4-Aminopyridine restores vertical and horizontal neural integrator function in downbeat nystagmus

Roger Kalla,1,2* Stefan Glasauer,1,3,* Ulrich Büttner,1,3 Thomas Brandt1,3 and Michael Strupp1

1Department of Neurology, Ludwig-Maximilian University, Munich, Germany, 2Institute of Cognitive Neuroscience, University College London, London, UK and 3Bernstein Center for Computational Neuroscience Munich, Germany

*These authors contributed equally to the work.

Correspondence to: Michael Strupp, MD, Department of Neurology, Ludwig-Maximilian University, D-81377 Munich, Germany
E-mail: Michael.Strupp@med.uni-muenchen.de

Downbeat nystagmus (DBN), the most common form of acquired fixation nystagmus, is often caused by cerebellar degeneration, especially if the vestibulo-cerebellum is involved. The upward ocular drift in DBN has a spontaneous and a vertical gaze-evoked component. Since cerebellar involvement is suspected to be the underlying pathomechanism of DBN, we tested in 15 patients with DBN whether the application of the potassium-channel blocker 4-aminopyridine (4-AP), which increases the excitability of cerebellar Purkinje cells as shown in animal experiments, reduces the vertical ocular drift leading to nystagmus. Fifteen age-matched healthy subjects served as the control group. 4-AP may affect spontaneous drift or gaze-evoked drift by either enhancing visual fixation ability or restoring vision-independent gaze holding. We therefore recorded 3D slow-phase eye movements using search coils during attempted fixation in nine different eye positions and with or without a continuously visible target before and 45 min after ingestion of 10mg 4-AP. Since the effect of 4-AP may depend on the associated etiology, we divided our patients into three groups (cerebellar atrophy, n = 4; idiopathic DBN, n = 5; other etiology, n = 6). 4-AP decreased DBN during gaze straight ahead in 12 of 15 patients. Statistical analysis showed that improvement occurred predominantly in patients with cerebellar atrophy, in whom the drift was reduced from $-4.99 \pm 1.07 \text{ deg/s}$ (mean $\pm$ SE) before treatment to $-0.60 \pm 0.82 \text{ deg/s}$ afterwards. Regression analysis of slow-phase velocity (SPV) in different eye positions revealed that vertical and horizontal gaze-evoked drift was significantly reduced independently of the patient group and caused perfect gaze holding on the average. Since the observed improvements were independent of target visibility, 4-AP improved fixation by restoring gaze-holding ability. All in all, the present study demonstrates that 4-AP has a differential effect on DBN: drift with gaze straight ahead was predominantly reduced in patients with cerebellar atrophy, but less so in the remaining patients; 4-AP on the average improved neural integrator function, i.e. gaze-evoked drift, regardless of etiology. Our results thus show that 4-AP was a successful treatment option in the majority of DBN patients, possibly by increasing Purkinje cell excitability in the cerebellar flocculi. It may work best when DBN is associated with cerebellar atrophy. Furthermore, 4-AP may be a promising treatment option for patients with a dominant gaze-evoked component of nystagmus, regardless of its etiology.

Keywords: downbeat nystagmus; 3D-search coil; neural integrator; 4-aminopyridine, cerebellum

Abbreviations: ANOVA = analysis of variance; DBN = downbeat nystagmus; EA-2 = episodic ataxia type 2; FMRI = functional magnetic resonance imaging; HHT = head-thrust test developed by Halmagyi and Curthoys; PC = Purkinje cell; SCC = semicircular canals; SE = standard error of mean; SPV = slow-phase velocity; VOR = vestibulo-ocular reflex; 3.4-DAP = 3.4-diaminopyridine; 4-AP = 4-aminopyridine


Introduction

Downbeat nystagmus (DBN) is the most frequent form of acquired persisting fixation nystagmus. DBN manifests with oscillopsia due to involuntary retinal slip and postural instability (Balo and Spooner, 1981; Bronstein, 2004; Sprenger et al., 2005; Leigh and Zee, 2006; for review see Pierrot-Deseilligny and Milea, 2005). In humans it appears...
mainly in cases of cerebellar degeneration (Bronstein et al., 1987; Baloh and Yee, 1989; Leigh and Zee, 2006); however, no underlying pathology is found in ~40% of the patients (Halmagyi et al., 1983). The nystagmus consists of a spontaneous upward drift that is constant velocity, increasing velocity or decreasing velocity, and a fast phase downward during gaze straight ahead. Additional ocular motor signs such as gaze-evoked nystagmus, deficient smooth pursuit eye movements, as well as deficient visual cancellation of the vestibulo-ocular reflex (VOR) are often associated with DBN and indicate a cerebellar dysfunction (Halmagyi et al., 1983; Straumann et al., 2000; Glasauer et al., 2003, 2004, 2005b; Leigh and Zee, 2006). Animal studies in monkeys have shown that bilateral ablation of the cerebellar flocculus and paraflocculus result in DBN and an integrator deficit (Zee et al., 1981), lasting deficits in pursuit eye movements, impaired horizontal VOR adaptation (Lisberger et al., 1984; Rambold et al., 2002), and impaired visual suppression of caloric nystagmus (Takemori and Cohen, 1974).

The upward drift of DBN consists of a gaze-evoked drift, which is hypothesized to be due to an impaired neural integrator function, and a spontaneous upward drift during gaze straight ahead (Straumann et al., 2000; Glasauer et al., 2003). Three different pathomechanisms are thought to cause the spontaneous upward drift: (i) a tone imbalance of the central vestibular pathways of the vertical eye movements (Balogh and Spooner, 1981; Halmagyi et al., 1983; Brandt and Dieterich, 1995; Böhmer and Straumann, 1998), including otolith pathways as suggested by the finding that DBN is gravity dependent (Marti et al., 2002; Sprenger et al., 2006), (ii) an imbalance of the vertical smooth pursuit tone in which the imbalance of upward visual velocity commands results in spontaneous upward drift (Zee et al., 1974) and (iii) a mismatch in the 3D neural coordinate system for vertical saccade generation due to a defect of the neural velocity-to-position integrator for gaze holding (Glasauer et al., 2003).

Marti et al. (2005) proposed a mechanism by which floccular deficiency causes DBN. They suggest that the distribution of on-directions of vertical gaze-velocity Purkinje cells (PCs) is inherently asymmetrical. These cells are predominantly activated with ipsiversive and downward gaze velocity, but only ~10% of them show on-directions for upward-gaze velocity (Partsalis et al., 1995; Krauzlis and Lisberger, 1996). Using functional magnetic resonance imaging (fMRI) and F-fluorodeoxyglucose-positron emission tomography, it was recently shown that patients with DBN have diminished activation/metabolism of both floccular lobes (Bense et al., 2006; Kalla et al., 2006). This supports the view that a functional deficiency of the flocculi causes not only a defect in downward pursuit but also DBN (Marti et al., 2005).

GABAergic substances such as the GABA-A agonist clonazepam (Chambers et al., 1983; Currie et al., 1986) and the GABA-B agonist baclofen (Dieterich et al., 1991, Averbuch-Heller et al., 1997), and cholinergic drugs such as trihexyphenidyl (Leigh et al., 1991) and scopolamine (Barton et al., 1994) have been used to treat DBN, but with only moderate success (for review see Straube et al., 2004; Strupp and Brandt, 2006). It was recently demonstrated that 3,4-diaminopyridine (3,4-DAP) (Strupp et al., 2003; Halmchen et al., 2004) and the related agent 4-aminopyridine (4-AP) (Kalla et al., 2004), effectively suppressed DBN. In patients with idiopathic cerebellar ataxia, 3,4-DAP was shown to reduce the gravity dependence of the vertical drift component (Sprenger et al., 2006). In animal experiments 4-AP, a non-selective blocker of the Kv family of voltage-gated potassium channels (Hille, 2001) increased PC excitability (Etzion and Grossman, 2001). It was assumed that such enhancement of PC activity could restore the inhibitory influence of the cerebellar cortex on vertical eye movements to normal levels (Leigh, 2003).

Here, we investigated for the first time the effect of 4-AP on a larger group of 15 patients with DBN due to different etiologies. We specifically asked which mechanism, if any, would improve the nystagmus. To differentiate between improvement of spontaneous drift in gaze straight ahead and improvement of gaze-evoked drift, we recorded eye movements in different eye positions. The experiment was performed with a continuously visible target and with a flashed target in darkness to determine if improvement of fixation ability with 4-AP was due to an increase in visual fixation suppression, as was shown for a case of upbeat nystagmus (Glasauer et al., 2005a), or was independent of visual input. Finally, to investigate whether the efficacy of 4-AP on DBN depends on the underlying etiology, patients were assigned to three groups according to their diagnosis (DBN associated with cerebellar atrophy, idiopathic DBN and DBN of other etiology). We hypothesized that if 4-AP increased the sensitivity of PCs involved in neural integration as shown in animal experiments, the drug would mainly decrease the gaze-evoked part of DBN independently of visual input.

**Methods**

**Subjects**

Fifteen patients with DBN due to different etiologies (aged 23–79 years, mean 61.7 ± 16.6 years; for details see Table 1) participated in the study. The exclusion criteria were the following (Hayes et al., 2003): patients with a history of seizure, heart attack, angina pectoris, stroke, hypertension or asthma; a history or the documentation of cardiac arrhythmias or QT-lengthening on electrocardiogram; a history of diseases or conditions that could substantially affect the pharmacokinetics of aminopyridines (e.g. renal, hepatic or gastrointestinal disease); women who were lactating or pregnant, or of childbearing age and not using approved birth control methods. No patient was taking medication that could affect the vestibular or ocular motor systems at the time of measurements. All patients underwent a complete neurological, neuro-ophthalmological and neuro-otological examination, electronystagmography (including caloric irrigation),
# Table 1: Clinical data of the patients with DBN

<table>
<thead>
<tr>
<th>No./Sex/Age</th>
<th>Additional neuro-ophthalmologic findings</th>
<th>MRI findings</th>
<th>Etiology</th>
<th>Time</th>
<th>SPV Pre mean ± SE</th>
<th>SPV Post mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. I-1W, 66</td>
<td>Disturbed visual fixation suppression of VOR, incomplete ocular tilt reaction, impaired smooth pursuit, rebound nystagmus</td>
<td>Cerebellar atrophy</td>
<td>Idiopathic cerebellar ataxia</td>
<td>4y</td>
<td>−6.03 ± 0.08</td>
<td>−0.86 ± 0.08</td>
</tr>
<tr>
<td>No. I-2W, 75</td>
<td>Impaired visual fixation suppression of VOR; impaired horizontal and vertical pursuit; pathological HTT bilaterally</td>
<td>Cerebellar atrophy</td>
<td>Cerebellar degeneration</td>
<td>9y</td>
<td>−8.60 ± 0.19</td>
<td>−1.73 ± 0.11</td>
</tr>
<tr>
<td>No. I-3 M, 64</td>
<td>Impaired visual fixation suppression of VOR; impaired vertical pursuit</td>
<td>Cerebellar atrophy</td>
<td>Cerebellar degeneration</td>
<td>6m</td>
<td>−1.75 ± 0.04</td>
<td>−0.03 ± 0.04</td>
</tr>
<tr>
<td>No. I-4W, 64</td>
<td>Horizontal hypermetric saccades; disturbed visual fixation suppression of VOR</td>
<td>Cerebellar atrophy</td>
<td>Cerebellar degeneration</td>
<td>3y</td>
<td>−1.82 ± 0.08</td>
<td>0.49 ± 0.12</td>
</tr>
<tr>
<td>No. II-5 M, 67</td>
<td>Impaired downward smooth pursuit, hypometric upward saccades</td>
<td>Normal</td>
<td>Unknown</td>
<td>9y</td>
<td>−3.94 ± 0.09</td>
<td>−0.17 ± 0.04</td>
</tr>
<tr>
<td>No. II-6 W, 73</td>
<td>Impaired smooth pursuit, strabismus sursaadductorius</td>
<td>Normal</td>
<td>Unknown</td>
<td>1y</td>
<td>−2.11 ± 0.06</td>
<td>−1.23 ± 0.05</td>
</tr>
<tr>
<td>No. II-7 W, 79</td>
<td>Disturbed visual fixation suppression of VOR, incomplete ocular tilt reaction, impaired smooth pursuit; pathological HTT bilaterally</td>
<td>Normal</td>
<td>Unknown</td>
<td>5y</td>
<td>−2.10 ± 0.06</td>
<td>−0.67 ± 0.06</td>
</tr>
<tr>
<td>No. II-8 M, 68</td>
<td>Disturbed visual fixation suppression of VOR, impaired smooth pursuit</td>
<td>Normal</td>
<td>Unknown</td>
<td>8y</td>
<td>−2.10 ± 0.07</td>
<td>−1.51 ± 0.05</td>
</tr>
<tr>
<td>No. II-9 W, 58</td>
<td>Impaired smooth pursuit</td>
<td>Normal</td>
<td>Unknown</td>
<td>7y</td>
<td>−3.46 ± 0.05</td>
<td>−3.77 ± 0.06</td>
</tr>
<tr>
<td>No. III-10 M, 72</td>
<td>Disturbed visual fixation suppression of VOR, impaired smooth pursuit, hypermetric saccades</td>
<td>Normal</td>
<td>Microangiopathy</td>
<td>5y</td>
<td>−6.91 ± 0.06</td>
<td>−6.20 ± 0.09</td>
</tr>
<tr>
<td>No. III-11 M, 55</td>
<td>Rebound nystagmus; impaired visual fixation suppression of VOR; impaired horizontal and vertical pursuit</td>
<td>Microangiopathy</td>
<td>Vascular</td>
<td>1.5y</td>
<td>−1.99 ± 0.07</td>
<td>−3.80 ± 0.06</td>
</tr>
<tr>
<td>No. III-12 W, 26</td>
<td>Impaired smooth pursuit in all directions, hypermetric upward saccades, downward and lateral gaze-holding deficit, impaired optokinetic nystagmus in all directions, bilaterally disturbed visual suppression of VOR</td>
<td>Normal</td>
<td>Episodic ataxia type 2 (EA2)</td>
<td>12y</td>
<td>−4.58 ± 0.08</td>
<td>−3.29 ± 0.07</td>
</tr>
<tr>
<td>No. III-13 W, 23</td>
<td>Impaired smooth pursuit, impaired upward optokinetic nystagmus</td>
<td>Several supra-tentorial small white matter lesions</td>
<td>Multiple sclerosis</td>
<td>2.5y</td>
<td>−5.22 ± 0.17</td>
<td>−4.68 ± 0.18</td>
</tr>
<tr>
<td>No. III-14 W, 77</td>
<td>Disturbed visual fixation suppression of VOR</td>
<td>Upper right cerebellar peduncle</td>
<td>Infarction of cerebellum</td>
<td>4y</td>
<td>−2.15 ± 0.05</td>
<td>−4.24 ± 0.08</td>
</tr>
<tr>
<td>No. III-15 W, 59</td>
<td>Disturbed visual fixation suppression of horizontal VOR, rebound nystagmus, impaired smooth pursuit, incomplete ocular tilt reaction</td>
<td>Meningioma</td>
<td>Cerebellar meningioma</td>
<td>2y</td>
<td>−1.24 ± 0.05</td>
<td>−0.76 ± 0.05</td>
</tr>
</tbody>
</table>

Data arranged according to the assumed diagnosis (given in the fourth row). The 15 patients included in the study are characterized by abbreviations of their sex, age, additional neuro-ophthalmological findings, MRI findings, etiology of DBN due to cerebellar atrophy (I), unknown etiology (II) or other etiologies (III), time since diagnosis of DBN, SPV of DBN pre- and 45-min post-medication of 10 mg 4-AP. VOR = vestibulo-ocular reflex; HHT = head-thrust test developed by Halmagyi and Curthoys (1988).
laboratory tests (including vitamin B12 and Mg²⁺), MRI (including high resolution MRI of the brainstem and cerebellum) and electrocardiogram. DBN was associated with cerebellar atrophy (4 patients), episodic ataxia type 2 (1), multiple sclerosis (1), infarction (1), cerebellar meningioma (1), microangiopathy (2) or of unknown etiology (5) (Table 1). In none of the patients was DBN caused by drugs or metabolic disorders. The mean duration of DBN was 4.9 years (range: 6 months–12 years, see Table 1).

Fifteen healthy persons (7 men, 8 women) without a history of vestibular or central-nervous disorder were recruited for two separate control groups, because of the wide range of the ages within the patient group (group 1 \([n=7]\), elderly \([>55]\): aged 56–66 years, mean 60 ± 3.8 years; group 2 \([n=8]\), young \([≤45]\): aged 22–45 years, mean 36 ± 8.1 years). All subjects gave their consent to participate in the study after being informed of the experimental procedure. The recordings were approved by the local ethics committee of the Medical Faculty of the University of Munich and done in accordance with the Helsinki II Declaration.

### Procedure and experimental paradigms

The subjects were seated in the centre of a field-coil system consisting of a cubic aluminium frame (side length 140 cm) that produced three orthogonal magnetic fields (Remmel Systems, Ashland, MA, USA). During data acquisition, the head of the subject was immobilized in an upright position by an adjustable chin rest. Rotation of the eye around the horizontal \((z\text{-axis})\), vertical \((y\text{-axis})\) and the torsional \((x\text{-axis})\) axes was recorded with a dual search coil (Skalar, Delft, the Netherlands), which was placed on the left eye after topical anesthesia with oxybuprocaine-HCl. The signals were digitized with a 12 bit analog–digital converter at a sampling rate of 1 kHz. The average 3D direction of the eye movement and the slow-phase velocity (SPV) of the nystagmus were evaluated in nine different horizontal–vertical eye positions in the dark while showing a continuously visible red laser dot, size 0.1° or a flashed target (visible for 750 ms before and after the target jump, dark period 2.5 s) in order to avoid visual interference by fixation of the target. This target was situated on a screen placed 140 cm in front of the subject ±18° from the centre on the horizontal and vertical meridians and ±25.46° on the diagonal meridians. An additional search coil fixed on the subject’s forehead monitored immobility. The 3D eye movement recordings were performed before and 45 min after ingestion of 10 mg 4-AP. The data of one patient (III-13) under the flashed condition were lost due to technical problems.

### Calibration

The exact procedure has been described elsewhere (Glasauer et al., 2003). Immediately before recording, the coil was moved manually within the coil frame to determine the magnitude and relative orientation of the three magnetic fields and the gain of the directional coil. Then, the offsets and the gain of the torsional coil as well as the relative orientation of the coil were determined on the basis of data recorded while the subject was wearing the coil and looking at different target positions. The resulting eye positions were expressed as rotation vectors (Haustein et al., 1989).

### Data analysis

Three-dimensional eye movements (right-handed coordinates: downward positive, upward negative) were analysed off-line in Matlab (The Mathworks). The calibrated data were low-pass filtered using a digital Gaussian filter with a bandwidth of 30 Hz. Saccade and fast phase were automatically detected and removed from the data using a combined velocity–acceleration criterion in interactive software so that detection errors could be detected manually. Slow phases shorter than 25 ms were discarded. For each remaining slow phase, the median slow-phase component velocity and eye position were used for analysis. Slow phases longer than 200 ms were divided into two or more parts of equal length to avoid collecting fewer data points for periods without frequent fast phases. Drift during gaze straight and eye-position sensitivity of drift (corresponding to the negative inverse drift time constant) were computed by linear regression, i.e. by expressing 2D SPV as function of 2D eye position (for details, see Glasauer et al., 2003). For statistical analysis, a repeated measures ANOVA (Statistica 6.1, Statsoft) with post hoc Scheffe tests was performed. Pearson correlation analysis was used to investigate relationships between spontaneous vertical drift and eye-position sensitivity in patients.

### Control group

A separate repeated measures ANOVA was performed for the control subjects to test possible effects of the medication. The ANOVA design was composed of AGE as continuous covariate and two within-subjects factors (LIGHT: light/dark; SESSION: pre, post 45 min).

### Patient + control groups

A repeated measures ANOVA was performed to test for the effects of 4-AP on vertical spontaneous drift and gaze-dependent SPV of DBN (=eye position sensitivity) in DBN. To evaluate the effects of a single dose of 4-AP in different forms of DBN, patients were divided into groups with DBN associated with cerebellar degeneration (DBN I; 4 patients), DBN with unknown etiology (DBN II; 5 patients), and DBN due to other etiologies (DBN III; 6 patients) (for details see Table 1). The ANOVA design was thus composed of one between-subjects factor (GROUP: normal/ cerebellar/unknown/other) and two within-subjects factors (LIGHT: light/dark; SESSION: pre/post 45 min).

### 4-AP

Capsules with 10 mg 4-AP were used. This treatment dosage is in line with findings in human trials, which have reported good tolerance to 4-AP at dosages of up to 50 mg per day and short-term exposure (Hayes et al., 2003; DeForge et al., 2004; Hayes, 2004). The capsules were manufactured and delivered by the pharmacy of the University of Munich (Klinikum Grosshadern). The generic drug was delivered by Synopharm GmbH, Pharmaceutical company, Barsbuettel, Germany, to our pharmacy. Two subjects reported transient, minor perioral and digital paresthesia and one patient nausea and headache. No other side effects were observed.

### Results

Raw vertical eye movement traces from all patients are presented in Fig. 1 (see Supplementary Material for the same data in higher resolution). For some subjects, the
positive effect of 4-AP on DBN was clearly visible from the raw data (e.g. I-1, I-2 and II-5). It was less obvious for others, and even increases of DBN were observed (III-11, III-14). A detailed analysis of vertical and horizontal eye movements is given below.

Effects of 4-AP on spontaneous vertical drift in DBN

The ANOVA on spontaneous vertical drift revealed a significant main effect of SESSION \[ F(1,25) = 25.4, P < 0.0001 \], which was due to reduction of mean SPV of DBN with gaze straight ahead after medication (Fig. 2 (left) and Fig. 3). In patients, SPV was reduced from \(-4.31 \pm 0.58\) deg/s (mean ± SE) before treatment to \(-2.52 \pm 0.44\) deg/s after ingestion of 4-AP. Improvement was found in 12 of 15 patients regardless of target visibility (DBN I: 4/4, DBN II: 4/5, DBN III: 4/6). The main effect of GROUP \[ F(3,25) = 25.9, P < 0.0001 \] was due to a difference between controls and all patients (post hoc Scheffé \( P < 0.002 \)), while there was no significant difference between patient groups. An interaction of SESSION × GROUP \[ F(3,25) = 16.3, P < 0.0001 \] revealed that the reduction in SPV was group-dependent: only patients with cerebellar atrophy showed a significant reduction in drift due to pre \((-4.99 \pm 0.72\) deg/s) versus post medication \((-0.60 \pm 0.56\) deg/s) (post hoc Scheffé \( P < 0.0001 \)) (see Fig. 3). There was a further significant main effect for LIGHT \[ F(1,25) = 47.2, P < 0.0001 \] due to lower SPV when the target was visible. An interaction of LIGHT × GROUP \[ F(3,25) = 13.9, P < 0.0001 \] confirmed that this fixation suppression was only visible in patients (light: \(-2.72 \pm 0.47\) deg/s; dark: \(-4.11 \pm 0.48\) deg/s). Post hoc tests showed that fixation suppression was significant for patient groups DBN II and III (both Scheffé \( P < 0.015 \)), but not for control subjects or cerebellar DBN patients.

Effects of 4-AP on spontaneous horizontal drift in DBN

In most cases, DBN is not a purely vertical nystagmus (Glasauer et al., 2003). We therefore assessed spontaneous horizontal drift as the absolute horizontal SPV component during gaze straight ahead. Medication had no significant effect on the horizontal nystagmus component. There was, however, an effect of GROUP \[ F(3,25) = 3.2, P = 0.04 \]; the largest horizontal drifts were found in cerebellar patients (0.68 ± 0.16 deg/s). In addition the drift became smaller when the target was visible during fixation [LIGHT \[ F(1,25) = 11.2, P = 0.002 \], thus demonstrating fixation suppression.

Vertical eye position dependence of DBN

Multiple regression analysis of vertical SPV in all DBN patients in different eye positions revealed that before treatment SPV depended on eye position according to Alexander’s law; the eye position sensitivity (negative inverse drift time constant) of SPV was \(-0.083 \pm 0.009\) deg/s/deg vertical (corresponding to a drift time constant of 11.7 s vertical). This implies gaze-evoked vertical nystagmus. The eye position sensitivity decreased to \(-0.007 \pm 0.008\) deg/s/deg vertical after administration of 4-AP (i.e. time constants increased to above 100 s after 45 min) (Fig. 4). A change of vertical eye position sensitivity towards zero [i.e. improvement of gaze-evoked nystagmus, Fig. 2 (right)] was found in 13 of 15 patients.
Accordingly, an ANOVA on vertical eye position sensitivity revealed a significant main effect of SESSION \( [F(1,25) = 197.3, \ P < 0.0001] \) and GROUP \( [F(3,25) = 12.2, \ P < 0.0001] \). The effect of GROUP was due to the difference between patients and controls. An interaction of SESSION/GROUP \( [F(3,25) = 25.57, \ P < 0.0001] \) revealed that medication had an effect in all patient groups (post hoc Scheffé, all \( P < 0.005 \)), and that after medication the average time constant of patients was not different from those of the controls. Prior to medication, the vertical eye position sensitivity depended on target visibility (interaction SESSION/LIGHT \( [F(1,25) = 9.6, \ P = 0.0047] \)), with a higher eye position sensitivity in the dark. No other effects became significant.

**Eye position dependence of horizontal nystagmus**

The horizontal component of nystagmus also showed an eye position dependence which, on the average, complied with Alexander’s law. Treatment significantly \( [F(1,25) = 157.2, \ P < 0.0001] \) reduced the horizontal eye position dependence from \( -0.072 \pm 0.012 \text{deg/s/deg} \) (mean ± SEM) to \( -0.014 \pm 0.010 \text{deg/s/deg} \) (patients).
As expected, the patients showed higher eye position sensitivity than controls [GROUP \( F(3,25) = 8.0, P = 0.0007 \)] (Fig. 5). The effect of medication differed for the subject groups [GROUP \( \times \) SESSION \( F(3,25) = 28.2, P < 0.0001 \)]. Post hoc tests showed that drift was significantly reduced in patient groups DBN I (Scheffé, \( P < 0.0001 \)) and DBN III (\( P = 0.0002 \)), but not in DBN II (\( P = 0.057 \)). Additionally, drift was lower with the visible target [LIGHT \( F(1,25) = 11.8, P = 0.0021 \)], which shows fixation suppression of horizontal gaze-dependent drift. A 3-way interaction (SESSION \( \times \) GROUP \( \times \) LIGHT) was due to group-specific differences in fixation suppression ability: on the average, DBN II patients profited from medication only with a visible target, but still showed considerable drifts in darkness (Fig. 5).

**Fig. 4** Vertical eye position sensitivity (=inverse drift time constant) in control subjects and DBN patients (groups I–III), before (PRE) and after (POST) administration of 4-AP (grey circles: target visible; black squares: target blanked). Patients had significantly shorter drift time constants than did controls; they increased after medication to values close to normal. Similar effects were observed for all different patient groups. Error bars indicate 95% confidence intervals.

**Relations between changes in drift and sensitivity**

As expected from the close relation between spontaneous vertical drift in darkness and in light (Fig. 3), medication-induced changes (pre- versus post-medication) in observed drift correlated strongly in light and darkness (\( r = 0.96, P < 0.001 \)). The change of spontaneous drift relative to pre-medication is shown in Fig. 6 (left). This confirms again that medication did not primarily lead to better fixation suppression of ocular drift, but suppressed (or, in three cases, increased) the drift in general. A similar but weaker relation was found between changes (pre- versus post-medication) of eye position sensitivity in light and changes of eye position sensitivity in darkness [\( r = 0.65, P = 0.012; \)]...
Fig. 6 (right)]. Changes (pre- versus post-medication) in spontaneous drift were not significantly correlated with changes in eye position sensitivity of drift.

4-AP and healthy controls

Spontaneous drift and eye position sensitivity

Separate ANOVAs were performed on the data of control subjects to determine the possible effects of the medication and age-related effects. The ANOVA on ‘spontaneous vertical drift’ in healthy controls revealed a significant main effect of SESSION \( F(1,13) = 12.1, P = 0.004 \), which was due to reduction of a small downward drift after medication. This reduction depended on target visibility (interaction SESSION \( \times \) LIGHT \( F(1,13) = 13.8, P = 0.003 \)): in darkness, the drift was reduced from \( 0.30 \pm 0.13 \) deg/s (mean ± SE) before treatment to \( -0.03 \pm 0.13 \) deg/s after medication, while there was no change in light, because subjects could visually suppress the drift. Age did not significantly influence the results.

‘Spontaneous horizontal drift’ in healthy controls was only affected by target visibility \( F(1,13) = 6.8, P = 0.022 \), but not by age or medication.

The analysis of vertical eye position sensitivity showed a significant main effect of SESSION \( F(1,13) = 13.5, P = 0.0027 \), which was due to a small change in eye position sensitivity from \( -0.009 \pm 0.002 \) deg/s/deg (mean±SEM) before treatment to \( -0.0004 \pm 0.002 \) deg/s/deg after ingestion of 4-AP. This change in eye position sensitivity corresponds to an increase in gaze-holding ability and integrator function and was not age dependent. No other effects became significant.

‘Eye position sensitivity of horizontal drift’ was also significantly affected by medication \( F(1,13) = 11.8, P = 0.0044 \), but the effect was age-dependent as shown by an interaction AGE \( \times \) SESSION \( F(1,13) = 5.4, P = 0.037 \). Eye position sensitivity tended to increase to positive values for the elderly after medication from \( -0.004 \pm 0.003 \) deg/s/deg (mean ± SEM) before treatment to \( 0.014 \pm 0.002 \) deg/s/deg after ingestion of 4-AP, i.e. the drift violated Alexander’s law. Target visibility influenced the sensitivity \( F(1,13) = 8.0, P = 0.014 \).

Discussion

The present results show for the first time that the clinical improvements seen after intake of 4-AP are due to an improvement of fixation with gaze straight ahead independently of visual input as well as an improved gaze-holding ability. Previous work showed the clinical benefit of 3,4-diaminopyridine (3.4-DAP), but did not provide evidence towards the underlying mechanisms of the pharmacological intervention by Strupp et al. (2003). Our results also demonstrate for the first time in a patient group \( (n = 15) \), that 4-AP is a successful treatment option in more than 80% of DBN patients, with a probably higher success rate than 3.4-DAP.

Differential effects of 4-AP on the ocular motor system in DBN

The present study shows that 4-AP has differential effects on DBN depending on its etiology. After medication with 4-AP, the spontaneous upward drift during gaze straight ahead was reduced in 12 of 15 patients. The improvement of spontaneous drift was most prominent in patients with cerebellar atrophy (DBN I), but less so in patients with other (DBN III) or unknown (DBN II) etiologies. This is of clinical relevance, since the best efficacy of the drug can be
expected in those patients in whom DBN is associated with cerebellar atrophy. In contrast to its effect on spontaneous drift, 4-AP improved the ability to hold gaze in an eccentric position in the majority of patients, irrespective of etiology. Before treatment, nystagmus slow-phase velocity increased during eccentric gaze, i.e. gaze-evoked nystagmus (vertical and horizontal) was superimposed on the spontaneous drift, as was shown previously (Straumann et al., 2000; Glasauer et al., 2003). The reduction of SPV in eccentric positions after medication implies that 4-AP partially restored the function of the neural velocity-to-position integrator, since it was found irrespective of target visibility, and, therefore, was not caused by an improvement in fixation suppression of the nystagmus. This finding is in line with a previous single case study, where 4-AP reduced DBN independently of target visibility, whereas for an upbeat patient nystagmus in darkness was almost unaffected after medication (Glasauer et al., 2005a, b). Furthermore, our results show that patients (groups II and III) can partially suppress spontaneous drift by visual input. This ability depends on etiology: it was not found in patients with cerebellar atrophy (group I). Notably, 4-AP did not further improve visual drift suppression. Visual suppression of gaze-evoked nystagmus was also observed, specifically for vertical nystagmus. Again, 4-AP had no effect on the visual suppression ability, with the exception of patient group II, who showed better improvement of horizontal gaze-evoked nystagmus in light than in darkness (Fig. 5).

The question arises as to how the different observed changes in spontaneous upward drift and gaze-evoked drift of DBN can be explained. Spontaneous upward drift most likely corresponds to a bias in neural activity. It was proposed that this bias may correspond to a dysfunction of cerebellar PCs in the floccular lobe (Marti et al., 2005). PCs, the sole output neurons of the floccular complex (Ito, 1984), modulate their activity with eye movements, predominantly gaze velocity, and exhibit either roughly horizontal or roughly vertical directional sensitivities (Miles et al., 1980; Chubb and Fuchs, 1982; Partsalis et al., 1995; Krauzlis and Lisberger, 1996). While horizontal PCs with ipsi- and contraversive sensitivity are distributed symmetrically in both floccular lobes, about 90% of vertical floccular PCs increase their firing rates during downward eye movements, while only the remaining 10% do so during upward movement (Stone and Lisberger, 1990; Krauzlis and Lisberger, 1996). This asymmetry of the directional specificity of vertical floccular PCs in the monkey was also found in humans by comparing floccular activity during downward and upward pursuit with fMRI in healthy subjects (Stephan et al., 2005). A deficient flocculus with Purkinje cell loss due to, e.g. cerebellar degeneration, might thus cause the observed upward drift in DBN due to reduced or absent resting discharge activity of the asymmetrically distributed vertical gaze-velocity sensitive PC population (Marti et al., 2005). This is in line with the mechanism of DBN proposed by Pierrot-Deseilligny and Milea (2005), which favours a disinhibition of the superior vestibular nucleus - ventral tegmental tract pathway due to a floccular lesion. Thus the relative hyperactivity of the drive to the motoneurons of the eye elevator muscles results in an upward slow phase.

Gaze holding in darkness is mediated by a network consisting of the horizontal (medial vestibular nucleus and nucleus prepositus hypoglossi) and vertical (interstitial nucleus of Cajal) brainstem integrators. These integrators are inherently leaky, and their performance is significantly improved by a brainstem-cerebellar loop including the cerebellar floccular lobe (Zee et al.; 1981) and the paramedian tract neurons (Nakamagoe et al., 2000). The gaze-holding deficits observed in DBN have, therefore, also been attributed to failure of floccular PCs. However, it can be hypothesized that it is not the resting discharge that determines how well the integrator works, but the sensitivity of the PCs. If the PCs are unable to change their firing rate in response to eccentric gaze, they cannot contribute to gaze holding. The same holds for visual suppression of nystagmus: floccular gaze velocity PCs change their firing rate when vestibular nystagmus must be suppressed by fixation of a target moving with the head.

The differential effect of 4-AP on spontaneous drift and gaze holding may thus be explained by the specific action of the potassium channel blocker. In patients with cerebellar atrophy (DBN I), both spontaneous drift and integrator function improved. This implies that PC resting discharge and sensitivity could be restored in these patients. In contrast, the effect on spontaneous drift in the other patients (DBN II and III) was small or even absent (Fig. 3). However, 4-AP again led to an improvement in integrator function, which suggests that 4-AP mainly affected the excitability of the PCs in these patient groups, but not their resting discharge.

The improvement in the gravity-dependent part of the upward drift previously observed after medication with 3,4-DAP in DBN patients with idiopathic cerebellar ataxia (Sprenger et al., 2006) may be caused by improved gaze holding, since it was hypothesized that positional nystagmus is a result of damage to cerebellar pathways mediating neural integrator function (Glasauer et al., 2001).

Our results in control subjects support the findings in patients. Even though ocular drifts were small in the control group, significant effects of 4-AP were found for both suppression of spontaneous and gaze-evoked drift. This suppression was not caused by a visually dependent mechanism, but can be attributed to mechanisms restoring intrinsic balance of neural populations and integrator function.

All in all, our results suggest that 4-AP is to date the most effective agent to improve spontaneous upward drift and neural integrator function in DBN, and furthermore possibly a treatment option for patients with various types of gaze-evoked nystagmus.
The effects of cellular mechanisms of 4-AP on Purkinje cells

Administration of 4-AP for DBN is likely to increase the excitability of cerebellar PCs; this was observed in vitro (Etzion and Grossman, 2001). 4-AP and its structural analogues such as 3,4-DAP are non-selective blockers of the Kv family of the voltage-gated potassium channels (Coetzee et al., 1999). They block the intracellular opening of Kv channels and prolong depolarization by preventing the repolarizing effect of K⁺ efflux (Howe and Ritchie, 1991). When applied in low concentrations (1–10 μM) to guinea pig cerebellar PCs, 4-AP markedly shortened the duration of the slowly depolarizing potential, which reduces the latency for Ca²⁺ spikes (Etzion and Grossman, 2001).

In cerebellar slices, dendritic calcium spikes that were generated by the P/Q-type voltage-gated calcium channels terminated burst firing in PCs (Womack and Khodakhah, 2004). Partial blockade of P/Q-type channels concurrently eliminated dendritic calcium spikes and caused a switch from regular bursting to tonic firing or irregular bursting (Womack and Khodakhah, 2004). Thus, blocking Kv channels with 4-AP may increase the time that the P/Q-type calcium channels are open and restore regular patterns of PC firing in DBN due to cerebellar degeneration. This view of the pharmacological interaction is supported by recent findings in both tottering mice (Weisz et al., 2005), which exhibit the common characteristics of human episodic disorders resulting from a mutation within the gene encoding the α₂,1 subunit of the Caᵥ2.1 calcium channel (Fletcher et al., 1996), and in humans suffering from EA-2 (Strupp et al., 2004), in whom 4-AP relieves ataxic symptoms. The proposed interactions between the blocking of potassium channels and the opening of calcium channels are further supported by the finding that 4-AP may also evoke complex spikes in PCs similar to those elicited by climbing fibre stimulation (Cavelier et al., 2002). Finally, 4-AP may help restore the regularity and precision of intrinsic PC firing, which, according to the cerebellar pacemaking model proposed by Walter and colleagues (2006), is an essential component of cerebellar output and subsequent motor coordination.

Clinical implications of 4-AP

Our results suggest that 4-AP in a single dosage of 10 mg is an effective agent to improve spontaneous upward drift (in 80% of our DBN patients) and neural integrator function (in 87% of DBN patients). The related agent 3,4-DAP reduced DBN in ~50% of the patients with a single dosage of 20 mg (Strupp et al., 2003). Further cross-over trials should be conducted to compare the two compounds and to establish the appropriate dosages and dosage intervals.

Along with the above-mentioned cellular mechanisms of 4-AP that affect PCs, our results in DBN suggest that the aminopyridines might also be a promising treatment option for ocular motor disorders due to a genetic impairment of the PCs like EA-2. Further trials are required to test the clinical efficacy of 4-AP in these disorders.

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