The nucleus raphe interpositus in the Steele–Richardson–Olszewski syndrome (progressive supranuclear palsy)

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Summary
As the integrity of the omnipause neurons located in the nucleus raphe interpositus is a prerequisite of normal ocular motility, cell and neurofibrillary tangle densities were determined in 13 Steele–Richardson–Olszewski syndrome (SROS) cases [eight with supranuclear gaze palsy (SGP) and five without] and six controls. Compared with normal controls, cases with SGP were associated with ~50% nerve cell loss (P < 0.001), whereas data from cases without SGP were not significantly different (P = 0.18). Furthermore, cases with SGP had lower neuronal cell (P = 0.016) and higher neurofibrillary tangle densities than those without (P = 0.011). These results indicate that the involvement of the omnipause neurons, which are glycinergic, contributes to abnormal eye motility in SROS. Involvement of these glycinergic nerve cells suggests that the degeneration of brainstem structures in SROS affects neurochemically diverse systems; so far other brainstem nuclei concerned with eye motility, and known to be affected in SROS, are cholinergic. The results of this study provide evidence that clinically distinct subgroups of SROS may be differentiated histologically when adequate morphometric techniques are applied.

Keywords: Steele–Richardson–Olszewski syndrome; supranuclear gaze palsy; nucleus raphe interpositus; neuronal cell loss; neurofibrillary tangle

Abbreviations: SGP = supranuclear gaze palsy; SROS = Steele–Richardson–Olszewski syndrome

Introduction
Steele–Richardson–Olszewski syndrome or progressive supranuclear palsy is a late-onset, progressive neurodegenerative condition characterized in its classical form by SGP, axial dystonia, bradykinesia, dysarthria, pseudobulbar palsy and cognitive disturbances (Steele et al., 1964). Although SGP, in association with other signs, is regarded as one of the cardinal clinical features of SROS, it may be absent in a proportion of cases (Dubas et al., 1983). The histological hallmarks of SROS include neuronal cell loss, gliosis and neurofibrillary tangles (Steele et al., 1964). The degenerative changes characteristically affect the basal ganglia, brainstem nuclei and cerebellum, but some cerebral cortical areas are also involved (Takahashi et al., 1989; Hauw et al., 1990).

The ocular motor abnormality observed in SROS classically involves severe impairment of vertical saccades and, usually late in the disease process, of horizontal saccades with relative sparing of the vestibulo-ocular reflexes (Pfaffenbach et al., 1972). Supranuclear gaze palsy in SROS correlates with degeneration of a number of primarily cholinergic brainstem structures which include the rostral interstitial nucleus of the medial longitudinal fascicle, interstitial nucleus of Cajal, superior colliculus and nucleus pontis centralis caudalis (Juncos et al., 1991).

The premotor neural network concerned with saccades lies in the paramedian pontine reticular formation and from a functional viewpoint contains two neuronal cell types: (i) the burst neurons, which are active before saccades, and (ii) pause neurons, which pause before and during eye movements. A subset of pause neurons, which have an on-going firing rate and exert a tonic inhibition on burst neurons, pause before eye movements in all directions and are known as omnipause neurons (Fuchs et al., 1985). In both monkeys and humans the omnipause neurons have been shown to lie in the caudal pontine paramedian tegmentum in an anatomically well-
defined area, which has been designated the nucleus raphe interpositus (Büttner-Ennever et al., 1988). Despite its functional importance the nucleus raphe interpositus has only rarely been examined in pathological conditions (Ridley et al., 1987; Büttner-Ennever et al., 1990).

In this study we examined the nucleus raphe interpositus in 13 SROS cases; eight presented clinically with SGP while the other five did not. The diagnosis was confirmed by full neuropathological examination in all cases.

Material and methods
Thirteen neuropathologically confirmed cases of SROS were selected from the case collections of the Parkinson’s Disease Society Brain Tissue Bank and of the Department of Neuropathology, Institute of Neurology, London. Nine of these cases have been reported in detail elsewhere (Daniel et al., 1995). Supranuclear gaze palsy was documented in life in eight of the patients, while it was absent in the remainder. Brains from six age-matched individuals who died of non-neurological disease were used as controls. All the brains used for this study had been immersed in 10% formalin at post-mortem and were then transported to the Brain Tissue Bank or the Department of Neuropathology. The neuropathological examination was carried out 6 weeks later and the tissue blocks were processed using standard techniques.

For this study the paraffin blocks of the lower pons containing the sixth nerve nucleus and the area of the nucleus raphe interpositus were cut serially in 16 μm thick tissue sections. Initially every 10th section was stained with haematoxylin and eosin and the first section containing both the sixth nerve nucleus and its rootlets was identified under a dissecting microscope. Then neighbouring sections were sequentially stained in groups of four, one with luxol fast blue/cresyl violet, one with modified Bielschowsky’s silver impregnation for axons, one with antibodies to tau (Sigma, 1:1400) and one with glial fibrillary acidic protein (Dako, 1:400).

Anatomical definitions
As suggested by Büttner-Ennever et al. (1988), the presence of the sixth nerve nucleus and its rootlets are important anatomical landmarks of the nucleus raphe interpositus. The area of the nucleus raphe interpositus in the human brainstem

Fig. 1 (A) Plate XXIII from the second edition of Cytoarchitecture of the Human Brain Stem by Olszewski and Baxter (1982), with permission to reproduce by S. Karger AG, Basel, Switzerland, illustrating the nucleus raphe interpositus in the paramedian tegmentum of the caudal pons (arrowheads). (B) Case 3. Cell and neurofibrillary tangle densities were determined in the outlined area of the nucleus raphe interpositus in each case (Flo m = medial longitudinal fascicle, N VI = abducent nucleus, VI = abducent nerve, Gc = gigantocellular nucleus, Le m = medial lemniscus). Luxol fast blue/cresyl violet. Bar = 200 μm.
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Fig. 2 (A) Case 8. Neurofibrillary tangle-bearing fusiform nerve cell in the nucleus raphe interpositus. Haematoxylin and eosin. Bar = 5 μm. (B) A neuron with tau-positive neurofibrillary tangle. The open arrowheads point to the horizontally orientated dendrites. Tau immunohistochemistry. Bar = 5 μm.

is outlined, but not named by Olszewski and Baxter (1982). Dorsally, the nucleus is surrounded by the medial longitudinal fascicle and the sixth nerve nucleus, ventrally by the nucleus gigantocellularis, while the lateral border ends before the rootlets of the sixth nerve cross the tegmentum (Fig. 1). The nucleus raphe interpositus comprises omnipause neurons in a linear arrangement on either side of the midline in the dorsal part of the tegmentum. Characteristically each fusiform nerve cell soma and its well-developed dendrites are horizontally orientated (Fig. 2) (Büttner-Ennever et al., 1988; Horn et al., 1994).

**Morphometric studies**

For cell counts, coded slides were used and the principle of the optical dissector was applied (Gundersen et al., 1988a). For the purpose of morphometry a ×100 oil-immersion objective with a high numerical aperture (1.32), providing a depth of field of 0.24 μm (Williams and Rakic, 1988), was used on a Zeiss research microscope. The microscope was fitted with a length gauge (Heidenhain, MT12) and an electronic display unit (Heidenhain, VRZ 405) for monitoring the movements of the stage in the z (focusing) axis. An unbiased grid, calibrated at 0.07×0.07 mm, was projected into the microscopic field via a Zeiss drawing tube fitted to the microscope. A 10 μm high counting box was used and counting was carried out in every third microscopic field. In the area of the nucleus raphe interpositus, nucleolated nerve cells were counted if they were either situated fully inside the counting box or their nuclei did not cross any of the forbidden planes (Gundersen et al., 1988a). As the total volume of the nucleus raphe interpositus was unknown, neuronal cell densities were calculated. As cell densities may be distorted by tissue shrinkage due to the disease process, a correction factor was calculated for all three groups of cases on the assumption that tissue shrinkage resulting from the disease process was proportional to the decrease in the height of the nucleus raphe interpositus. To estimate this decrease, a ratio ($R$) was calculated for each case comparing the height of the nucleus raphe interpositus ($h_{NRI}$) with the whole height of the pons ($h_{pons}$):

\[ R = \frac{h_{NRI}}{h_{pons}} \]

Measurements were carried out on an image analyser (Colourmorph, Perceptive Instruments, UK). The mean ratios (SD; range) in the SROS groups with SGP ($R_{sgm}$) and
Table 1  Basic clinical data of 13 patients with SROS

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex</th>
<th>Age at death (years)</th>
<th>Disease duration (years)</th>
<th>Gaze palsy</th>
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<td>67</td>
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<td>88</td>
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</table>

The mean age (SD; range) was 67.3 years (8.9; 57–74 years) in the SROS patients and 72.7 years (8.9; 57–88 years) and 78.8 years (5.3; 72–85 years), respectively (P = 0.14, two-tailed Student’s t test). The unconnected mean neuronal cell density per mm³ in the SROS group with SGP and 81.4 years (5.8; 75–88 years) in the group without SGP (P = 0.001, two-tailed Student’s t test, significant at the 1% level allowing for multiple comparisons). At death, the SROS patients with SGP were also younger than the control individuals (P = 0.002, two-tailed Student’s t test, significant at the 1% level allowing for multiple comparisons), but those without SGP were not younger than the controls (P = 0.46, two-tailed Student’s t test). There was no difference in the duration of disease between the two groups of SROS cases (P = 0.662, two-tailed Student’s t test), the mean disease duration (SD; range) being 5.9 years (2.6; 2–10 years) in the SROS group with SGP and 6.6 years (2.5; 4–9 years) in the group without SGP.

Microscopical examination of the nucleus raphe interpositus

The tegmentum of the lower pons appeared shrunken in many of the SROS cases and particularly in those with SGP. Increased astrocytosis was demonstrated with the glial fibrillary acidic protein preparation in all SROS cases with SGP and in four cases without SGP. Many nerve cells in the nucleus raphe interpositus appeared to contain neurofibrillary tangles (Fig. 2) and there was only a single case without the clinical history of SGP in which this abnormality was absent.

Morphometry

The uncorrected mean neuronal cell density per mm³ (SD; range) was 1314.9 (492; 609.2–1998) in the SROS group with SGP (n = 8), 1627.4 (396.3; 1208.4–2248.4) in the group without SGP (n = 5) and 1888 (211; 1488–2102) in the six control cases. The difference between the neuronal cell densities of the SROS group with SGP and the control group was statistically significant (P = 0.014, two-tailed Student’s t test, significant at the 5% level allowing for multiple comparisons).

The mean adjusted neuronal cell density per mm³ (SD; range) was 946.8 (354.3; 438.6–1438.6) in the SROS group.
Discussion

In this study we have shown that the nucleus raphe interpositus is severely affected in SROS; the mean cell density in patients with SGP was ~ 50% of that in controls \((P < 0.001)\). Furthermore, cases with SGP had a greater cell loss \((P = 0.016)\) and larger mean neurofibrillary tangle density \((P = 0.011)\) than those without eye movement abnormality.

An inverse relationship between cell and neurofibrillary tangle densities in the nucleus raphe interpositus has also been established \((P = 0.04)\).

Neurofibrillary tangles, neuronal cell loss and gliosis occurring in certain subcortical brainstem and cerebellar structures are the classical pathological hallmarks of SROS (Steele et al., 1964; Jellinger and Bancher, 1992; Lantos, 1994). It has been suggested that the development of neurofibrillary tangles precedes neuronal degeneration and loss (Seitelberger, 1969), and areas with the most severe nerve cell depletion usually show the largest number of tangles in this condition (Lantos, 1994). The precise cellular mechanisms which initiate neurofibrillary tangle formation and finally cell death in SROS are not clear. However, the existence of an intimate relationship between tangle formation and cell death has been shown in Alzheimer’s disease, where the occurrence of neurofibrillary tangles, in general, appears to correlate with the number of nerve cell nuclei with DNA fragmentation, which is an important indicator of nerve cell death through apoptosis (Lassmann et al., 1995).

In typical SROS cases degeneration of midbrain cholinergic structures (including the rostral interstitial nucleus of the medial longitudinal fascicule, nucleus interstitial of Cajal and superior colliculus) appears to be crucial for the development of SGP in the vertical plane (Juncos et al., 1991). The cholinergic nucleus pontis centralis caudalis, which is coextensive with the caudal portion of the paramedian pontine reticular formation, is also severely affected with a loss of up to 60% of its neurons (Malessa et al., 1991). Degeneration of the nucleus pontis centralis caudalis may perhaps be related to abnormal horizontal saccades, which usually appear late in SROS (Troost and Daroff, 1977). The results of our study have shown that, in addition to cholinergic midbrain and pontine structures, omnipause neurons in the nucleus raphe interpositus are also affected in SROS. Omnipause neurons use glycine as a neurotransmitter and receive numerous contacts from GABAergic, glycinegic and glutaminergic afferents; these neurons act as a gating mechanism and exert tonic inhibition both on the horizontal saccadic burst neurons in the paramedian pontine reticular formation and on the vertical saccadic burst neurons in the rostral interstitial nucleus of the medial longitudinal fascicule (Büttner-Ennever et al., 1988; Nakao et al., 1989; Horn et al., 1994). The morphological and functional integrity of omnipause neurons is a prerequisite to normal saccades (Büttner-Ennever et al., 1988; Nakao et al., 1989, 1991; Langer and Kaneko, 1990; Horn et al., 1994). Our finding that omnipause neurons are severely depleted and affected...
by neurofibrillary tangle formation in cases of SROS with SGP, and only to a lesser degree in those without SGP, suggests that degeneration of the nucleus raphe interpositus contributes to the abnormal eye movements in this condition. Furthermore, our observation that the glycnergic omnipause neurons are severely depleted in the paramedian pontine tegmentum in SROS with SGP, combined with the above-mentioned studies of degeneration of cholinergic brainstem structures, indicates that the disease process is both biochemically and regionally diverse.

In recent years it has been confirmed that SROS is clinically a rather heterogeneous condition; atypical cases presenting without SGP (Dubas et al., 1983), with severe dementia (Davis et al., 1985) or pure akinesia (Matsuo et al., 1991) and also a familial form of the disease (Brown et al., 1993) have all been documented. It is possible that the differences in clinical presentation may delineate distinct subgroups in SROS; patients presenting without SGP appear to be older at the disease onset, have a longer disease duration and are more often females than males (de Bruin and Lees, 1992; Daniel et al., 1995). Histological heterogeneity of SROS has also been described here and a new classification recommended, which distinguishes three subgroups: (i) typical (type 1) cases with histological features corresponding to the original description; (ii) atypical (type 2) cases in which either the severity or the distribution of changes deviates from those in typical cases; and (iii) combined (type 3) cases in which the typical histological picture of SROS is associated with features of another neurodegenerative condition (Hauw et al., 1994; Lantos, 1994). Morphologically, however, such subtypes are difficult to discriminate particularly when they rely on qualitative assessment alone (Gearing et al., 1994; Daniel et al., 1995). Other attempts at histological subclassification have been made from a clinical perspective. Investigations in one such study of 17 SROS cases failed to show any histological differences when SGP was used as discriminating criterion (Daniel et al., 1995). We report the novel finding that if careful morphometric evaluation is applied to these cases, a histological distinction can be made. Furthermore, the results of this study extend our understanding of the morphological basis of eye movement abnormality in SROS.

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