

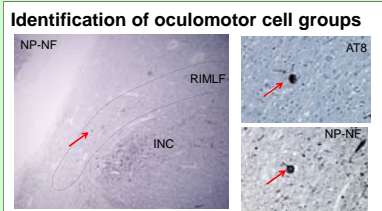
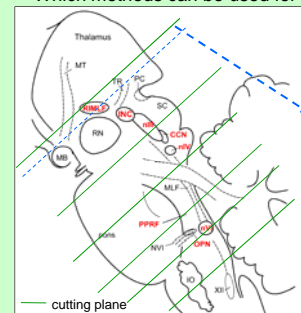
# Neuropathological correlates of ocular motor deficits in progressive supranuclear palsy (PSP)

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## Introduction

Progressive supranuclear palsy (PSP) is a tauo-pathy clinically characterized by eye movement disorders, e.g. slowing and loss of vertical saccades, later slowing and loss of horizontal saccades with intact VOR, ending up in a complete gaze palsy. Further symptoms include postural instability and early falls. With histochemical markers (e.g. non-phosphorylated neurofilaments, parvalbumin) the premotor brainstem cell groups of the saccadic and VOR system have been identified in human and now they are assessed in PSP cases from the brain bank to study degenerative changes. Here, specifically the questions are addressed:

- Is there a correlation between eye movement deficits and degeneration in premotor and/or motor neurons of the oculomotor system in PSP patients?
- Which methods can be used for quantification of degenerative changes?



**Fig. 2:** Functional oculomotor cell groups – here vertical saccadic burst neurons in the RIMLF – are identified by histochemical markers (non-phosphorylated neurofilament; NP-NF), degeneration as tau deposits are assessed in neighbouring sections stained for AT8 (red arrows).

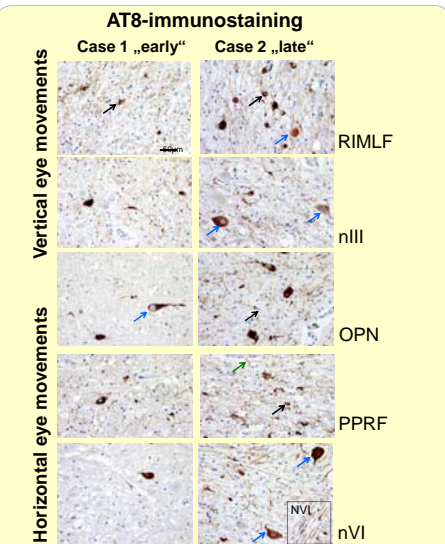
**Fig. 1:** A dissection protocol was developed to access all oculomotor-related brainstem regions.

## Methods

The clinical history of 45 archival and 4 new PSP cases from the brain bank the clinical reports were screened for history of oculomotor deficits. From 36 archival and 2 new cases with clinical reports the available tissue of so far 24 and 2 cases was screened for relevant brainstem regions, which in some cases had to be recovered from formalin and were embedded in paraffin for histological processing. Adjacent paraffin sections of oculomotor brain-stem regions were immunostained for histochemical markers identifying the relevant cell groups (parvalbumin, non-phosphorylated neurofilament) and abnormally phosphorylated tau protein (AT8) revealing degeneration. While still screening the remaining 14 cases a semi-automatic quantitative analysis of AT8-positive profiles was performed on 2 PSP cases with a trial version of HistoQuant-Software (3D Histotech) on scanned slides (Zeiss, MiraxMidi). AT8-positive neurons and glial cells were manually counted in defined areas.

Archival PSP cases / with clinical report	New PSP cases / with clinical report	For analysis	No or minor oculomotor signs "early"	Vertical gaze palsy, horizontal slowed "intermediate"	Complete gaze palsy "late"
45/36		24	4	16	4
	4/2	2			1

For a first quantitative analysis 2 PSP cases with oculomotor deficits at different degree were chosen: **Case 1:** female, 78 years, eye movements are possible, but slowed saccades, **no gaze palsy ("early")**. **Case 2:** male, 70 years, postural instability (wheel chair), **complete gaze palsy (late)**.



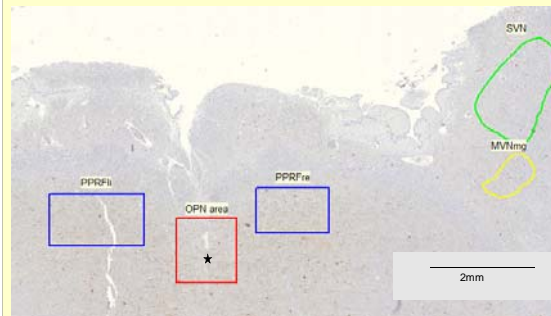
**Fig. 3:** In archival cases assessed so far AT8-immunostaining works with antigen retrieval methods. Strong AT8-staining in glial cells (black arrows) is present in the RIMLF (vertical gaze center) of both cases, but in addition involves neurons in the „late“ state (red arrows) including motoneurons (nIII, nVI).

## Results

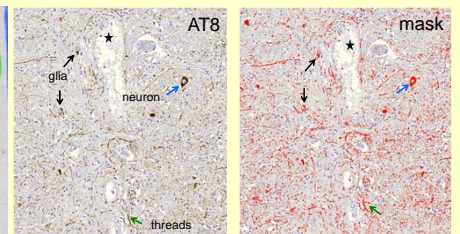
Both PSP cases show deposits of hyperphosphorylated tau protein (AT8-staining) in premotor areas for vertical and horizontal eye movements, but at different degree in different cellular compartments. Glial AT8-positive deposits and threads are present in premotor regions for horizontal (PPRF) and vertical (RIMLF) eye movements in both cases, but to a higher degree in the „late“ PSP case 2 (Fig. 3 and 6), where strong additional neuronal staining of premotor and even motoneurons is prominent. Although the semi-automatic quantitative method largely resembles the results of visual inspection of AT8-staining (Fig. 6A), the manual counting of affected glia and neurons is required to reflect the functional deficit.

## Results

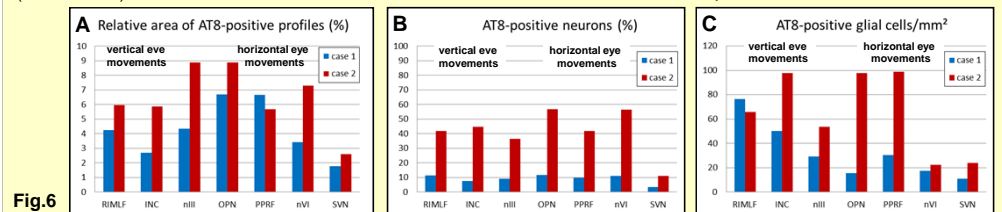
## Quantification of AT8-positive profiles



**Fig. 4:** Pontine section captured with a slide scanner (Mirax-Midi, Zeiss). Annotation areas were defined with Panoramic Viewer (3DHistotech).



**Fig. 5:** A mask of brown AT8-positive profiles (left) was determined (right; red) within the OPN area (see asterisks in Fig. 4 and 5) and analyzed with HistoQuant-Software (3DHistotech) (Fig. 6). In addition manual counting of glial cells and neurons within the annotated areas (see Fig. 4) defined for semi-automated quantification was conducted.



**Fig. 6**

**Abbreviations:** RIMLF – rostral interstitial nucleus of the medial longitudinal fascicle; INC – interstitial nucleus of Cajal; nIII – oculomotor nucleus; PPRF – paramedian pontine reticular formation; nVI – abducens nucleus; SVN – superior vestibular nucleus

## Conclusion

- Functional oculomotor cell groups can be identified in archival tissue and now be targeted for a further investigation of the tau-load.
- Tau-deposition in glia and neuronal processes precedes the affection of neuronal cytoplasm
- Tau-deposition in premotor areas precedes the involvement of motoneurons
- The extent of tau-pathology corresponds to the severity of oculomotor deficits.
- The results support the hypothesis that the disease PSP may progress along neuronal pathways in an anterograde fashion.
- The chosen parameters for quantification are critical for the interpretation of the results and have to be worked out carefully.