Published in final edited form as:

Ann N Y Acad Sci. 2011 September ; 1233: 1–7. doi:10.1111/j.1749-6632.2011.06169.x.

Is there any sense in the Palisade endings of eye muscles?

Karoline Lienbacher¹, Michael Mustari², Bernhard Hess³, Jean Büttner-Ennever¹, and Anja K.E. Horn¹

¹Institute of Anatomy I, Ludwig-Maximilians University of Munich, Germany. ²Washington National Primate Research Center, University of Washington, Seattle, Washington. ³Department of Neurology, University Hospital Zurich, Switzerland

Abstract

Palisade endings (PEs), which are unique to the eye muscles, are associated with multiply innervated muscle fibers. They lie at the myotendinous junctions and form a cap around the muscle fiber tip. They are found in all animals investigated so far, but their function is not known. Recently, we demonstrated that cell bodies of PEs and tendon organs lie around the periphery of the oculomotor nucleus in the C- and S-groups. A morphological analysis of these peripheral neurons revealed the existence of different populations within the C-group. We propose that a small group of round or spindle-shaped cells gives rise to PEs, and another group of multipolar neurons provide the multiple motor endings. If PEs have a sensory function, then their cell body location close to motor neurons would be in an ideal location to control tension in extraocular muscles; in the case of the C-group, its proximity to the preganglionic neurons of the Edinger–Westphal nucleus would permit its participation in the near response. Despite their unusual properties, PEs may have a sensory function.

Keywords

oculomotor nucleus; C-group

Introduction

The classical proprioceptors of skeletal muscle are muscle spindles to measure length, and Golgi tendon organs to measure force. Extraocular muscles (EOM) are a well-known exception to this, perhaps because they are characterized by embryological features, like immature skeletal muscles. They exhibit a wide variation in sensory receptors depending on species.^{1,2} The eye muscles of some vertebrate species such as monkeys have few tendon organs (no classical Golgi tendon organs and no muscle spindles);³ other species (e.g., human) have some muscle spindles that appear underdeveloped and all of them with striking anomalies compared to sheep spindles.⁴ In contrast, artiodactyls (e.g., sheep, goat, camel)

Address for correspondence: Karoline Lienbacher, Institute of Anatomy I, LMU Munich, Pettenkoferstrasse 11, D-80336 Munich, Germany. karoline.lienbacher@med.uni-muenchen.de.

Conflicts of interest

The authors declare no conflicts of interest.

have well-developed tendon organs and muscle spindles. But even in species without classical proprioceptors, such as monkeys, representations of eye position in the primary somatosensory cortex have been demonstrated.⁵ Furthermore, a stretch reflex in the EOMs of different species with sparse, diminutive, or even absent muscle spindles has been reported.⁶ The question considered in this article is, Which structure could provide this central proprioceptive information? One possibility is the palisade endings.

Palisade endings (PEs) are unique to eye muscles and have been found in all species investigated so far.^{4,7–12} Up to now PEs were assumed to be sensory on account of the location in the tendon⁸ and their similarity to immature Golgi tendon organs.¹³ However, recent studies have shown that they have some properties typical of motor terminals: they are cholinergic and a small portion of PE terminals (approximately 10%) are positive for α -bungarotoxin at the postsynaptic site.^{14,15} Furthermore, Lienbacher *et al.*¹⁶ and Zimmermann *et al.*¹⁷ have shown that PEs can be anterogradely filled by central tracer injections into the region of the oculomotor or abducens nucleus, respectively. In spite of these results, in this paper we will present evidence supporting the sensory function of PEs.

Tracer injections into the eye muscles of monkey retrogradely label sensory ganglion cells and motor neurons. The sensory pseudo-unipolar ganglion cells lie in the ophthalmic division of the trigeminal ganglion;^{18,19} but different populations of neurons were found both within and around the oculomotor, abducens, and trochlear nuclei. The neurons within the boundaries of the classical motor nuclei were shown to innervate the singly-innervated fibers, whereas the peripheral cell groups were assumed to innervate the multiply-innervated muscle fibers and possibly PEs. The peripheral cell groups for medial rectus and inferior rectus comprise the C-group and those for inferior oblique and superior rectus the S-group.²⁰ In this study, we have analyzed the peripheral cell group of the medial rectus of monkeys (C-group) in more detail with focus on size and morphology of neurons. A few neurons showed a morphology resembling that of trigeminal ganglion cells and may represent the cell bodies of sensory PEs.

Methods

All experimental procedures conformed to the state and university regulations for laboratory animal care, including the *Principles of Laboratory Animal Care* (NIH Publication 85-23, revised 1985), and they were approved by animal care officers and the Institutional Animal Care and Use Committees and were described in detail in a previous report.²⁰

Macaque monkeys were injected in the belly or the distal tip of the medial rectus muscle with cholera toxin subunit B (CTB) or wheat-germ-agglutinin horseradish peroxidase (WGA-HRP). After a survival time of three days, the animals were killed and transcardially perfused as described previously.²⁰ The brainstem and the orbits were removed and cut transversely at 20 μ m (eye muscles) and 40 μ m (brainstem).

For the immunocytochemical detection of CTB, free-floating brain sections were pretreated with 10% methanol and 3% H₂O₂ to suppress endogenous peroxidase activity and were then preincubated in 0.1M phosphate buffer (PB) at pH 7.4 containing 0.3% Triton X-100 with

5% normal rabbit serum for 1 h. Then, the tissue was processed with goat anti-CTB (1:40,000; List Biological Laboratories, CA) for 48 h at 4° C. The sections were washed in 0.1M PB three times and treated with biotinylated rabbit anti-goat (1:200; Vector Labs, Burlingame, CA) for 1 h at room temperature. Then the sections were washed in 0.1M PB three times and incubated in avidin–biotin complex (1:50; Vector Labs, Burlingame, CA) for one hour at room temperature. After two rinses in 0.1M PB and one rinse in 0.05M Tris buffer solution (TBS, pH 8.0), the antigenic sites were visualized with 0.05% diaminobenzidine (DAB) and 0.01% H₂O₂ in 0.05 M TBS (pH 8.0) for 5–10 minutes. The sections were mounted, air-dried, dehydrated, and cover-slipped in Depex. The WGA-HRP was visualized with the tetramethylbenzidine method as described previously.²⁰

Seven macaque monkeys with tracer injections (CTB or WGA) into the MR, and one additional macaque monkey with a tracer injection (CTB) into the distal part of the superior rectus muscle (SR) were carefully reanalyzed. The focus lay on the examination of the peripheral C-group neurons of the MR. We distinguished between C-group neurons in close proximity to the dorsomedial aspect of the oculomotor nucleus (nIII) (after belly injections) and those which extend far rostral to nIII encircling the preganglionic neurons of the Edinger–Westphal nucleus (EW_{pg}; after distal injections into the myotendinous junction).

The sections of a supplementary case containing nIII and immunostained for the cholinergic marker choline acetyltransferase (ChAT) were analyzed for the morphology of the peripheral neurons in the C-group. The cholinergic C-group neurons can be distinguished from preganglionic neurons of the EW_{pg} as another cholinergic neuron group in the periorbital region by their different histochemical properties, for example, lack of non-phosphorylated neurofilaments and cytochrome oxidase.^{21,22}

The slides were examined with a Leica microscope (DMRB; Leica, Bensheim, Germany) under bright field conditions. Micrographs were taken with a digital camera (Pixera Pro 600 ES; Klughammer, Markt Indersdorf, Germany), captured on a computer (Pixera Viewfinder software; Klughammer), and processed with image analysis software (Photoshop 11.0; Adobe Systems, Mountain View, CA). The images were arranged and labeled using drawing software (CorelDraw 11.0; Corel, Ottawa, Ontario, Canada). A morphometric analysis was performed on digitized images taken at a 40× magnification. Only those cells with a clearly visible nucleus were measured with ImageJ software. The mean diameter in micrometer was calculated in Excel 2007 by $[D_{\min} + D_{\max}]/2$.

Results

Morphological and morphometric analysis of the nIII peripheral groups revealed a heterogeneous cell population. Belly tracer injections into the MR led to retrogradely labeled twitch motor neurons within nIII and neurons in the periphery of nIII, with the largest accumulation within the C-group adjacent to the dorsomedial aspect of nIII. Very distal tracer injections into the myotendinous junction, mostly sparing muscle fibers, resulted in exclusive labeling of the peripheral neurons around nIII, some of them extending dorsally to nIII encircling the EW_{pg}²³ and also far rostrally to nIII.

After a systematic inspection of the peripheral either tracer-labeled or ChAT-labeled neurons, we found numerous multipolar neurons—a morphology typical for motor neurons (Fig. 1B). We also identified a small but consistent population of neurons that were round or bipolar—a morphology (Fig. 1A) resembling the sensory ganglion cells of the mesencephalic trigeminal nucleus (V_{mes} ; Fig. 1C). The population lay closer to the EW_{pg} , and distant from nIII; and, importantly, they were only back-labeled after very distal tracer injections. These two different morphological cell types were also seen in tracer labeled neurons of the S-group after a tracer injection into the myotendinous junction of the SR (Fig. 1D).

The morphometric analysis of the peripheral tracer-labeled neurons around nIII revealed a two-peak cell size profile (Fig. 2): (1) tracer-positive presumed twitch motor neurons within nIII after belly injections into the MR had a large mean diameter (22–34 μm), whereas (2) the mean diameter of tracer-labeled peripheral C-group neurons after belly injections ranged across a large cell size spectrum with addition small cells (14–38 μm). A population of smaller neurons (3) was seen within the tracer-labeled neurons far rostral to nIII after distal injections into the myotendinous junction of MR (14–22 μm).

Discussion

The eye muscles consist of two separate fiber types: the singly innervated (SIF) twitch muscle fibers in the global layer innervated by single *en plaque* terminals and the multiply innervated (MIF) non-twitch muscle fibers innervated by multiple *en grappe* terminals along the whole muscle length. The MIFs of the orbital layer are additionally innervated by *en plaque* endings at their central part.²⁴ The PEs represent special nerve endings unique to eye muscles, which are exclusively associated with MIFs at the myotendinous junctions of the muscle. PEs are found in all animals being investigated so far, and recently their cell bodies were shown to be located in or around the motor nuclei of EOMs.^{16,17} It is still not clear what function the PEs have and whether they are motor¹⁴ or sensory²⁵ organs. Recent findings, such as the association with cholinergic markers,¹⁴ and the fact that they could be tracer labeled from central tracer injections in or around the motor nuclei,^{16,17} suggest a motor function of these endings. Otherwise, the location of PEs at the myotendinous junctions and their continuity with tendon endings indicate a sensory function,¹⁶ putting the assumption of a pure motor function into question.

Tracer injections into primate eye muscles showed that the motor neurons of the *en plaque* endings innervating the SIFs lay within the motor nuclei and the cell bodies of the *en grappe* endings innervating the MIFs are located in the periphery of the motor nuclei.²⁰ Distal tracer injections into the eye muscle showed only the MIF neurons, whereas belly injections showed the SIF and the MIF neurons.²⁰ Until now the peripheral neurons around the motor nucleus have generally been considered as MIF neurons forming one population of neurons whose neuromuscular multiple nerve endings are distributed along the whole muscle fiber length reaching up to the distal and proximal ends of the eye muscle. However, these distal tracer injections also involve the PEs and tendon organs at the myotendinous junctions and in the tendon. In light of our recent findings that the cell bodies of PEs are located in the periphery of the motor nuclei, the peripheral cell groups, back-labeled after tracer injections

into the myotendinous junction, it must be considered that some are back-labeled also from PEs. Theoretically, there are two possibilities of organization as already suggested previously:²⁰ either the peripheral neurons around the motor nuclei form one homogenous population of neurons that give rise to multiple *en grappe* endings and PEs plus tendon organs, or there are at least two different neuron populations, for example, cell bodies of the motor *en grappe* endings and cell bodies of possible sensory PEs.

Our analysis of the peripheral neurons around nIII revealed two groups of neurons based on cell size but also morphological differences (Fig. 1). The findings of this reanalysis are in good accordance with previous data on the cell sizes comparing tracer-labeled peripheral and tracer-labeled presumed twitch-neurons within nIII after a large tracer injection into the muscle belly, where a bimodal distribution of cell sizes was noted, as well (Büttner-Ennever *et al.*, see Fig. 13A²⁰). As in the previous paper, no difference in cell sizes of retrogradely labeled neurons in the peripheral and central, presumed twitch-motoneurons, were noted after a tracer injection in the belly (Büttner-Ennever *et al.*,²⁰ see Fig. 2). We extended this morphometric analysis by comparing the cell sizes of peripheral cell groups around the EW_{pg} and those of peripheral cells adjacent to nIII only back-labeled after distal tracer injections and found a bimodal distribution, with the latter group being the larger cells.

Only tracer injections into the myotendinous junction—the location of PEs—revealed, in addition, a group of round or spindle-shaped neurons with a morphology resembling that of sensory ganglion cells²⁶ (Fig. 1). A similar attempt of classification based on morphological cell features of tracer-labeled neurons after EOM injections was undertaken in the pig.²⁷ Multipolar large cells were considered as alpha motoneurons corresponding to twitch motoneurons and small cells as γ -motoneurons, which may represent the non-twitch motoneurons providing multiple innervation and their spindle-shaped and round neurons—suggested as sensory cell bodies of spindle afferents—may represent the cell bodies of PEs. Although the possibility of the location of sensory neuronal cell bodies so close to the motor nuclei (Fig. 3), and not in a separate ganglion, does not seem obvious, there is at least one example of well-known sensory neurons in the brainstem. The large round ganglion-like neurons of the mesencephalic trigeminal nucleus (V_{mes}) located close to the fourth ventricle at pontine levels and as single neurons at the border of the periaqueductal gray at mesencephalic levels represent well-described sensory neurons innervating the muscle spindles of the jaw muscles.^{26,28} Furthermore, several studies indicate that at least part of them may innervate muscle spindle afferents of the eye muscles.^{29,30} Because we never saw tracer labeled V_{mes} cells after distal injections of the EOM,¹⁶ this nucleus was ruled out as a source of PEs. In addition, tracer injections into the medullary part of V_{mes} did not result in anterogradely labeled PEs.¹⁷

Based on the findings that only tracer injections into the myotendinous junction of MR revealed two populations of peripheral neurons—differing in their cell sizes and/or morphology—we propose that the round neurons within the peripheral cell groups represent sensory cell bodies of the PEs. These sensory cells in the periphery of nIII are assumed to give rise to sensory terminals of PEs, and the multipolar neurons in the periphery of nIII to represent MIF motor neurons, innervating the non-twitch muscle fibers via multiple *en grappe* endings (Fig. 4). Only active MIFs would provide a tension on the tendon thereby

transferring the information to sensory palisade ending terminals. A central circuit between sensory and motor pathways would stimulate the MIF motor neurons thereby modulating the MIF activity.

In conclusion, our results show that the peripheral cell groups (S- and C-group) of nIII contain separate populations of neurons with different morphology and with different cell size profile. We suggest that one cell population of neurons with a multipolar shape represent the cell bodies of the *en grappe* motor terminals innervating non-twitch fibers, and the other group of round neurons closer to EW_{pg} are likely to represent the cell bodies of sensory palisade endings (Fig. 4). Further recording studies on the different populations in the nIII periphery, or selective small tracer-injections into both groups, are necessary to prove this hypothesis.

Acknowledgments

This work was supported by Deutsche Forschungsgemeinschaft DFG HO 1639/4-3 (K.L., J.B., A.K.E.H.), National Institutes of Health EY020744; RR00166 (M.M.), Swiss National Science Foundation Grant 3100A0-110802, and the Betty and David Koetser Foundation for Brain Research (B.H.).

References

1. Cilimbaris PA. Histologische Untersuchungen über die Muskelspindeln der Augenmuskeln. Arch. mikro. Anat. und Entwicklungsgeschichte. 1910; 75:692–747.
2. Harker DW. The structure and innervation of sheep superior rectus and levator palpebrae extraocular eye muscles. II: Muscle spindles. Invest. Ophthalmol. Vis. Sci. 1972; 11:970–979.
3. Maier A, DeSantis M, Eldred E. The occurrence of muscle spindles in extraocular muscles of various vertebrates. J. Morph. 1974; 143:397–408. [PubMed: 4277714]
4. Ruskell GL. Extraocular muscle proprioceptors and proprioception. Prog. Retin. Eye Res. 1999; 18:269–291. [PubMed: 10192514]
5. Wang X, Zhang M, Cohen IS, Goldberg ME. The proprioceptive representation of eye position in monkey primary somatosensory cortex. Nat. Neurosci. 2007; 10:640–646. [PubMed: 17396123]
6. Dancause N, Taylor MD, Plautz EJ, et al. A stretch reflex in extraocular muscles of species purportedly lacking muscle spindles. Exp. Brain Res. 2007; 180:15–21. [PubMed: 17216145]
7. Dogiel AS. Die Endigungen der sensiblen Nerven in den Augenmuskeln und deren Sehnen beim Menschen und den Säugetieren. Arch. mikro. Anat. 1906; 68:501–526.
8. Alvarado-Mallart RM, Raymond M, Pincon. The palisade endings of cat extraocular muscles: a light and electron microscope study. Tissue Cell. 1979; 11:567–584. [PubMed: 494241]
9. Billig I, Buisseret-Delmas C, Buisseret P. Identification of nerve endings in cat extraocular muscles. Anat. Rec. 1997; 248:566–575. [PubMed: 9268145]
10. Ruskell GL. The fine structure of innervated myotendinous cylinders in extraocular muscles in rhesus monkey. J. Neurocytol. 1978; 7:693–708. [PubMed: 104011]
11. Blumer R, Lukas JR, Wasicky R, Mayr R. Presence and structure of innervated myotendinous cylinders in sheep extraocular muscle. Neurosci. Lett. 1998; 248:49–52. [PubMed: 9665661]
12. Eberhorn AC, Horn AKE, Eberhorn N, et al. Palisade endings in extraocular eye muscles revealed by SNAP-25 immunoreactivity. J. Anat. 2005; 205:307–315. [PubMed: 15733303]
13. Zelená J, Soukup T. The development of Golgi tendon organs. J. Neurocytol. 1977; 6:171–194. [PubMed: 853311]
14. Konakci KZ, Streicher J, Hoetzenecker W. Palisade endings in extraocular muscles of the monkey are immunoreactive for choline acetyltransferase and vesicular acetylcholine transporter. Invest. Ophthalmol. Vis. Sci. 2005; 46:4548–4554. [PubMed: 16303947]
15. Blumer R, Konakci KZ, Pomikal C, et al. Palisade endings: cholinergic sensory organs or effector organs? Invest. Ophthalmol. Vis. Sci. 2009; 50:1176–1186. [PubMed: 18936148]

16. Lienbacher K, Mustari M, Ying HS, et al. Do palisade endings in extraocular muscles arise from neurons in the motor nuclei? *Invest. Ophthalmol. Vis. Sci.* 2011; 52:2510–2519. [PubMed: 21228383]
17. Zimmermann L, May PJ, Pastor AM, et al. Evidence that the extraocular motor nuclei innervate monkey palisade endings. *Neurosci. Lett.* 2011; 489:89–93. [PubMed: 21138754]
18. Porter JD, Guthrie BL, Sparks DL. Innervation of monkey extraocular muscles: localization of sensory and motor neurons by retrograde transport of horseradish peroxidase. *J. Comp. Neurol.* 1983; 218:208–219. [PubMed: 6604075]
19. Fackelmann K, Nouriani A, Horn AK, Büttner-Ennever JA. Histochemical characterisation of trigeminal neurons that innervate monkey extraocular muscles. *Prog. Brain Res.* 2008; 171:17–20. [PubMed: 18718277]
20. Büttner-Ennever JA, Horn AKE, Scherberger H, D'Ascanio P. Motoneurons of twitch and nontwitch extraocular muscle fibers in the abducens, trochlear, and oculomotor nuclei of monkeys. *J. Comp. Neurol.* 2001; 438:318–335. [PubMed: 11550175]
21. Eberhorn AC, Ardelenanu P, Büttner-Ennever JA, Horn AKE. Histochemical differences between motoneurons supplying multiply and singly innervated extraocular muscle fibers. *J. Comp. Neurol.* 2005; 491:352–366. [PubMed: 16175553]
22. Horn AK, Eberhorn A, Härtig W, et al. Perioculomotor cell groups in monkey and man defined by their histochemical and functional properties: reappraisal of the Edinger-Westphal nucleus. *J. Comp. Neurol.* 2008; 507:1317–1335.
23. Kozicz T, Bittencourt JC, May PJ, et al. The Edinger-Westphal nucleus: a historical, structural, and functional perspective on a dichotomous terminology. *J. Comp. Neurol.* 2011; 519:1413–1434. [PubMed: 21452224]
24. Spencer RF, Porter JD. Biological organization of the extraocular muscles. *Prog. Brain Res.* 2006; 151:43–80. [PubMed: 16221585]
25. Büttner-Ennever JA, Eberhorn AC, Horn AKE. Motor and sensory innervation of extraocular eye muscles. *Ann. N.Y. Acad. Sci.* 2003; 1004:40–49. [PubMed: 14662446]
26. Johnston JB. The radix mesencephalica trigemini. *J. Comp. Neurol.* 1909; 19:593–644.
27. Kubota K, Matsuyama S, Kubota M, et al. Localization of proprioceptive neurons innervating the muscle spindles of pig extraocular muscles studied by horseradish peroxidase labelling. *Anat. Anz.* 1988; 166:117–131. [PubMed: 3263819]
28. Alvarado-Mallart MR, Batini C, Buisseret-Delmas C, Corvisier J. Trigeminal representations of the masticatory and extraocular proprioceptors as revealed by horseradish peroxidase retrograde transport. *Exp. Brain Res.* 1975; 23:167–179. [PubMed: 1081052]
29. Wang N, May PJ. Peripheral muscle targets and central projections of the mesencephalic trigeminal nucleus in macaque monkeys. *J. Comp. Neurol.* 2008; 291:974–987.
30. Bortolami R, Lucchi ML, Pettorossi VE, et al. Localisation and somatotopy of sensory cells innervating the extraocular muscles of lamb, pig and cat. Histochemical and electrophysiological investigation. *Arch. Ital. Biol.* 1987; 125:1–15. [PubMed: 3606296]

Figure 1.

(A, B) The immunoperoxidase staining for choline acetyltransferase (ChAT) of peripheral C-group neurons in the monkey. (C) Ganglion cells within the mesencephalic trigeminal nucleus immunostained for nonphosphorylated neurofilament (NP-NF). Note the similarity to the peripheral C-group neuron in A. (D) Retrogradetracer-labeled (WGA-HRP) spindle-shaped (closed arrow) and multipolar (open arrow) neuron in the S-group after a distal tracer injection into the superior rectus muscle of a monkey.

Figure 2.

Cell size profile of internal and peripheral neurons of nIII. Histogram demonstrating the cell size profile of labeled neurons after tracer injections into the distal or central part of the medial rectus muscle (MR). Note that no major differences of the cell size profile of peripheral and central neurons are present after belly injection. However, small-sized cells in the peripheral cell groups are only present after tracer injections into the distal muscle (myotendinous junction).

Figure 3.

The oculomotor nuclei (III) with their peripheral cell groups. (A) The oculomotor nucleus containing the motor neurons of the medial rectus (MR) with A- and B-groups, inferior rectus (IR), inferior oblique (IO), and superior rectus (SR) muscle. The nIII has different peripheral groups: the C containing neurons of IR and MR—and S-group containing neurons of SR and IO. (B) The peripheral neurons of the superior oblique muscle form a cap dorsal to the trochlear nucleus (IV). (C) The peripheral neurons of the lateral rectus muscle are located around the medial and dorsal aspect of the abducens nucleus (VI).

Figure 4. Schematic drawing of the myotendinous junction and its innervation illustrating a hypotheses for palisade ending (PE) function. Two functionally different sets of neurons within the peripheral MIF-motor neuron groups provide innervation of the myotendinous junction. Sensory neurons give rise to PEs and motor neurons provide multiple innervation by *en grappe* endings. A stretch of the myotendinous junction would transmit a sensory signal via the PEs centrally to activate MIF-motor neurons.