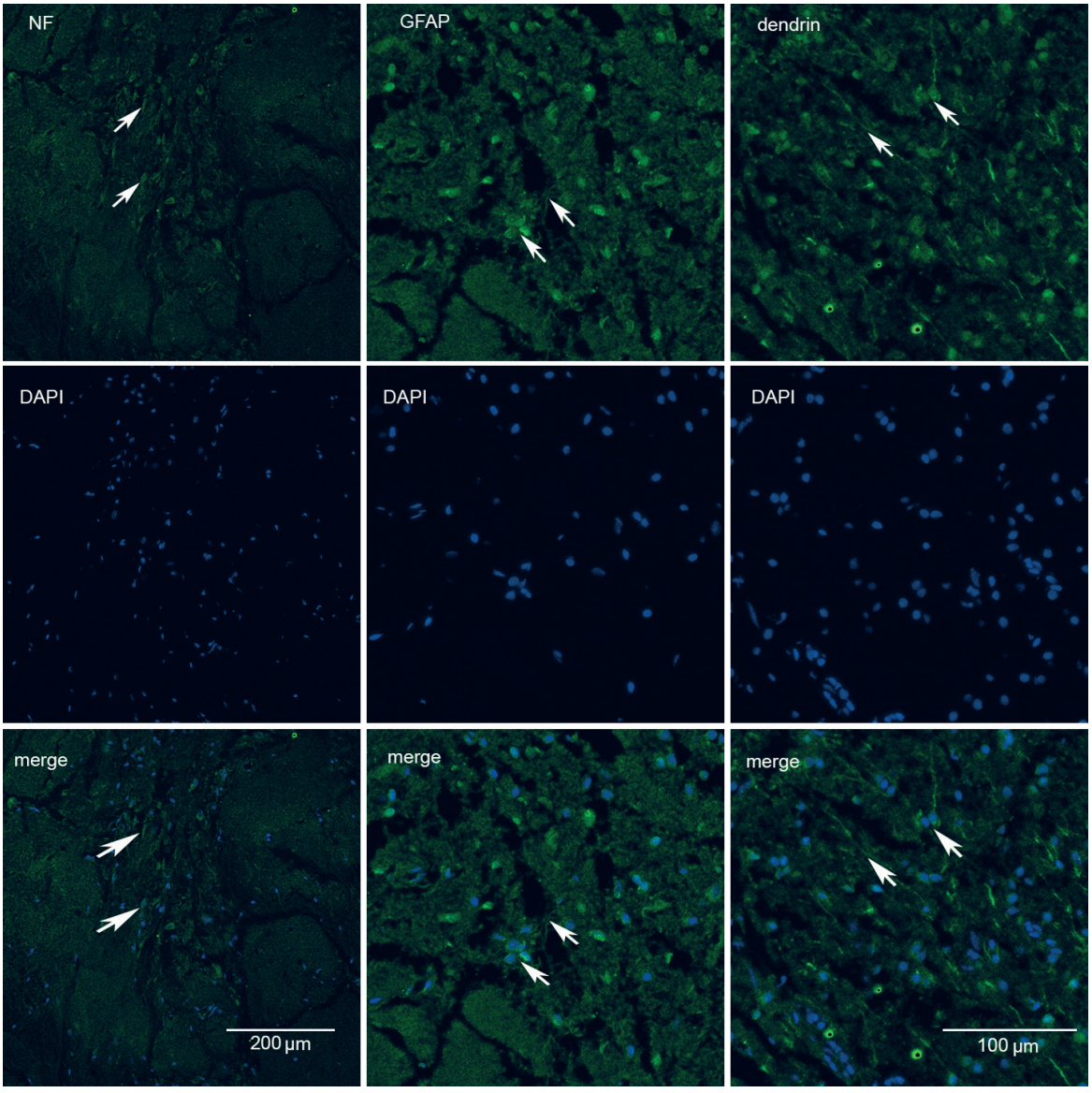

Figure 3. *Expression of NF, GFAP and dendrin in the olfactory lobes of the dogfish. Arrows represent localization of NF, GFAP and dendrin.*

were presented as mean±standard deviation (M±SD). Statistical significance was considered at $p < 0.05$. The analysis was performed in GraphPad Prism 8.0.1 (GraphPad Software, Inc., CA, USA).

RESULTS

Basic histology of the dogfish brain

The forebrain (telencephalon and olfactory lobes), midbrain (mesencephalon), and hindbrain (cerebellum and medulla oblongata) were defined as three basic regions of the adult elasmobranches brain. A diffuse cell architecture was noticed in the telencephalon and olfactory lobes of the forebrain. Mostly, there are neuronal and glial cell bodies surrounded by a mass of neuropil composed of a meshwork of axons, dendrites, synapses, glial cell processes, and blood vessels (Figures 1A, B). In the midbrain, bipolar neurons and pyramidal cells are the predominant cells in the inner layers, while in the outer layers, only a few neurons can be found (Figure 1C). In the hindbrain, three layers of the cerebellar grey matter were well distinguished: the outer molecular layer, the middle Purkinje cells layer, and the inner granular layer (Figure 1D). The inner granular layer is thick, containing neuronal cells of different sizes and glial cells. The axons of the Purkinje cells also enter this layer. The abundance of the Purkinje cells can be seen in the middle layer. The molecular layer contains numerous small neurons of stellate appearance. The granular and Purkinje layers are mainly composed of granule cells. Cells of different sizes with long, branched dendrites could be found

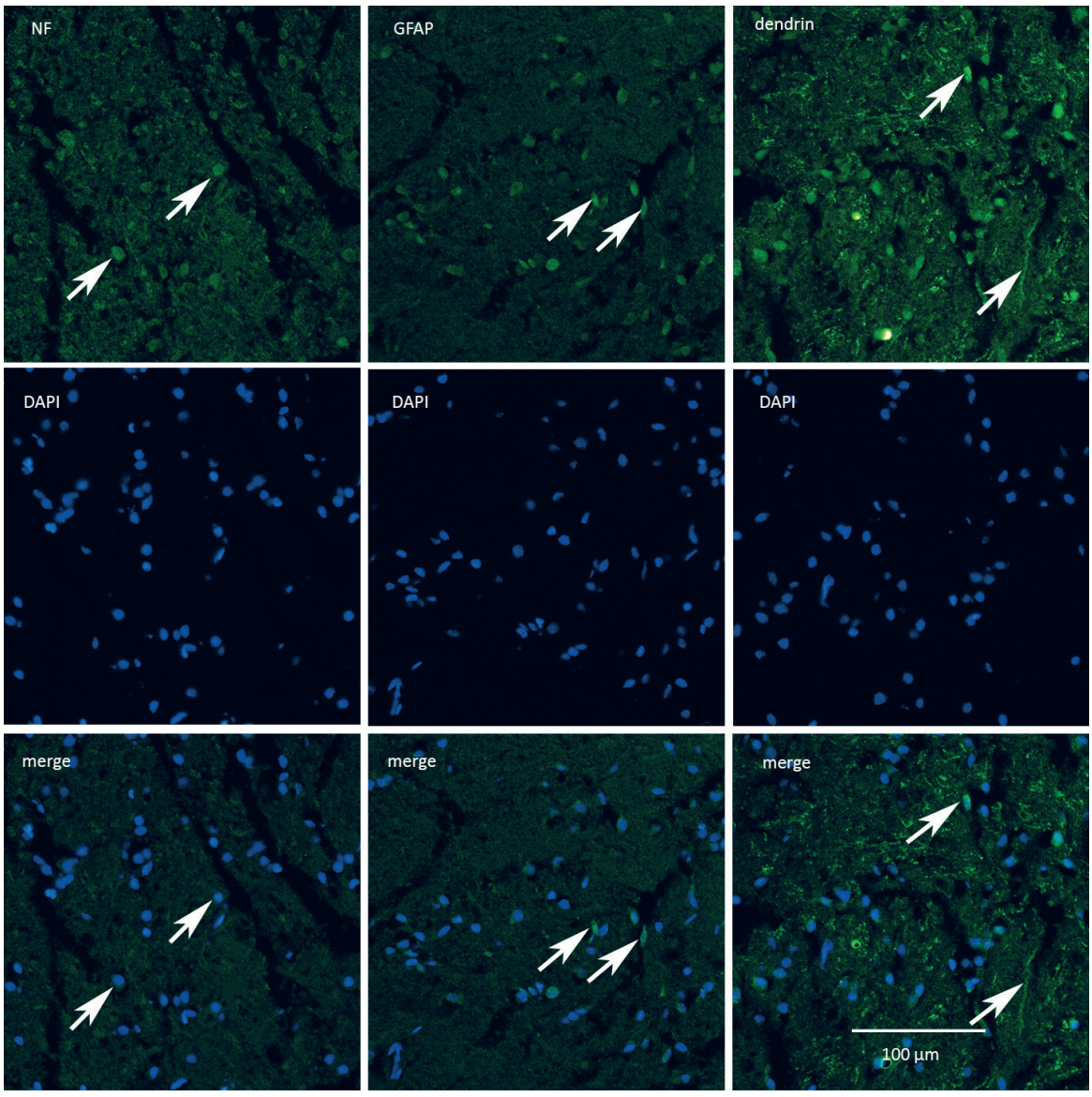

Figure 4. *Expression of NF, GFAP and dendrin in the midbrain of the dogfish. Arrows represent localization of NF, GFAP and dendrin.*

in the brain stem (*medulla oblongata*) of the hindbrain (Figure 1E). Some of these cells contact the blood supply and lie adjacent to large blood vessels or have capillaries entwined around them.

Immunolabelling of dendrin, NF and GFAP in the dogfish brain

Immunolabelling has shown the expression of NF, GFAP, and for the first time, the expression of dendrin in the different regions of the dogfish brain. In the forebrain, in the telencephalon region, dendrin was strongly expressed along the dendrites of neuronal cells, while glial cells were labelled by GFAP (Figure 2). Although a weak expression of NF was observed in the telencephalon (Figure 2), in the total percentage area, its expression was not significant (Figure 7A, $p > 0.05$). In fact, the expression of dendrin significantly differed in comparison to those of GFAP and NF in the telencephalon of dogfish *S. canicula* (Figure 7A, $p < 0.001$). The percentage of dendrin expressed area was noticeably larger than those of GFAP and NF. In the region of olfactory lobes, dendrin was also expressed along the dendrites of neuronal cells, and glial cells strongly expressed GFAP, while the expression of NF was noticeably weak (Figure 3). The dendrin and GFAP expression percentage area in the olfactory lobes were significantly larger than in the telencephalon (Figure 7B, $p < 0.01$).

In the midbrain, the expression of dendrin was even stronger in the dendrites of neuronal cells than those in the forebrain (Figure 4). GFAP expression in the glial cells

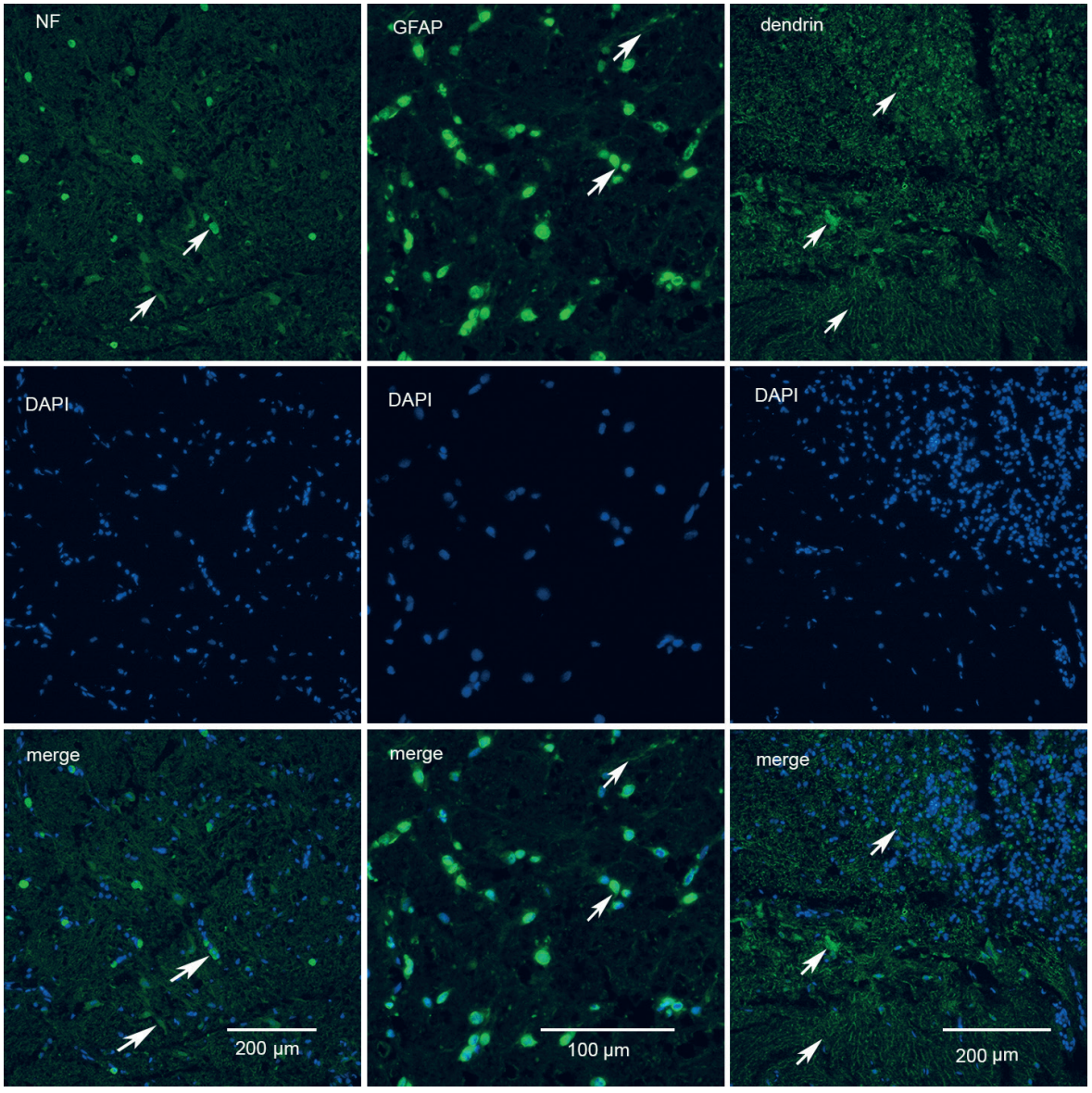

Figure 5. *Expression of NF, GFAP and dendrin in the cerebellum of the dogfish. Arrows represent localization of NF, GFAP and dendrin.*

was weaker than in the forebrain, while expression of NF showed negligible variation (Figure 4). Regarding the percentage area of expression, the expression of dendrin was significantly increased compared to the expression of GFAP and NF (Figure 7C, $p < 0.01$).

Immunolabelling in the cerebellar part of the hindbrain has shown an increased expression of GFAP and NF compared to those in the forebrain and midbrain (Figure 5). The percentage area of GFAP expression in the total area of expression significantly differed from those of dendrin and NFs (Figure 7D). The increased GFAP expression was confirmed by statistical analysis with a significant statistical difference of $p < 0.05$. The expression of dendrin in the cerebellum was similar to those in the anterior regions of the dogfish brain.

Although the expression of dendrin and GFAP in the brain stem was weaker than those in the cerebellum (Figure 6), the percentage area of their expression in the total area of expression was almost the same as in the cerebellum (Figure 7E). Analysis revealed a significantly higher percentage of the GFAP expressed area than those of dendrin and NF (Figure 7E, $p < 0.01$) The expression of NF in the brain stem was very weak and a significant statistical difference in the percentage of its expressed area compared to those of dendrin (Figure 7E, $p < 0.01$) and GFAP was shown (Figure 7E, $p < 0.05$).

When comparing the distribution of all investigated proteins (dendrin, GFAP and NFs) in different regions of the dogfish *S. canicula* brain (Figure 8A-C) it was noticed that dendrin is distributed in all investigated

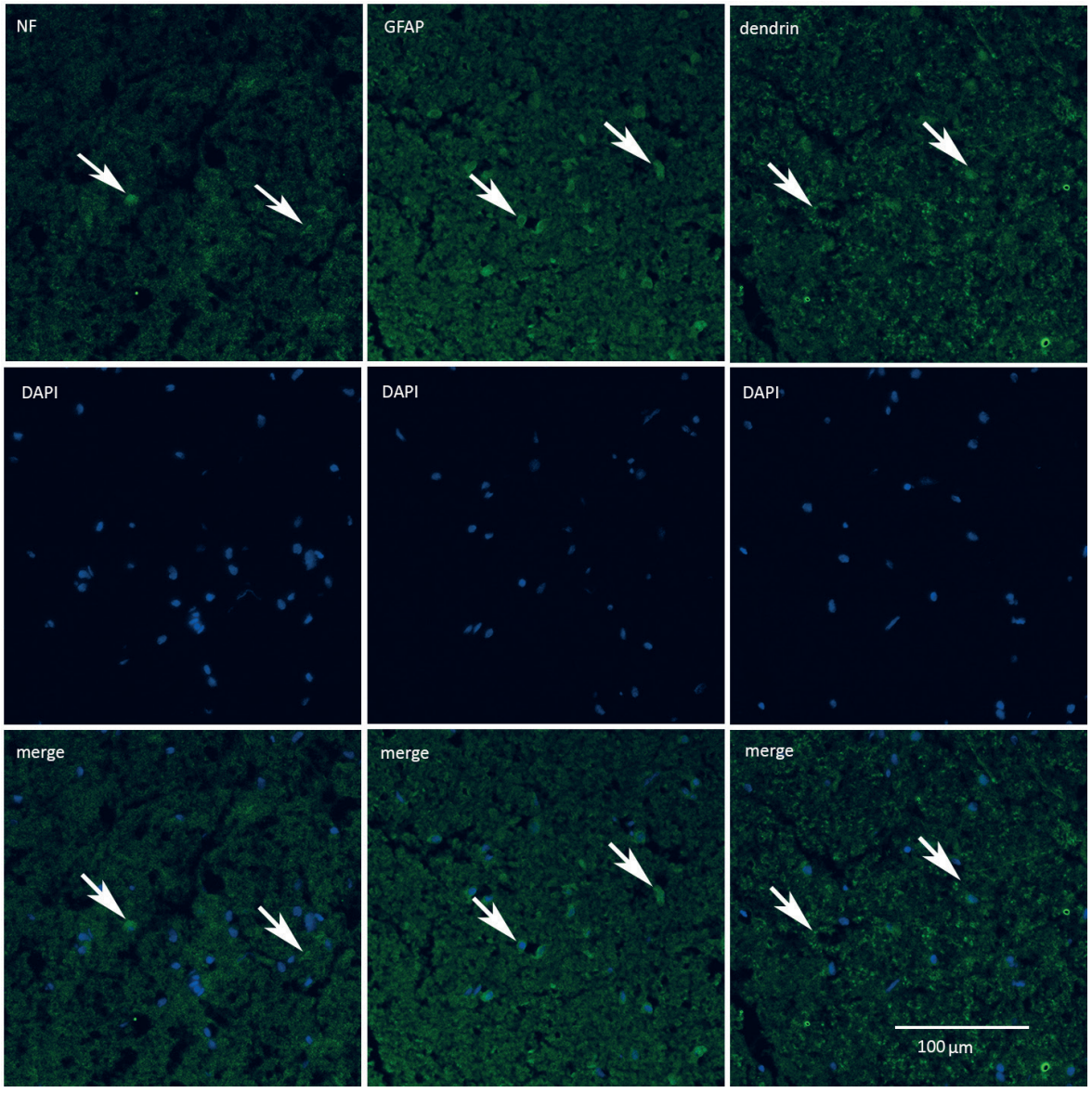

Figure 6. *Expression of NF, GFAP and dendrin in the brain stem of the dogfish. Arrows represent localization of NF, GFAP and dendrin.*

brain regions (Figure 8A). A statistically significant difference in percentage of the dendrin expressed area ($p < 0.01$) was observed between the mesencephalon and medulla oblongata. On the other side, GFAP, despite its distribution in all brain regions, is predominantly distributed in the cerebellum and brain stem with no statistically significant difference (Figure 8B, $p > 0.05$) in comparison to its distribution in the mesencephalon ($p < 0.01$), telencephalon ($p < 0.001$) and lobus olfactorius ($p < 0.01$; $p < 0.001$). Compared to dendrin and GFAP, the NF was poorly distributed in all brain regions (with no significant difference between these brain parts) except the cerebellum were compared to other parts of the brain, a statistically significant increase in its expression was observed (Figure 8C, $p < 0.01$).

DISCUSSION

In the present study, we aimed to investigate dendrin expression in the dogfish brain since this newly discovered protein was never reported in fish. Despite its specific features *(26)*, the brain of cartilaginous fish has many similarities to those of mammals *(27,33)*. For example, the formation of the telencephalon in cartilaginous fish is more similar to those of jawed vertebrates than those of teleost fish *(27,33)*. Regarding the similarities of the cartilaginous fish brain in comparison to those of the mammal's brain, as well as the fact that dendrin as a novel protein was first described in the mammal's brain *(1)*, we investigated the expression and distribution of dendrin in comparison to the expression of glial (GFAP) and neuronal (NF) proteins which have already been described in

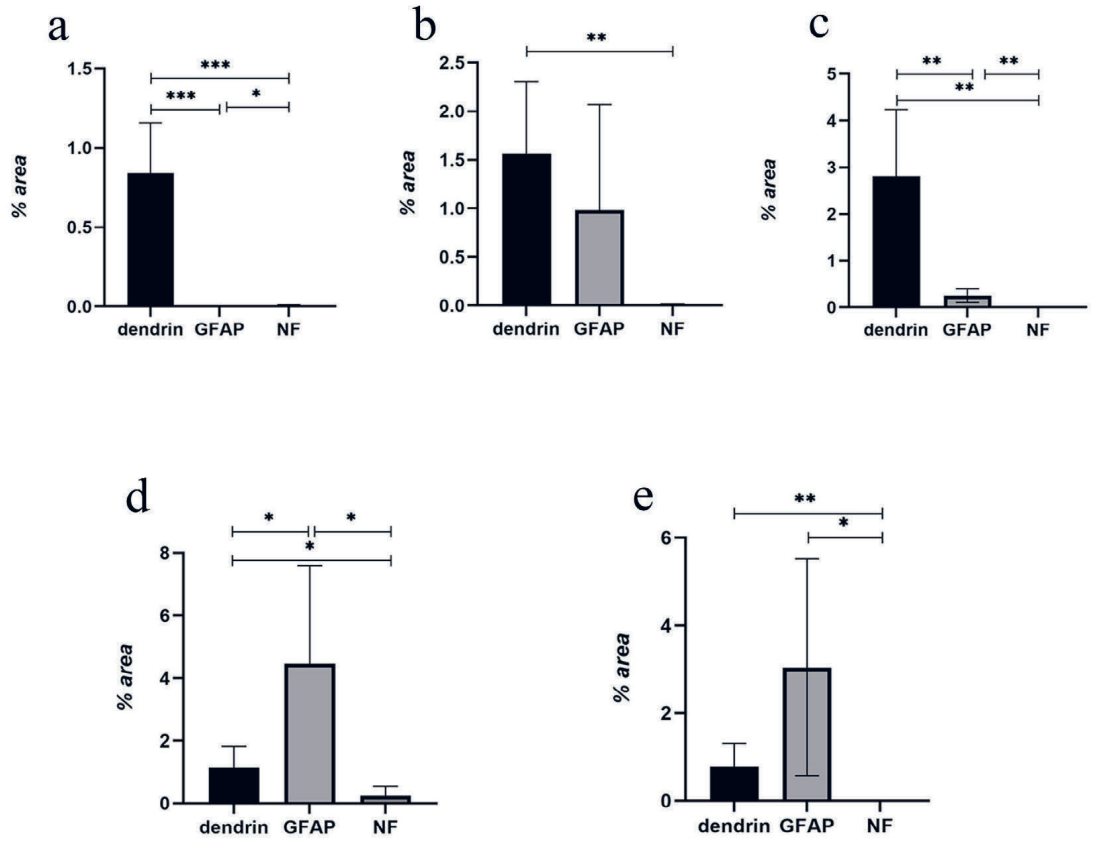

Figure 7. *Percentage area of dendrin, NF and GFAP expression in the: a – telencephalon; b – olfactory lobes; c - midbrain; d – cerebellum and e - brain stem of the dogfish S. canicula. Asterisks denote a statistically significant difference in expression of measured fluorescence percentage area (% area) of dendrin, NF and GFAP. * p < 0.05; ** p < 0.01; *** p < 0.001.*

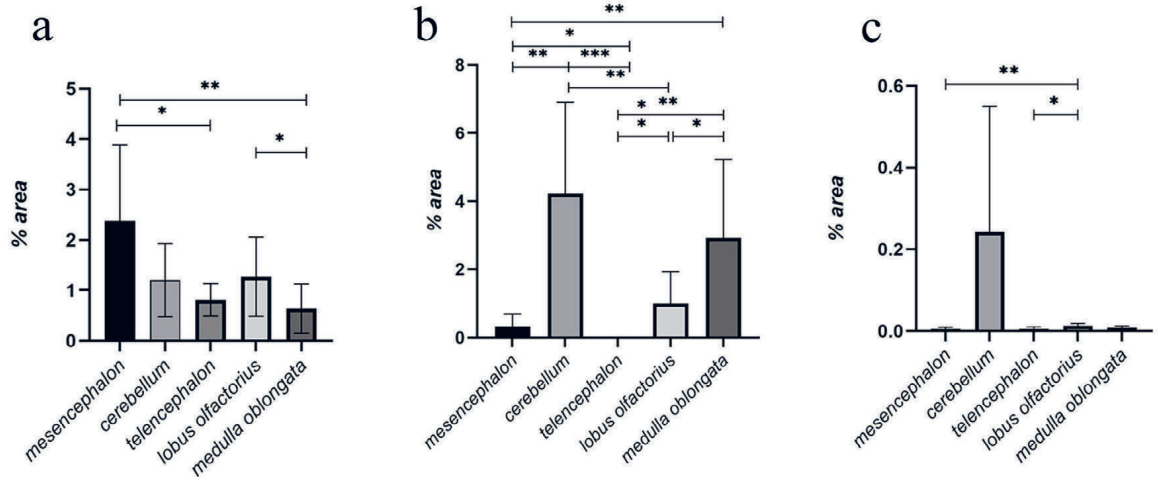

Figure 8. *Distributions of dendrin, GFAP and NF in the different regions of the dogfish brain. Asterisks denote a statistically significant difference in expression of measured fluorescence percentage area (% area) of dendrin (a), GFAP (b) and NF (c) throughout the dogfish brain. * p < 0.05; ** p < 0.01; *** p < 0.001.*

the dogfish brain tissue *(23,41–46)* as well as in the brain of other fish *(21,47–50)*.

Our results have shown that dendrin is expressed in all observed parts of the dogfish brain. In the forebrain, both observed parts (telencephalon and olfactory lobes) expressed dendrin. The same results were reported on rat brain *(2)* where dendrin was also expressed in forebrain structures particularly in neocortex, olfactory bulb, hip-

pocampus, caudate putamen, and limbic system. In rat forebrain dendrin was exclusively expressed in the neuronal cell bodies and their dendrites *(2)*. These findings are actually in accordance with our findings on dendrin in dogfish forebrain. Regarding the percentage area of dendrin expression in the dogfish forebrain, this protein was expressed more in olfactory lobes than in the telencephalon. Compared with GFAP and NF expression, the expression of dendrin significantly differs in both parts of the dogfish forebrain. The highest dendrin expression was noticed in the dogfish midbrain. These results correspond to those of Neuner-Jehle et al. *(1)*. In dogfish midbrain, the difference in expression of dendrin in comparison to those of GFAP and NF was even more significant. The percentage area of dendrin expression in both parts of the hindbrain (*cerebellum* and *medulla oblongata*) was smaller than those in the forebrain and midbrain, while the percentage area of intermediate filaments GFAP and NF were significantly higher in the same area.

But, contrary to our findings, when first characterized in rat's brains, dendrin was not found in the cerebellum, neither in total protein fractions using Western blot nor on brain sections when immunolabelling with polyclonal rabbit anti-dendrin antibody was used *(1)*. According to present data, the findings on dendrin expression in brain, not only in fish but also in the brain of other organisms, are sparse and still remain to be investigated.

Although it is known that dendrin is dendritically translated, its function is largely unknown as well *(51)*. In the brain of mammals, most synapses are located on dendritic spines whose shape can change within seconds due to their microfilament architecture *(52,53)*. Last years, the spatially restricted translation of mRNA in dendrites has been considered as a key mechanism contributing to synaptic plasticity *(54)*. At this moment, with the lack of data on dendrin in fish brain, it is hard to discuss dendrin function in the dogfish brain, we can only assume that it could be at least connected to synaptic plasticity.

CONCLUSION

So far, dendrin has been described as a brain and kidney protein of mammals. Although recently discovered, dendrin is probably a highly conserved protein since it has also been confirmed in Chondrychthyans in the present study. The present study has shown the expression of dendrin in the dogfish brain for the first time. Dendrin has been found in all investigated parts of the dogfish brain, especially in the midbrain, where the expression was even stronger compared to those of GFAP and NF, which were previously described in the dogfish brain as markers for glial and neuronal cells. The present study indicates the presence of dendrin in the dongfish brain, but the role and function of this newly discovered protein still remain to be investigated in the fish brain.

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REFERENCES

1. NEUNER-JEHLE M, DENIZOT JP, BORBELY AA AND MALLET J 1996 Characterization and sleep deprivation-induced expression modulation of dendrin, a novel dendritic protein in rat brain neurons J. Neurosci 46(2): 138–151. https://doi.org/10.1002/(SICI)1097-4547(19961015)46:2<138::AID-JNR2>3.0.CO;2-I
2. HERB A, WISDEN W, CATANIA MV, MARECHAL D, DRESSE A, SEEBURG PH 1997 Prominent dendritic localization in forebrain neurons of a novel mRNA and its product, dendrin. Mol Cell Neurosci 8(5): 367–374. https://doi.org/10.1006/mcne.1996.0594
3. JURIĆ M, BALOG M, IVIC V, BOSKOVIC M, BENZON B, RACETIN A, VUKOJEVIC K, BOCINA I, KEVIC N, RESTOVIC I, SZUCS KF, GASPAR R, HEFFER M, VARI SG, FILIPOVIC N 2021 Increased expression of dendrin in the dorsal horn of the spinal cord during stress is regulated by sex hormones. Neuropeptides 86. https://doi.org/10.1016/j.npep.2021.102126
4. DUNER F., PATRAKKA J, XIAO Z, LARSSON J, VLAMISGARDIKAS A, PETTERSSON E, TRYGGVASON K, HULTENBY K, WERNERSON A 2008 Dendrin expression in glomerulogenesis and in human minimal change nephrotic syndrome. Nephrol, 23(8), 2504–2511. https://doi.org/10.1093/ndt/gfn100
5. SCHOCHET TL, BREMER QZ, BROWNFIELD MS, KELLEY AE, LANDRY CF 2008 The dendritically targeted protein Dendrin is induced by acute nicotine in cortical regions of adolescent rat brain." Eur. J. Neurosci 28(10): 1967–1979. https://doi.org/10.1111/j.1460-9568.2008.06483.x
6. KREMERSKOTHEN J, KINDLER S, FINGER I, VELTEL S, BARNEKOW A 2006 Postsynaptic recruitment of Dendrin depends on both dendritic mRNA transport and synaptic anchoring. J. Neurochem 96(6): 1659–1666. https://doi.org/10.1111/j.1471-4159.2006.03679.x
7. JI ZY, LI H, YANG Z, HUANG X, KE X, MA SH, LIN ZJ, LU YM, ZHANG MJ 2019 Kibra Modulates Learning and Memory via Binding to Dendrin. Cell Rep 26(8): 2064–2077. https://doi.org/10.1016/j.celrep.2019.01.097
8. EHRLICH ME, SOMMER J, CANAS E, UNTERWALD EM 2002 Periadolescent mice show enhanced DeltaFosB upregulation in response to cocaine and amphetamine. J. Neurosci 22: 9155–9159. https://doi.org/10.1523/JNEUROSCI.22-21-09155.2002
9. LESLIE FM, LOUGHLIN SE, WANG R, PEREZ L, LOTFIPOUR S, BELLUZZIA JD 2004 Adolescent development of forebrain stimulant responsiveness: insights from animal studies. Ann. N. Y. Acad. Sci 1021: 148–159 https://doi.org/10.1196/annals.1308.018
10. SCHOCHET TL, KELLEY AE, LANDRY CF 2005 Differential expression of arc mRNA and other plasticity-related genes induced by nicotine in adolescent rat forebrain. Neuroscience 135: 285–297. https://doi.org/10.1016/j.neuroscience.2005.05.057
11. SHRAM MJ, FUNK D, LI Z, LEˆ AD 2007 Acute nicotine enhances c-fos mRNA expression differentially in reward-related substrates of adolescent and adult rat brain. Neurosci 418: 286–291. https://doi.org/10.1016/j.neulet.2007.03.034
12. RAMIREZ-AMAYA V, VAZDARJANOVA A, MIKHAEL D, ROSI S, WORLEY PF, BARNES CA 2005 Spatial exploration-induced arc mRNA and protein expression: evidence for selective, network-specific reactivation. J. Neurosci 25: 1761–1768. https://doi.org/10.1523/JNEUROSCI.4342-04.2005

13. ASANUMA K, AKIBA-TAKAGI M, KODAMA F, ASAO R, NAGAI Y, LYDIA A, FUKUDA H, TANAKA E, SHIBATA T, TAKAHARA H, HIDAKA T, ASANUMA E, KOMINAMI E, UENO T, TOMINO Y 2011 Dendrin Location in Podocytes Is Associated with Disease Progression in Animal and Human Glomerulopathy. Am. J. Nephrol 33(6): 537–549. https://doi.org/10.1159/000327995
14. PATRAKKA J. XIAO Z, NUKUI M, TAKEMOTO M, HE L, ODDSSON A, PERISIC LJ, KAUKINEN, A., AL-KHALILI SZIGYARTO C, UHLE´N M, JALANKO H, BETSHOLTZ C, TRYGGVASON K 2007 Expression and Subcellular Distribution of Novel Glomerulus-Associated Proteins Dendrin, Ehd3, Sh2d4a, Plekhh2, and 2310066E14Rik. JASN 18: 689–697. https://doi.org/10.1681/ASN.2006060675
15. KODAMA F, ASANUMA K, TAKAGI M, HIDAKA T, ASANUMA E, FUKUDA H, SEKI T, TAKEDA Y, HOSOE-NAGAI Y, ASAO R, HORIKOSHI S, TOMINO Y 2013 Translocation of dendrin to the podocyte nucleus in acute glomerular injury in patients with IgA nephropathy. Nephrol 28: 1762–1772. https://doi.org/10.1093/ndt/gfs500
16. WEINS A, WONG JS, BASGEN JM, GUPTA R, DAEHN I, CASAGRANDE L, LESSMAN D, SCHWARTZMAN M, MELIAMBRO K, PATRAKKA J, SHAW A, TRYGGVASON K, HE JC, NICHOLAS SB, MUNDEL P, CAMPBELL KN 2015 Dendrin ablation prolongs life span by delaying kidney failure. Am J Pathol. 185(8): 2143–57. https://doi.org/10.1016/j.ajpath.2015.04.011
17. MIZDRAK M, VUKOJEVIĆ K, FILIPOVIĆ N, ČAPKUN V, BENZON B, GLAVINA DURDOV M 2018 Expression of dendrin in several glomerular diseases and correlation to pathological parameters and renal failure – preliminary study. Diagn13:90 2018) 13:90 https://doi.org/10.1186/s13000-018-0767-z
18. EMPITU MA, MAKINO SI, YAMADA H, KADARISWANTININGSIH IN, AIZAWA M, ASANUMA K 2022 Dendrin's subcellular localization modulates the resilience of podocyte. Kidney Int. Rep 7(2) Supplement S164.
19. MESCHER AL 2013 Junqueira's Basic histology. Text and Atlas. 13th Edition The Mc Graw Hill Companies, pp. 160–187. USA
20. VAN BODEGRAVEN EJ, SLUIJS JA, TAN AK, ROBE PAJT, HOL EM 2021 New GFAP splice isoform (GFAP mu) differentially expressed in glioma translates into 21 kDa N-terminal GFAP protein. FASEB Journal 35(3): e21389 https://doi.org/10.1096/fj.202001767R
21. JACOBS AJ, KAMHOLZ J, SEIZER ME 1995 The single lamprey neurofilament subunit (NF-180) lacks multiphosphorylation repeats and is expressed selectively in projection neurons. Brain Res. Mol. Brain Res 29(1): 43–52. https://doi.org/10.1016/0169-328x(94)00227-6
22. SONSKA A, KUCERA T, ENTLICHER G 2004 Neurofilaments – The intermediate filaments of neural cells. A review. Collecti 69(3): 511–534. https://doi.org/10.1135/CCCC20040511
23. WASOWICZ M, WARD R, REPÉRANT J 1999 An investigation of astroglial morphology in Torpedo and Scyliorhinus. J. Neurocytol. 28: 639–653. https://doi.org/10.1023/a:1007004714712
24. DOCAMPO-SEARA A, SANTOS-DURÁN GN, DIAZ MAR 2019 Expression of radial glial markers (GFAP, BLBP and GS) during telencephalic development in the catshark (Scyliorhinus anicular). Brain Struct 224(1): 33–56. https://doi.org/10.1007/s00429-018-1758-2
25. ARI C AND KÁLMÁN M 2008 Evolutionary changes of astroglia in Elasmobranchii comparing to Amniotes: A study based on three immunohistochemical markers (GFAP, S-100, glutamine synthetase). Brain Behav 71: 305–324. https://doi.org/10.1159/000129654
26. KALMAN M. 1998 Astroglial architecture of the carp (Cyprinus carpio) brain as revealed by immunohistochemical staining against glial fibrillary acidic protein (GFAP). Ital. J. Anat. Embryol 120(5): 409–433. https://doi.org/10.1007/s004290050193
27. SMEETS WJAJ, NIEUWENHYUS R, ROBERTS BL 1983 The central nervous system of cartilaginous fishes. Springer, Berlin
28. HUSSEIN MN, CAO X 2018 Brain anatomy and Histology in Teleosts. BVMJ 35(2): 446–463.
29. ZUPANC GKH AND SIRBULESCU RF 2009 Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish. Eur. J. Neurosci 34(6): 917–929. https://doi.org/10.1111/j.1460-9568.2011.07854.x
30. ZUPANC, G K H 2001 Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish. Brain Behav 58(5): 250–275. https://doi.org/10.1016/j.jphysparis.2008.10.007
31. KIZIL C, KASLIN J, KROEHNE V, BRAND M 2012 Adult neurogenesis and brain regeneration in zebrafish. Dev. Neurobiol 72(3): 429–461. https://doi.org/10.1002/dneu.20918
32. YOPAK KE, LISNEY TJ, COLLIN SP 2015 Not all sharks are "swimming noses": variation in olfactory bulb size in cartilaginous fishes. Brain Structu 220(2): 1127–1143. https://doi.org/10.1007/s00429-014-0705-0
33. QUINTANA-URZAINQUI I. RODRIGUEZ-MOLDES I, MAZAN S, CANDAL E 2015 Tangential migratory pathways of subpallial origin in the embryonic telencephalon of sharks: evolutionary implications." Brain Struct 220(5): 2905–2926. https://doi.org/10.1007/s00429-014-0834-5
34. ZACCONE D, LO CASCIO P, LAURIANO R, PERGOLIZZI S, SFACTERIA A, MARINO F 2011 Occurrence of neuropeptides and tyrosine hydroxylase in the olfactory epithelium of the lesser-spotted catshark (Scyliorhinus canicula Linnaeus, 1758). Acta Histochem 113(7): 717–722. https://doi.org/10.1016/j.acthis.2010.09.010
35. DOCAMPO-SEARA A, PEREIRA-GULDRIS S, SANCHEZ-FARIAS N, MAZAN S, RODRIGUEZ MA, CANDAL E 2020 Characterization of neurogenic niches in the telencephalon of juvenile and adult sharks. Brain Struct 225(2): 817–839. https://doi.org/10.1007/s00429-020-02038-1
36. SPEARE DJ AND FRASCA S Jr 2006 Nervous system. In Systemic Pathology of Fish (H. W. Ferguson, ed.), 2nd ed., pp. 219–43. London, UK: Scotian Press.
37. ANADON R, FERREIRO-GALVE S, SUEIRO C, GRANA P, CARRERA I, YANEZ J, RODRIGUEZ-MOLDES 2009 Calretinin-immunoreactive systems in the cerebellum and cerebellum-related lateral-line medullary nuclei of an elasmobranch, Scyliorhinus canicula. J. Chem. Neuroanat 37(1): 46–54. https://doi.org/10.1016/j.jchemneu.2008.09.003
38. NICHOLSON C, LLINAS R, PRECHT W 1969 Neural elements of the cerebellum in elasmobranch fishes; structural and functional characteristics. In Neurobiology of Cerebellar Evolution and Development (ed. R. Llinas), pp. 215–243. Chicago: AMA-ERF Institute for Biomedical Research.
39. ALVAREZ-OTERO R, REGUEIRA SD, ANADON R 1993 new structural aspects of the synaptic contacts on Purkinje-cells in an elasmobranch cerebellum. J. Anat 182: 13–21.
40. CAJA, SR 1911 Histologie du Systeme Nerveux de l'Homme et des Vertebres, vol. 2, Paris, reprinted by C.S.I.C, Madrid (1952). https://doi.org/10.1097/00005072-199809000-00011
41. KALMAN M AND GOULD RM 2001 GFAP-immunopositive structures in spiny dogfish, Squalus acanthias, a little skate, Raia erinacea, brains:differences have evolutionary implications. Ital. J. Anat. Embryol 204(1): 59–80. https://doi.org/10.1007/s004290100180
42. INOUE Y, SAITO T, ENDO M, HARUTA C, NAKAI T, MORITOMO T, NAKANISHI T 2005 Molecular cloning and preliminary expression analysis of banded dogfish (Triakis scyllia) CC chemokine cDNAs by use of suppression subtractive hybridization.

Immunogenetics 56(10): 722–734.
https://doi.org/10.1016/j.fsi.2007.08.005

43. SUEIRO C, CARRERA I, FERREIRO S, MOLIST P, ADRIO F, ANADON R, RODRIGUEZ-MOLDES, I. 2007 New insights on saccus vasculosus evolution: A development and immunohistochemical study in elasmobranchs. Brain Behav 70(3): 187–204. https://doi.org/10.1159/000104309

44. QUINTANA-URZAINQUI I, RODRIGUEZ-MOLDES I, CANDAL E 2014 Developmental, tract-tracing and immunohistochemical study of the peripheral olfactory system in a basal vertebrate: insights on Pax6 neurons migrating along the olfactory nerve. Brain Struct 219(1): 85–104.
https://doi.org/10.1007/s00429-012-0486-2

45. DOCAMPO-SEARA A, LAGADEC R, MAZAN S, RODRÍGUEZ M, QUINTANA-URZAINQUI I, CANDAL E 2018 Study of pallial neurogenesis in shark embryos and the evolutionary origin of the subventricular zone. Brain Struct 223(8) 3593–3612 https://doi.org/10.1007/s00429-018-1705-2

46. DOCAMPO-SEARA A., CANDAL E, RODRIGUEZ MA 2022 Study of the glial cytoarchitecture of the developing olfactory bulb of shark using immunochemical markers of radial glia. Brain Struct 227(3): 1067–1082. https://doi.org/10.1007/s00429-021-02448-9

47. VELASCO A, BRAGADO MJ, JIMENO D, CAMINOS E, LILLO C, AIJON J, LARA JM 2000 Growing and regenerating axons in the visual system of teleosts are recognized with antibody RT97. Brain Res 88381): 98–106.
https://doi.org/10.1016/S0006-8993(00)02783-9

48. MATHIEU M, MENSAH-NYAGAN AG, VALLARINO M, DOREGO JL, BEAUJEAN D, VAUDRY D, LUU-THE V, PELLETIER G, VAUDRY H 2001 Immunohistochemical localization of 3 beta-hydroxysteroid dehydrogenase and 5 alpha-reductase in the brain of the African lungfish Protopterus annectens. J. Comp. Neurol 438(2): 123–135. https://doi.org/10.1002/cne.1304

49. CHAPMAN GB, TARBOUSH R, CONNAUGHTON VP 2012 The effects of rearing light level and duration differenceson the optic nerve, brain, and associated structures in developing zebrafish larvae: a light and transmission electron microscope study. Anat Rec 295(3): 515–531. https://doi.org/10.1002/ar.22403

50. LIAO ML, PENG WH, KAN D, CHIEN CL 2019 Distribution patterns of zebrafish neuronal intermediate filaments inaa and inab. J. Neurosci 97(2): 202–214. https://doi.org/10.1002/jnr.24347

51. KAWATA A, LIDA J, IKEDA M, SATO Y, MORI H, KANSAKU A, SUMITA K, FUJIWARA N, ROKUKAWA C, HAMANO M, HIRABAYASHI S, HATA Y 2006 CIN85 is localized at synapses and forms a complex with S-SCAM via Dendrin. Biochem. J 139(5): 931–939. https://doi.org/10.1093/jb/mvj105

52. HERING H AND SHENG M (2001) Dendritic spines: structure, dynamics and regulation. Nat Rev. Neurosci. 2(12): 880–888. https://doi.org/10.1038/35104061

53. CARLISLE HJ AND KENNEDY MB (2005) Spine architecture and synaptic plasticity. Trends Neurosci. 28: 182–187.
https://doi.org/10.1016/j.tins.2005.01.008

54. SUTTON MA AND SCHUMAN EM (2005) Local translocation control in dendrites and its rolein long-term synaptic plasticity. J Neurobiol 64: 116–131. https://doi.org/10.1002/neu.20152