Published in final edited form as:

J Neurol. 2017 March ; 264(3): 583–585. doi:10.1007/s00415-017-8397-z.

Identification of secondary vestibulo-ocular neurons in human based on their histochemical characteristics found in monkey

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Dear Sir or Madam,

The vestibular system serves a variety of functions i.e. postural control, spatial orientation and gaze stabilization during head and body movements. Thereby the vestibulo-ocular reflex (VOR) provides an efficient rapidly acting three neuron arc transmitting signals about angular and linear head motions to the extraocular muscles in order to elicit compensatory eye movements [1]. Phylogenetically the VOR represents one of the oldest types of eye movements, and the secondary vestibulo-ocular connections seem to be organised similarly in all vertebrates [2], i.e. secondary vestibular neurons in the vestibular nuclei receive primary canal afferents via the vestibular nerve and project to motoneurons in the abducens, trochlear and oculomotor nuclei. The rather similar cytoarchitecture of the vestibular complex in all vertebrates allows four main nuclei composed of small to large-sized neurons of variable shape to be distinguished [3]: the superior (SV), medial (MV), lateral (LV) and descending (DV) vestibular nuclei, though specific functions and histochemical features are not necessarily confined to these nuclear borders [4–6]. For example contralateral excitatory vestibulo-ocular projections arise from central magnocellular regions of the medial (MVm) and superior vestibular nucleus (SV), whereas the smaller cells surrounding this more magnocellular core in MVm and SV seem to represent mainly commissural and intrinsic connections [7].

For a clinicopathological analysis of cases with oculomotor or vestibular disorders it is important to identify functional cell groups of the oculomotor/vestibular system in order to

Ethical standards

From all human control subjects written consent from next of kin was obtained and approved by the Local Research Committees of the Ludwig-Maximilians University in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

be able to correlate their damage with the observed deficits. Based on combined tract-tracing and immunohistochemical studies in monkeys histochemical markers for saccadic burst neurons or omnipause neurons have been established and were applied to human brainstem sections to identify the homologue cell groups [8–11]. Here, it was our aim to find a histochemical marker for the secondary vestibular neurons in humans. The reanalysis of monkey brainstem sections immunostained for non-phosphorylated neurofilaments (NPNF) revealed similar neuron populations as those seen of retrogradely labelled vestibulo-ocular neurons after a tract-tracer injection into the oculomotor nucleus (nIII) (Fig. 1A, B) [12]. The combined immunofluorescence staining for the tracer (choleratoxin subunit B; CTB) and NPNF (monoclonal anti NPNF 1: 1000; SMI32, Sternberger monoclonals) revealed that the vast majority of tracer-labelled neurons in the vestibular nuclei express NPNF thereby confirming a previous preliminary observation (Fig. 1C, D) [12]. The large-sized NPNF-positive neurons in the magnocellular part of the medial vestibular nucleus (MVm) and core region of the SV are considered the secondary vestibular neurons (Fig. 1B).

The transfer to human tissue was carried out on three control human brainstems (age: 62-75; immersion fixation in 10% formalin) obtained 24 – 72h after death from bodies donated to the Anatomical Institute and from the Neurobiobank Munich approved by the ethics committee of the Ludwig Maximilians University. Series of frozen or paraffin sections were stained with Luxol Fast Blue (LFB) for cytoarchitecture or immunostained for NPNF as described previously [13]. In accordance with the work by Baizer and colleagues all vestibular nuclei in human contained NPNF-positive neurons [14]. As in monkeys a group of large-sized NPNF-positive neurons was located at the border of the MVm and LV and in the SV (Fig. 1E,F). The large non-secondary vestibular Deiters' neurons in the LV also expressing NPNF-immunoreactivity could easily be distinguished from putative secondary vestibular neurons by their plump shape and high lipofuscin content [3].

Based on location, morphological and histochemical criteria attained from the monkey experiments the large-sized NPNF-immunoreactive neurons in the MVm and SV are considered to be the secondary vestibular neurons in human, also going along with the concept that NPNF-positive neurons represent long-range projection neurons rather than local interneurons [14]. Thereby a physiologically well characterized cell group that mediates the VOR forming the link between vestibular inputs and motoneurons of the extraocular muscles can be identified in the human vestibular nuclei complex. This allows specific investigations of this cell group on a cellular level for additional characterization, e.g. transmitter inputs, receptors, ion channels, in human control cases. It further enables the assessment of secondary vestibular neurons in clinical cases involving or relatively sparing the VOR, such as progressive supranuclear palsy, and could therefore help gain a better understanding of the functional neuroanatomy underlying the oculomotor and vestibular system in humans.

Acknowledgments

We thank C. Unger and A. Messoudi for excellent technical assistance. The work was supported by the German Federal Ministry of Education and Research (IFBLMU01EO0901, Brain-Net-01GI0505), German Research Council (Ho 1639/4-4) and National Institutes of Health EY06069; ORIP-P510D010425; Research to Prevent Blindness.

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Fig. 1.

Photographs of transverse brainstem sections at the level of the caudal abducens nucleus (nVI) to demonstrate the vestibular nuclei in monkey (A-D) and human (E-G). A: Distribution pattern of retrogradely labelled vestibuloocular neurons following a WGA-HRP injection into the oculomotor nucleus in a macaque monkey. B: Distribution pattern of vestibular neurons immunopositive for non-phosphorylated neurofilaments (NPNF). C,D: Detailed view of retrogradely labelled large-sized vestibulo-ocular neurons in the magnocellular medial vestibular nucleus (MVm) (C) expressing NPNF-immunoreactivity (D; arrows). E: Luxol Fast Blue staining to demonstrate the location and cytoarchitecture of

the vestibular nuclei in human. F: Neighbouring section showing the distribution of NPNF-positive neurons. G: Overview and detailed view (inset) of MVm to demonstrate the morphology of NPNF-positive putative secondary vestibular neurons in human. The stars in E-G point to two blood vessels as landmarks. Scale bar = 500µm in A, B; 30µm in C,D; 1mm in E,F; 200µm in G; 30µm in inset of G