Annals of Anatomy 243 (2022) 151955

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

Research article

Synaptophysin is a selective marker for axons in human cutaneous end organ complexes

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ARTICLE INFO

Article history:
Received 10 January 2022
Received in revised form 4 April 2022
Accepted 24 April 2022
Available online 17 May 2022

Keywords:
Synaptophysin
PIEZO2
Meisner corpuscles
Pacinian corpuscles
Merkel cell- neurite complexes
Immunohistochemistry
Human

ABSTRACT

Background: Small clear synaptic-like vesicles fill axon terminals of mechanoreceptors. Their functional significance is controversial and probably includes release of neurotransmitters from afferent axon terminals. Synaptophysin, a major protein of the synaptic vesicle membrane, is present in presynaptic endings of the central and peripheral nervous systems. It is also expressed in mechanosensory neurons which extend into skin forming sensory corpuscles. Nevertheless, synaptophysin occurrence in these structures has never been investigated.

Methods: Here we used immunohistochemistry to detect synaptophysin in adult human dorsal root ganglia, cutaneous Meissner and Pacinian corpuscles and Merkel cell-neurite complexes from foetal to elderly period. Moreover, we analyzed whether synaptophysin co-localizes with the mechano-gated protein PIEZO2.

Results: Synaptophysin immunoreactivity was observed in primary sensory neurons (36 ± 6%) covering the entire soma size ranges. Axons of Meissner's and Pacinian corpuscles were positive for synaptophysin from 36 and 12 weeks of estimated gestational age respectively, to 72 years old. Synaptophysin was also detected in Merkel cells (from 14 weeks of estimated gestational age to old age). Additionally in adult skin, synaptophysin and PIEZO2 co-localized in the axon of Meissner and Pacinian corpuscles, Merkel cells as well as ~~in~~ some axons of Merkel cell-neurite complexes.

Conclusion: Present results demonstrate that a subpopulation of primary sensory neurons and their axon terminals forming cutaneous sensory corpuscles contain synaptophysin, a typical presynaptic vesicle protein. Although the functional relevance of these findings is unknown it might be related to neurotransmission mechanisms linked to mechanotransduction.

1. Introduction

Synaptophysin (SYN), also known as protein p38, is a major integral glycoprotein of the synaptic vesicle membrane encompassing the 8% of total protein (Valtorta et al., 2004; Evans and Cousin, 2005). It is involved in synaptic vesicle formation and recycling (Alder et al., 1992, 1995; Shibaguchi et al., 2000; Daly and Ziff, 2002; Horikawa et al., 2002), synapse formation (Tarsa and Goda, 2002) and synaptic transmission (Valtorta et al., 2004; Kovacs, 2017). Consistently with these functions, SYN is supposedly present at all presynaptic endings and widely distributed in all brain regions (De Camilli et al., 1988). In the peripheral nervous system, SYN has been detected in synaptic profiles of sympathetic and parasympathetic ganglia (Diaz and Diana, 1992; Luckensmeyer and Keast, 1995; Roudenok and Kühnel, 2002), as well as in the enteric nervous system (see Parathan et al., 2020). SYN in rat is also expressed in about ~~the~~ 80% of total DRG neurons ~~in rat~~ (including all large neurons and Aβ-fibers) and afferent fibers to III–IV laminae of spinal

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https://doi.org/10.1016/j.aanat.2022.151955
0940-9602/

dorsal horn (Sun et al., 2006). Most large primary sensory neurons/Aβ-fibers work as low-threshold mechanoreceptors (LTMRs) (Abraira and Ginty, 2013), which extends into skin to form sensory corpuscles or cutaneous end organ complexes (CEOC; Handler and Ginty, 2021). In fact, axons of LTMRs in dermis are associated with either specialized terminal glial cells forming sensory corpuscles (Meissner, Ruffini, and Pacinian corpuscles), either specialized epithelial cells (Merkel cells) forming Merkel cell-axon complexes, or hair follicles cells forming piloneural complexes (Rice and Albercht, 2008; Zimmerman et al., 2014; Cobo et al., 2021; Suazo et al., 2022).

Since LTMRs expressing SYN project into skin, it might be hypothesized that axon terminals of CEOC contain SYN. According to this supposition, Bewick (2015) conducted a superb review highlighting the occurrence of synaptic-like vesicles, synaptic vesicle-associated proteins (including synaptophysin), Ca^{2+}-binding proteins, and a synaptic glutamatergic system in axon terminals of mechanosensory afferents of muscle spindle and lanceolate endings from hairy skin. Furthermore, clear synaptic-like vesicles have been found in axon terminals supplying Meissner and Pacinian corpuscles by using transmission electron microscopy (see Munger and Idé, 1988; Zelená, 1994), as well as reported indirect evidence for glutamatergic neurotransmission in Pacinian corpuscles (Pawson et al., 2007, 2009). Varga et al. (2020) have also recently observed SYN-immunoreactivity in the axon of Pacinian corpuscles.

Interestingly, synaptic-like vesicles in mechanosensory terminals are functionally linked to ion channels involved in mechanotransduction, like degenerin-epithelial Na^+ channel (DEG/ENa$^+$C) subunits or acid-sensing ion channels (ASIC; see for a review Cobo et al., 2020). However, as far as we know the only ion channel that fulfills conditions to be considered as a mechanotransducer is PIEZO2 (Coste et al., 2010), which is present in CEOC (Cobo et al., 2020) including human Merkel cell-neurite complexes, and Meissner and Pacinian corpuscles (García-Mesa et al., 2017; García-Piqueras et al., 2019).

Therefore, the present study was designed to investigate SYN occurrence in the human cutaneous sensory corpuscles and Merkel cell-axon complexes and analyse age-related changes from prenatal to elderly life periods. Moreover, co-localization between SYN and the mechanotransducer ion channel PIEZO2 was examined.

2. Material and Methods

2.1. Materials and tissue treatment

Experiments were conducted on skin samples from the palmar side of the distal phalanx, belonging to the histological collection of SINPOs Research Group at the University of Oviedo (Registro Nacional de Biobancos, Sección colecciones, Ref. C-0001627). Skin pieces were obtained from amputated fingers (n = 12) or necropsies of free neurological-disease subjects (n = 8) within 6–12 h after accident or death at the Department of Pathology of HUCA (Oviedo, Spain). The age range of those subjects was 8–72 years old. Furthermore, same-location skin samples were extracted from either amputated supernumerary fingers (n = 3) or fingers obtained during autopsy of fetuses and perinatally deceased children (n = 34). Fetuses and children age was ranged from 11 WEGA (weeks of estimated gestational age) to 3 years old. These materials proceeded from the Departments of Pathology of Hospital Universitario Central de Asturias (HUCA, Oviedo, Spain), Hospital Universitario Donostia (San Sebastián, Spain), Hospital José Molina Orosa (Lanzarote, Spain) and Complejo Hospitalario Universitario de Salamanca (Salamanca, Spain). On the other hand, human lumbar dorsal root ganglia (DRG; n = 8) were obtained from 4 healthy adult males who died in traffic accidents (age-ranged from 35 to 56 years old), during organ removal for transplantation (HUCA, Oviedo, Spain).

All used tissues were obtained in compliance with the Spanish Law (RD 1301/2006; Ley 14/2007; DR 1716/2011; Orden ECC 1414/2013) and the study was approved by the Ethical Committee for Biomedical Research of the Principality of Asturias, Spain (Cod. CElm, PAst: Proyecto 266/18).

Specimens were fixed in 4% formaldehyde in 0.1 M phosphate buffer saline (pH 7.4) for 24 h, dehydrated and embedded in paraffin. Paraffin-embedded tissues were cut into 10-μm-thick sections perpendicularly to skin surface and mounted on gelatine-coated microscope slides. Presence of sensory corpuscles in skin samples was ensured using haematoxylin-eosin staining.

2.2. Immunohistochemistry

Deparaffinized and rehydrated sections were processed for SYN immunodetection using EnVision antibody complex detection kit (DakoCytomation, Copenhagen, Denmark) and following supplier's instructions. Briefly, endogenous peroxidase activity and non-specific binding were blocked, and sections were then incubated with a mouse monoclonal antibody against SYN (1:200 dilution; clone DAK-SYNAP; DAKO, Glostrup, Denmark; reference M7315) overnight at 4 °C. Subsequently, sections were rinsed, and incubated with Dako EnVision System labeled polymer-HR anti-mouse IgG (DakoCytomation) for 30 min at room temperature. Finally, sections were washed, and immunoreaction visualized using 3–3′-diaminobenzidine as a chromogen. To ascertain structural details, some sections were counterstained with Mayer's haematoxylin, dehydrated, and mounted with Entellan® (Merk, Dramstadt, Germany).

2.3. Double immunofluorescence

Sections were processed for simultaneous detection of SYN and neurofilament proteins (NFP; polyclonal, raised in rabbit; Invitrogen, Massachusetts, USA; reference ab204893, EPR20020), S100 protein (S100P; polyclonal, raised in rabbit; DAKO, Glostrup, Denmark; reference IS504), or PIEZO2 (polyclonal, raised in rabbit; Sigma Aldrich, Saint Louis, MO, USA; reference PA5–72976, amino acid sequence FEDEN-KAAVRIMAGDNVEICMNLDAASFSQHNP). NFP and S100P label axons and lamellar cells respectively, within sensory corpuscles (Vega et al., 2009; Cobo et al., 2021).

Double immunostaining was performed on 10-μm-thick deparaffinized and rehydrated sections. Non-specific binding was reduced by incubation with a solution of 1% bovine serum albumin in tris buffer solution (TBS) for 30 min. Sections were later incubated with a 1:1 mixture of mouse anti-SYN and rabbit anti-NFP antibodies (both 1:100 diluted in the blocking solution), or mouse anti-SYN and rabbit anti-S100 protein antibodies (1:100 and 1:1000 diluted respectively), or mouse anti-SYN and rabbit anti-PIEZO2 antibodies (1:100 and 1:200 diluted respectively) overnight at 4ºC in a humid chamber. After rinsing with TBS, slides were incubated with Alexa fluor 488-conjugated goat anti-rabbit IgG (1:100 diluted in TBS containing 5% mouse serum; Serotec, Oxford, UK), then rinsed again and incubated with Cy™3-conjugated donkey anti-mouse antibody (1:100 diluted in TBS; Jackson-ImmunoResearch, Baltimore, MD, USA) for 1 h. Both steps were performed at room temperature in a dark humid chamber. Finally, sections were counterstained with DAPI (10 ng/ml) to label the nuclei, washed, dehydrated and mounted with Fluoromount Gold (ThermoFisher, Runcoen, UK).

Double immunofluorescence was detected using a Leica DMR-XA automatic fluorescence microscope coupled with a Leica Confocal Software (version 2.5; Leica Microsystems, Heidelberg GmbH, Germany), and captured images were processed using Image J software (version 1.43 g; Master Biophotonics Facility, Mac Master University Ontario; www.macbiophotonics.ca).

For control purposes, representative sections were processed in the same way as described above but either using non-immune

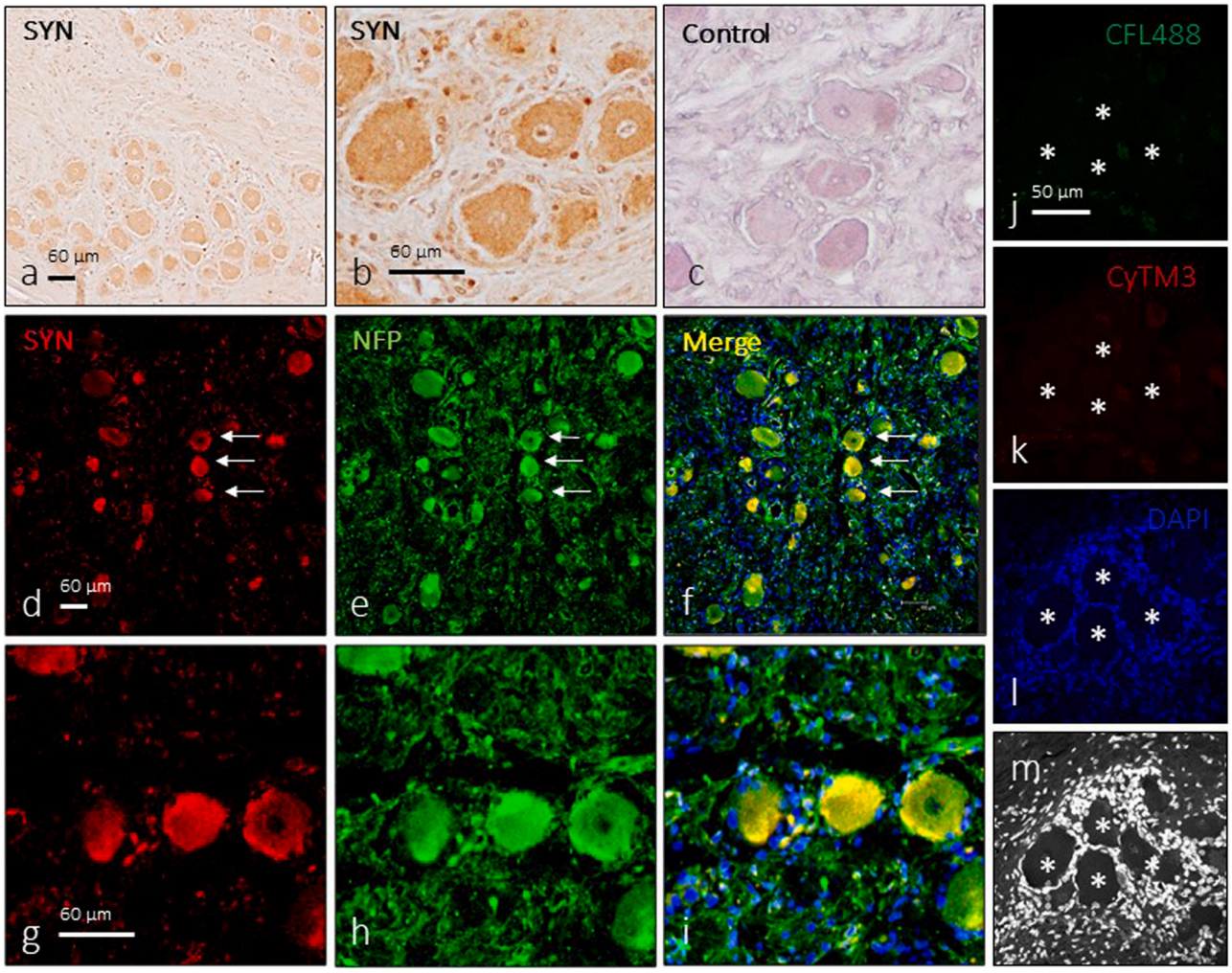

Fig. 1. Immunohistochemical detection of synaptophysin in human lumbar dorsal root ganglia (**a**,**b**) Double immunofluorescence for synaptophysin (SYN, red; **d** and **g**) and neurofilament proteins (NFP, green; **e** and **h**) in human lumbar dorsal root ganglia. SYN co-localized with NFP (yellow; **f** and **i**) in the soma of DRG primary sensory neurons of different sizes. In sections in which the primary antibody was omitted neither immunoreactivity nor immunofluorescence (**j-m**) was observed. **d-f** objective 40X/1.25 oil, pinhole 1.00, XY resolution 156 nm and Z resolution 334 nm; **g-i** objective 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rabbit or mouse sera instead of primary antibodies or omitting primary antibodies when incubation. No positive immunostaining was observed under these conditions (data not shown).

2.4. Quantitative study

A quantitative image analysis was carried out in adult SYN-positive DRG using an automatic image analysis system (Quantimet 550, Leika, QWIN Program). Percentage and size (mean diameter in µm) of immunoreactive neurons were evaluated. Measurements were made on three sections per specimen, 200 µm apart between them to avoid measure the same neuron twice, evaluating five randomly selected fields per section (2.5 mm^2). For evaluation of cell body size just neuronal profiles with apparent nuclei were considered, and neurons were divided into 3 size classes according to their diameter: ≤ 20 µm (small neurons), 21–50 µm (intermediate neurons), and > 50 µm (large neurons).

Moreover, percentage of Meissner and Pacinian corpuscles displaying SYN immunoreactivity was calculated in 10 sections per specimen, 100 µm apart. Total number of sensory corpuscles in an entire section was determined by counting the number of sensory corpuscles displaying S100P-immunoreactivity (for details see García-Piqueras et al., 2019).

3. Results

3.1. Dorsal root ganglia

The first step of this research was to investigate SYN occurrence in DRG. SYN was detected in neurons of all analyzed samples, covering the entire soma area (Fig. 1a and d), as well as in intraganglionic axon profiles (Fig. 1a-b; 1d and 1 g). These SYN-positive neurons were too immunoreactive for NFP (Fig. 1e and h), and both proteins co-localized (Fig. 1f and i). Within the neuronal body, the immunostaining pattern was cytoplasmic, and sometimes observed more remarkably on the periphery, close to the plasma membrane. Contrary, satellite glial cells lacked SYN-immunoreactivity. SYN-positive neuronal bodies were about 36 ± 6% (242/672 evaluations) of total counted neurons and distributed ~~in~~ across the above preestablished size-categories as follows: 11% small neurons (74/672), 23% intermediate neurons (155/672), and 2% (13/672) large neurons. No immunoreactivity nor immunofluorescence for SYN was observed in control sections (Fig. 1c, j-m).

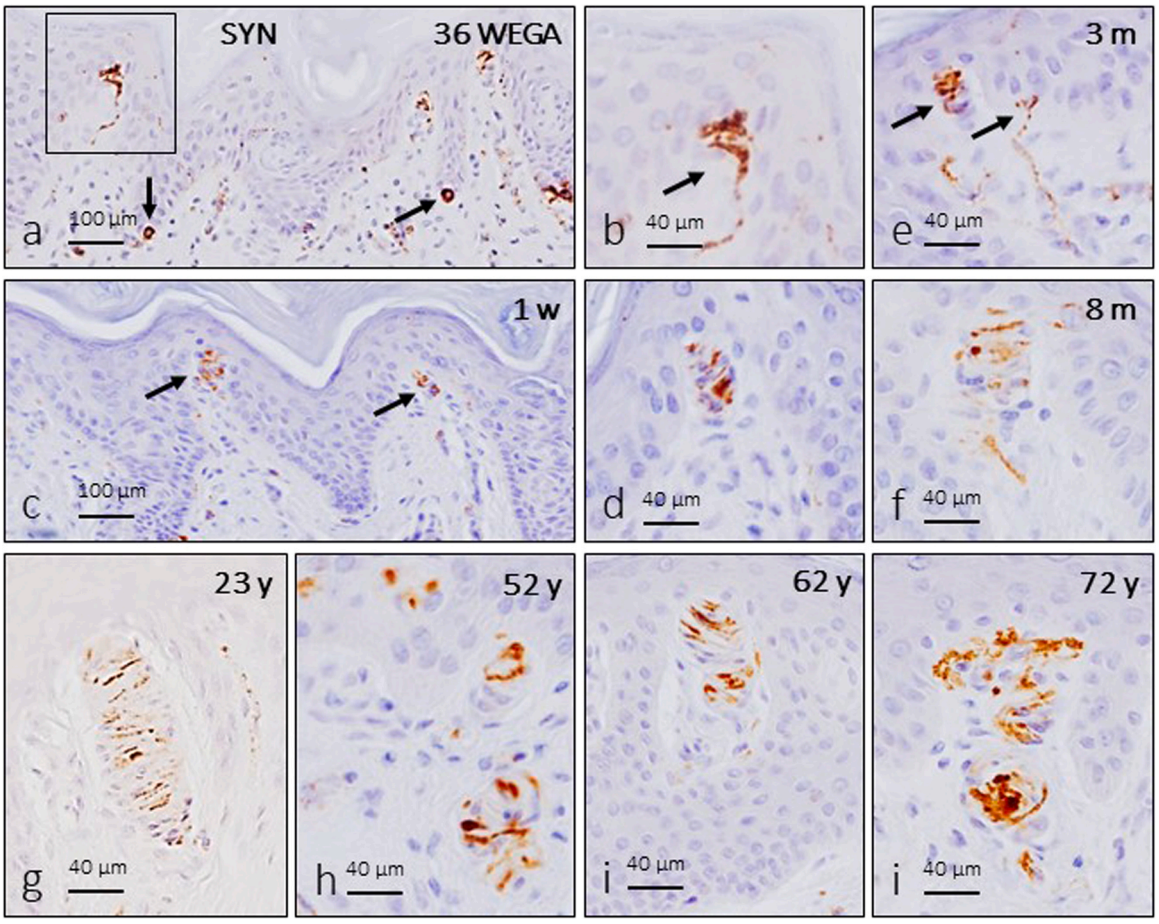

Fig. 2. Immunohistochemical detection of synaptophysin in axonal profiles of Meissner corpuscles in the digital skin at different ages: 36 WEGA (**a**, **b**), 1 week (**c**, d), 3 months (**e**), 8 months (**f**), and 23, 52, 62 and 72 years (**g** to **i**, respectively). Synaptophysin-positive Merkel cells were detected (arrows in **a**).

3.2. Meissner's corpuscles

Axonal profiles of Meissner's corpuscles were positive for SYN independently of age, from very early (36 weeks WEGA) to 72 years old (Fig. 2). Immunohistochemistry for NFP and SYN in consecutive sections suggested axonal localization of SYN in Meissner's corpuscles (Fig. 3). To confirm that, double immunofluorescence was performed (Fig. 4): SYN was shown to co-localize with NFP (Fig. 4a-d) but no with S100P (Figs. e-g). However, a few Meissner's corpuscles seemed to have different distribution of SYN-NFP along their axonal terminal, showing a preference of SYN for the corpuscular apical pole, opposite to the basal NFP immunostaining (Fig. 4d).

Regarding sampled subject ages, the number of Meissner corpuscles displaying SYN immunoreactivity was as follows: 81 ± 11% at 36 WEGA, 88 ± 9% at 1 week, 79 88 ± 6% from 8 to 10 months, 100% from 8 to 65 years old, 76 ± 14% over 65 years old. Thus, no significant age-dependent differences were noted.

3.3. Pacinian corpuscles

As well as in Meissner's corpuscles, SYN immunoreactivity was visualized in the axon of all identified Pacini's corpuscles. Despite Pacinian corpuscles starting development at 12 WEGA, ~~and~~ the first SYN occurrence in their axon was detected at 18 WEGA, and then retained over all studied time periods (Fig. 5) in the 100% of ~~the~~ Pacinian corpuscles. Although SYN distribution pattern within the corpuscle was clearly axonal, double immunofluorescence for NFP and SYN verified this, since both co-localized (Fig. 5i).

3.4. Merkel cell-neurite complexes

SYN was also detected in Merkel cells and apparently, in some axonal profiles contacting them (Fig. 6). SYN-positive structures were observed unequally distributed between the most external cells of immature epidermis at 14 WEGA (Fig. 6a-b). Once the epidermis acquires its multilayered structure (between 22 and 36 WEGA), clusters of SYN-positive cells appeared in the basal layer (Fig. 6c-f). In adult tissues, SYN was detected in isolated cells within the basal layer of the epidermis (Fig. 6g-i) forming immunoreactive rings, which were identified as Merkel cells; this typical immunohistochemical pattern seemed to become disorganised in the elderly (Fig. 6i). Definitive identification of those epidermal cells as Merkel cells was based on co-localization between SYN and CK-20 (data not shown).

In regard to sample ages, percentage of Merkel cells displaying SYN immunoreactivity (considering CK20-positive cells as a reference) was as follows: 93 ± 11% at 22 WEGA, 85 ± 9% at 36 WEGA, 72 ± 12% at 1 week, 74 ± 8% from 8 to 10 months, 72 ± 4% at 3 years old, 49 ± 6% at 23 years old, 51 ± 7 at 45 years old, and 41 ± 11% over 65 years old. Thus, density of SYN-positive Merkel cells was higher during fetal life first postnatal years, followed by a progressive decrease with age.

3.5. SYN and PIEZO2 co-localize in Meisner corpuscles, Pacinian corpuscles, and Merkel cells

Since synaptic-like vesicles and mechanosensory axon terminals are functionally related to ion channel participating in

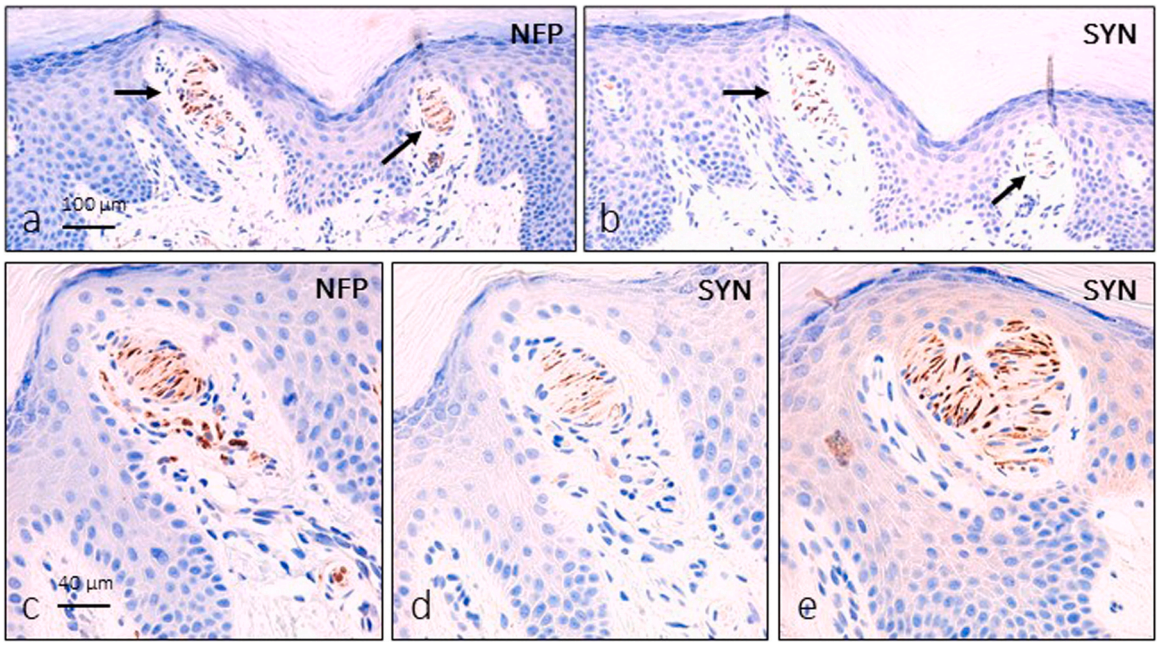

Fig. 3. Immunohistochemical detection of synaptophysin (SYN) and neurofilament proteins (NFP) in serial sections (**a** and **b**; **c** and **d**) of Meissner corpuscles of digital skin, corresponding to one subject 23 years old (**e**), 35 years old (a and b) and one subject 48 years old. Both synaptophysin and NFP show similar patterns of localization.

mechanotransduction, we analyzed whether SYN co-localizes with the mechanogated ion channel PIEZO2 in axon terminals of sensory corpuscles. Using double immunofluorescence for SYN and PIEZO2 we observed partial co-localization in axons of Meissner (Fig. 7a-c) and Pacinian corpuscles (Fig. 7f). Despite full co-localization between SYN and PIEZO2 in some Merkel cells (Fig. 7d), some others lacked PIEZO2 immunoreactivity, in which case it was observed in axons supplying them (Fig. 7e).

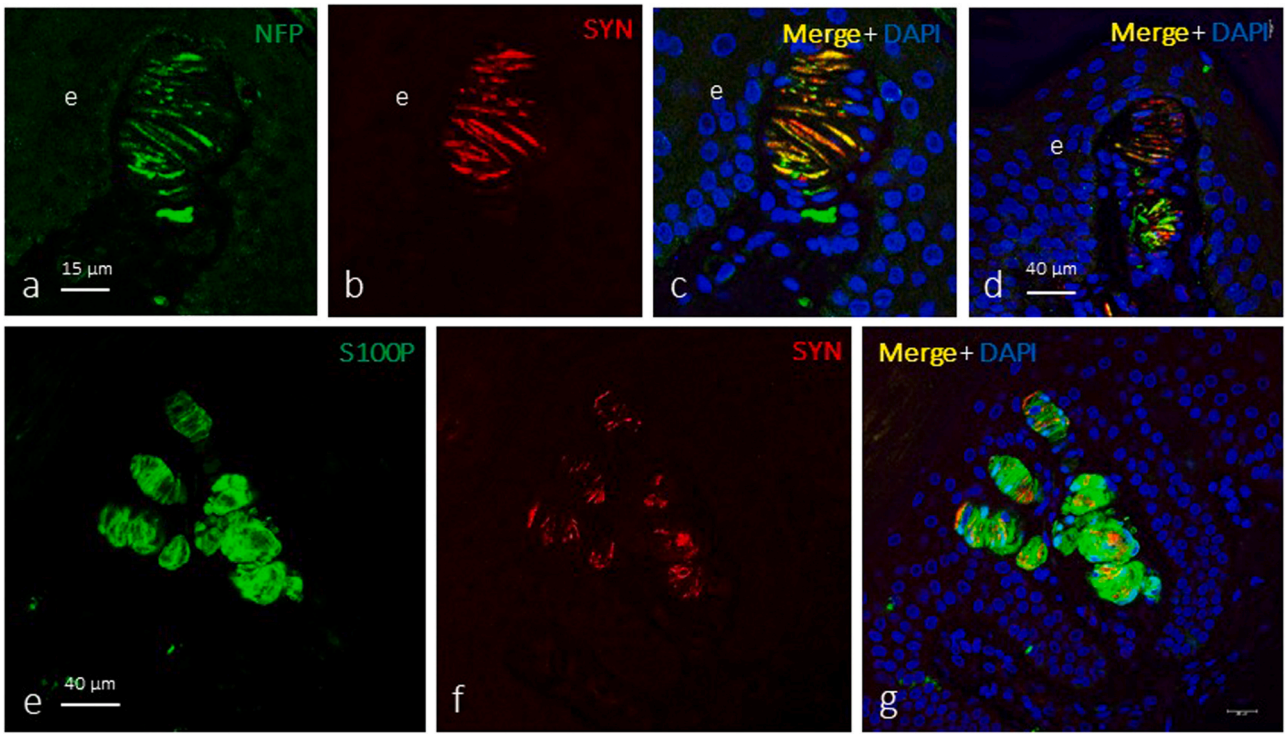

Fig. 4. Confocal dual immunofluorescence for synaptophysin (SYN, red) and neurofilament proteins (NFP) or S100 protein (S100P, green) in human digital Meissner's corpuscles. SYN totally or in part co-localize with NFP (**a-c** and **d**) but did not co-localize with S100 protein (**e** to **g**) when merged (**c**, **d** and **9**) but. **a-c** objective 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm; **d-e** and **g** objective 40X/1.25 oil, pinhole 1.00, XY resolution 156 nm and Z resolution 334 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

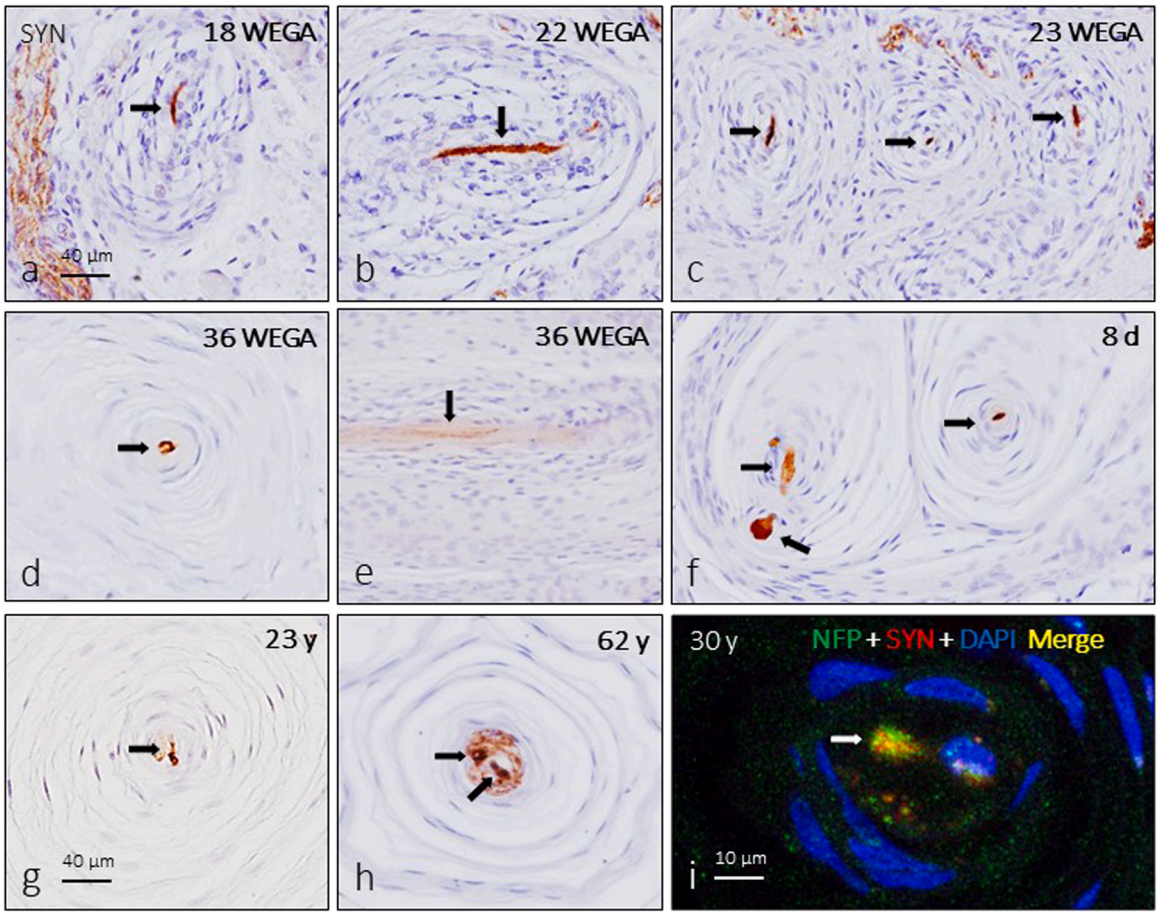

Fig. 5. Immunohistochemical detection of synaptophysin (SYN) in human cutaneous corpuscles of Pacini at different ages: 18, 22, 23 and 36 WEGA (**a** to **e**), 8 days (**f**) 23 years (**g**) and 62 years (**h**). SYN was found in the axon of Pacinian corpuscles (arrows in a to h) at all studied ages. Picture in **i** shows confocal dual immunofluorescence for SYN (red) and NFP (green) showing the co-localization of both proteins in the axon. Objective 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. Other cutaneous structures

SYN immunoreactivity was also detected in nerve plexuses surrounding dermal blood vessels (Fig. 8a) and sweat glands (Fig. 8b-e). In nerve trunks (Fig. 7d and e), SYN immunofluorescence was detected in a subpopulation of axons, preferentially those large and surrounded by Schwann cells, which demonstrated the same organized structure than in sensory corpuscles (Fig. 7f).

4. Discussion

The present research was aimed to investigate the occurrence of SYN, a synaptic vesicle membrane protein (Valtorta et al., 2004) virtually present in all presynaptic terminals (De Camilli et al., 1988), in both human DRG and ~~the~~ axon terminals of CEOC. Secondly, co-localization of SYN with the mechanotransducer ion channel PIEZO2 was studied. The research covers a wide age-range (from 11 WEGA to 72 years old) and was performed using immunohistochemistry and double immunofluorescence.

The motivation to investigate this topic was based on the following facts: a) SYN expression in DRG, including LTMRs (Sun et al., 2006); b) sensory corpuscle axon terminals are typically filled with small clear synaptic-like vesicles (see for review Malinovský and Pack, 1982; Munger and Idé, 1988; Zelená, 1994); c) SYN occurrence in afferent nerve endings of muscle spindles and tendon sensory organs (De Camili et al., 1988), as well as in Pacinian corpuscles (Varga et al., 2020); and, d) PIEZO2 expression in axons of human CEOC (García-Mesa et al., 2017).

No information exists about the presence of SYN in human DRG. Our results on primary sensory neurons basically agree with previous studies conducted in rat DRGs by Sun et al. (2006). Here we report that approximately the 25% of neurons, including intermediate and large size ranges, were SYN-positive. Considering that axon terminals of these neurons form CEOCs on the periphery (Li et al., 2011; Abraira and Ginty, 2013; Handler and Ginty, 2021), some of them (if not all) are expected to display axonal SYN immunoreactivity. According to this supposition, our findings on human digital skin demonstrate that axon terminals of Pacini's and Meissner corpuscles show SYN immunoreactivity at all examined ages, from fetal period to old age. Therefore, we conclude that SYN presence in axons of these two types of CEOC is a common and permanent characteristic. On the other hand, although SYN-positive spinal terminals from rat DRG display morphological characteristics of endings of myelinated primary mechanosensitive afferents rather than nociceptive unmyelinated afferents (Lu et al., 2003), these findings were not confirmed and SYN was detected in both Aβ and Aδ/C spinal terminals (Chung et al., 2019).

A particularity of LTMR terminal axons is their capability to form Merkel-neurite cell complexes, since they contact with differentiated specialized epithelial cells (i.e., Merkel cells; Zimmerman et al., 2014; Cobo et al., 2021), but do not with specialized peripheral glial cells (the so-called terminal glia; Reed et al., 2021). Merkel cells contain synapse-like vesicles at their cytoplasmic axonal pole (Moll et al. 2005; Boulais and Missery, 2007), which expresses SYN immunoreactivity (Ortonne et al., 1988; see Maksimovic et al., 2013). Our results show that SYN is present in Merkel cell cytoplasm from

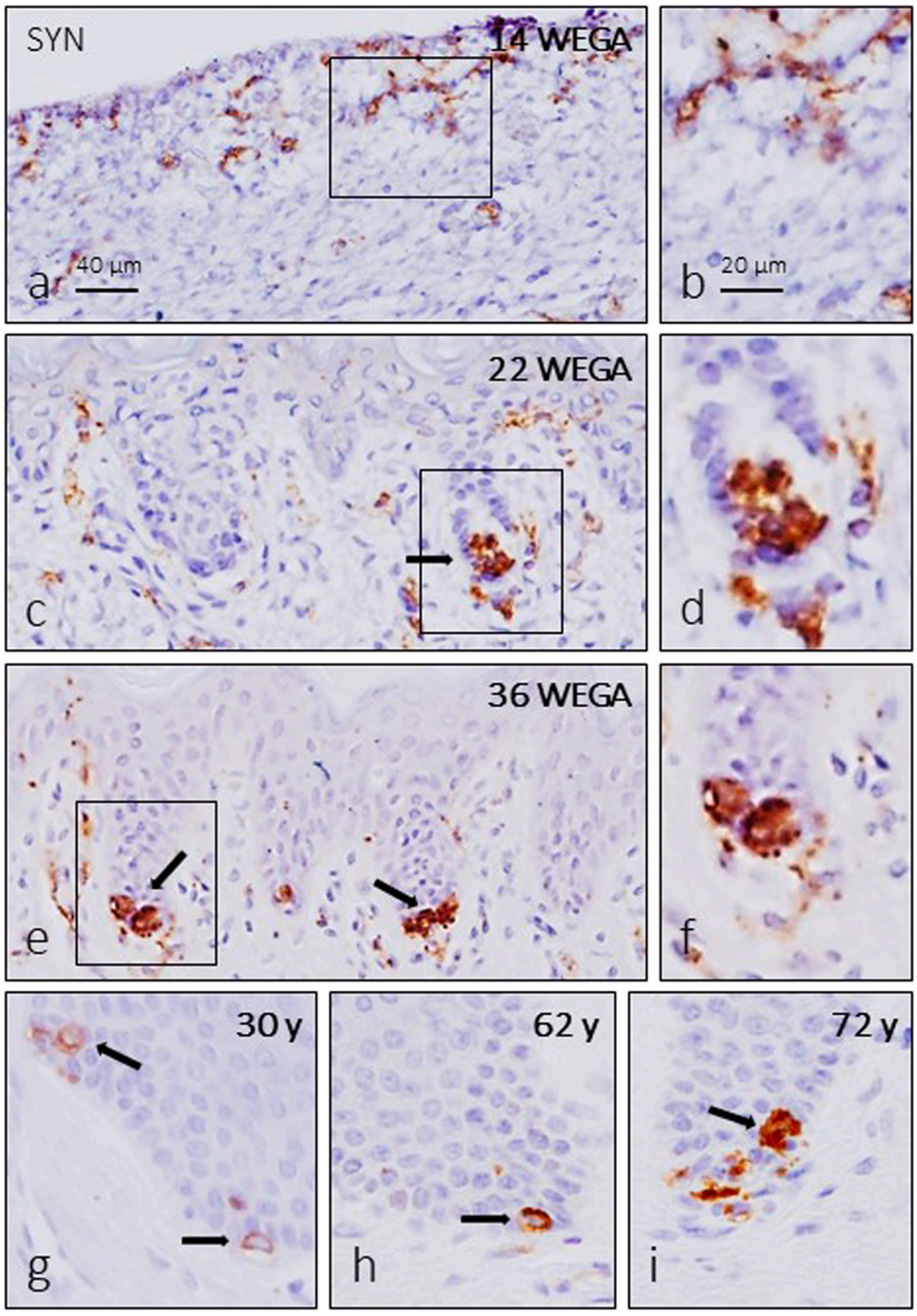

Fig. 6. Immunohistochemical detection of synaptophysin in human digital skin focusing on Merkel cells-neurite complexes at different ages: 14 WEGA (**a**, **b**), 22 WEGA (**c**, **d**; arrows), 36 WEGA (**e**, **f**; arrows). Individual Merkel cells in the basal layer of the epidermis were selectively SYN-positive in adults: 30 years (**g**), 62 years (**h**) and 72 years (**i**).

the beginning of their development ~~and~~ to throughout adult life, although not all Merkel cells express it in adult digital skin. Additionally, we have observed SYN immunoreactivity in some axon terminals contacting Merkel cells, thus confirming which confirms the occurrence of SYN in LTMR afferents.

The prompt observations of synaptic-like vesicles in CEOC axon terminals using electron microscopy had controversial interpretation about their role, including likely release of neurotransmitters (Malinovský and Pac, 1982; Munger and Idé, 1988; Zelená, 1994). De Camilli et al. (1988) suggested possible functional similarities between small synaptic vesicles at presynaptic nerve endings and small clear vesicles of CEOC, and Bewick et al. (2005) posteriorly affirmed that synaptic-like vesicles in sensory terminals of primary mechanosensory neurons resemble the synaptic vesicles of chemical synapses. Our findings on human CEOC and Merkel cell-neurite complexes lend support to a possible neurotransmission within these structures. A possible GABA-ergic/glutamatergic neurotransmission in Pacinian corpuscles (Pawson et al., 2007, 2009), palisade ending and muscle spindles (Bewick et al., 2005; Bewick, 2005) has been suggested, but this remains to be proven as a common characteristic of mechanoreceptors. In that case, the presence of synaptic-like vesicles in CEOC axon terminals might have an explanation.

It is now well known that Final del formulario mechanotransduction depends on the mechanical sensitivity of certain ion channels, which may be mechanically gated, or may show

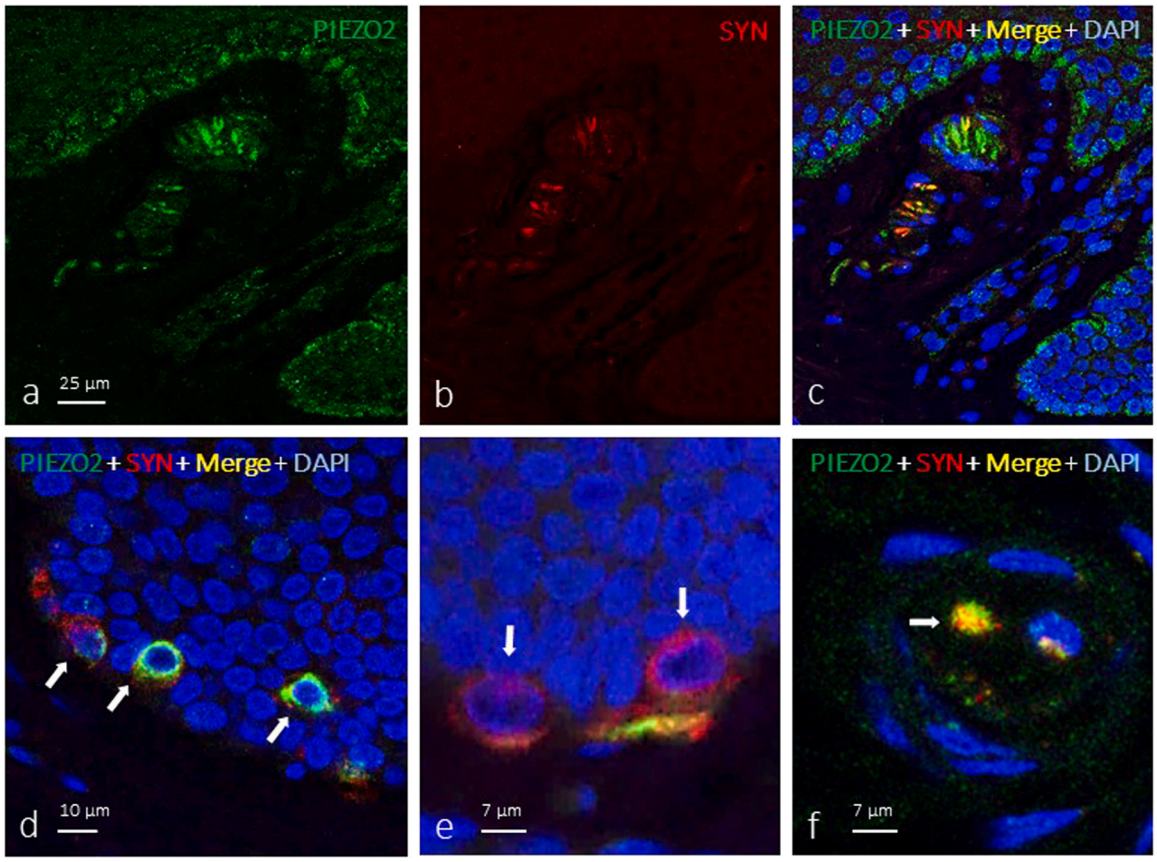

Fig. 7. Double immunofluorescence for synaptophysin (SYN, red) and PIEZO2 (green) in human cutaneous Meissner corpuscles (**a-c**), Merkel cell-neurite complexes (**d** and **e**), and Pacinian corpuscles (**f**). SYN and PIEZO2 co-localized partially and irregularly with PIEZO2 the axon of Meissner and Pacinian corpuscles. In Merkel cells PIEZO2 co-localized with SYN in most cases (arrows in d), but Merkel cells SYN positive and PIEZO2 negative were observed. In some cases, only the nerve afferent of the Merkel cell-neurite complexes showed co-localization (**e**). **a-c** objective 40X/1.25 oil, pinhole 1.00, XY resolution 156 nm and Z resolution 334 nm; **d-f** objective 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

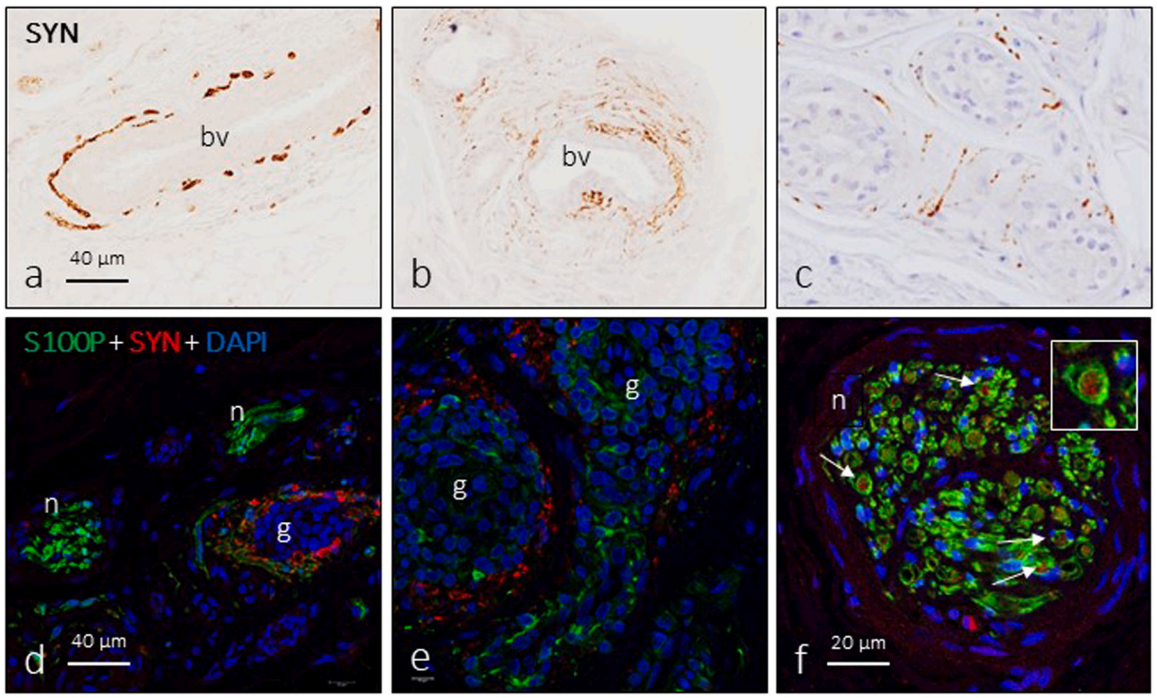

Fig. 8. Single immunohistochemistry (**a-c**) and double immunofluorescence (synaptophysin and S100 protein) for synaptophysin (SYN) in distinct anatomical structures of skin dermis. SYN was found in nerve profiles around blood vessels (bv; **a**, **b**) and sweat glands (g; **c**, **d**, **f**). In dermal nerve trunks large axons displayed SYN immunofluorescence (arrows in **f**). **d** and **e** objective 40X/1.25 oil, pinhole 1.00, XY resolution 156 nm and Z resolution 334 nm; **f** objective 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm.

mechanical sensitivity (see Cobo et al., 2020). To date, the only channel that fulfills the properties of a mechanotransducer is PIEZO2. In the present study, we have confirmed previous results from our lab reporting PIEZO2 immunodetection in different morphotypes of human CEOC and Merkel cell-neurite complexes (García-Mesa et al., 2017, 2021a,b; García-Piqueras et al., 2019). Furthermore, we observed that PIEZO2 and SYN fully co-localize in CEOC axon terminals. To the best of our knowledge, PIEZO2 is present in the axonal membrane but not in vesicles. Therefore, the functional significance of these findings, if any, remains to be demonstrated. SYN deficient mice are viable and do not show defects in neurotransmitter release (Eshkind and Leube, 1995; McMahon et al., 1996). In any case, our data suggest that SYN is a reliable marker for peripheral axon terminals of primary mechanosensory neurons forming CEOC.

Ethical statement

This study was approved by the Ethical Committee for Biomedical Research of the Principality of Asturias, Spain (Cod. CElm, PAst: Proyecto 266/18).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgements

This study was supported in part by a grant from Gerencia Regional de Salud de Castilla y León to JF and JAV (GRS 1882/A/18). Y. G-M. (PA-18-PF-BP17-044) and P.C. (PA-21-PF-BP20-122) were supported by Grants “Severo Ochoa Program” from the Govern of the Principality of Asturias.

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