Effects of Nucleus Prepositus Hypoglossi Lesions on Visual Climbing Fiber Activity in the Rabbit Flocculus

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Arts, M. P., C. I. De Zeeuw, J. Lips, E. Rosbak, and J. I. Simpson. Effects of nucleus prepositus hypoglossi lesions on visual climbing fiber activity in the rabbit flocculus. J Neurophysiol 84: 2552–2563, 2000. The caudal dorsal cap (dc) of the inferior olive is involved in the control of horizontal compensatory eye movements. It provides those climbing fibers to the vestibulocerebellum that modulate optimally to optokinetic stimulation about the vertical axis. This modulation is mediated at least in part via an excitatory input to the caudal dc from the pretectal nucleus of the optic tract and the dorsal terminal nucleus of the accessory optic system. In addition, the caudal dc receives a substantial GABAergic input from the nucleus prepositus hypoglossi (NPH). To investigate the possible contribution of this bilateral inhibitory projection to the visual responsiveness of caudal dc neurons, we recorded the climbing fiber activity (i.e., complex spikes) of vertical axis Purkinje cells in the flocculus of anesthetized rabbits before and after ablative lesions of the NPH. When the NPH ipsilateral to the recorded flocculus was lesioned, the spontaneous complex spike firing frequency did not change significantly; but when both NPHs were lesioned, the spontaneous complex spike firing frequency increased significantly. When only the contralateral NPH was lesioned, the spontaneous complex spike firing frequency decreased significantly. Neither unilateral nor bilateral lesions had a significant influence on the depth of complex spike modulation during constant velocity optokinetic stimulation or on the transient continuation of complex spike modulation that occurred when the constant velocity optokinetic stimulation stopped. The effects of the lesions on the spontaneous complex spike firing frequency could not be explained when only the projections from the NPH to the inferior olive were considered. Therefore we investigated at the electron microscopic level the nature of the commissural connection between the two NPHs. The terminals of this projection were found to be predominantly GABAergic and to terminate in part on GABAergic neurons. When this inhibitory commissural connection is taken into consideration, then the effects of NPH lesions on the spontaneous firing frequency of floccular complex spikes are qualitatively explicable in terms of relative weighting of the commissural and caudal dc projections of the NPH. In summary, we conclude that in the anesthetized rabbit the inhibitory projection of the NPH to the caudal dc influences the spontaneous firing frequency of floccular complex spikes but not their modulation by optokinetic stimulation.

INTRODUCTION

The inferior olive (IO) is the sole source of the climbing fibers (CFs) that innervate Purkinje cells in the cerebellar cortex. In the adult animal, the dendritic tree of each Purkinje cell is innervated by only one CF, which produces a powerful excitation generating a complex spike (CS) (Eccles et al. 1966; Thach 1967). Because the CS is an all-or-none response, it directly reflects the activity of an individual IO neuron. The activity pattern of olivary neurons is substantially influenced by their intrinsic membrane and coupling properties. Olivary neurons have a unique combination of membrane conductances that causes them to fire at a low average frequency of about 1 spike/s (Crill 1970). In the slice preparation or with administration of tremorgenic drugs like harmaline, these conductances allow olivary neurons to oscillate and to fire rhythmically (Llinás and Volkind 1973; Llinás and Yarom 1981a,b). Further, olivary neurons are electrotonically coupled by dendrodendritic gap junctions and tend to fire synchronously (Llinás et al. 1974; Sotelo et al. 1974). This electrotonic coupling has been demonstrated to be dynamic (Llinás and Sasaki 1989; Welsh et al. 1995). For example, the GABAergic terminals that directly surround the electrotonically coupled olivary dendrites can influence the level of coupling (De Zeeuw et al. 1989, 1990; Lang et al. 1996).

Another major role of the GABAergic input to the IO may be to modulate the firing frequency of its neurons (Barmack et al. 1989, 1993b). A useful system for addressing this role of the GABAergic input to the IO is the dorsal cap (dc) because for this olivary subdivision, the sources of both its excitatory and inhibitory inputs have been identified and many characteristics of the responses of dc neurons to natural visual stimulation are known (e.g., De Zeeuw et al. 1994b; Leonard et al. 1988). The dc, together with the ventrolateral outgrowth (VLO) of the IO, provide the great majority of CFs to the flocculus of the vestibulocerebellum (Tan et al. 1995). The flocculus is involved in controlling the gain and phase dynamics of the vestibuloocular reflex (VOR) and optokinetic reflex (De Zeeuw et al. 1995; Ito 1982; Lisberger et al. 1994; Stahl and Simpson 1995). It contains at least four Purkinje cell zones that are involved in the control of compensatory eye movements (De Zeeuw et al. 1994b; Van der Steen et al. 1994). The CFs of these floccular zones respond optimally to optokinetic stimuli rotating about particular axes in space, and they are derived from particular olivary subnuclei. The CFs that respond optimally to rotation about the horizontal axis perpendicular to the
ipsilateral anterior semicircular canal are derived from the VLO and the rostral dc, while those that respond optimally to rotation about the vertical axis (VA) are derived from the caudal dc (De Zeeuw et al. 1994a; Leonard et al. 1988; Tan et al. 1995). To a large extent, the modulation of the neurons in these different olivary subnuclei is already encoded in the descending projections from the accessory optic system and the nucleus of the optic tract of the pretectum. The VLO and rostral dc receive a major input from the medial terminal nuclei via the ipsilateral visual tegmental relay zone, whereas the caudal dc receives a major input from the ipsilateral dorsal terminal nucleus and the pretectal nucleus of the optic tract (Giolli et al. 1984; Maekawa and Takeda 1977; Simpson 1984; Simpson et al. 1988; Soodak and Simpson 1988). These projections are non-GABAergic and presumably excitatory (Horn and Hoffmann 1987; Mizuno et al. 1974; Nunes-Cardozo and Van der Want 1990). The major inhibitory input to the VLO and dc is from the hindbrain, as is also the case for the other olivary subnuclei (De Zeeuw et al. 1993, 1994a). The VLO and rostral dc receive their predominant GABAergic input from the contralateral ventral dentate nucleus and dorsal group y, whereas the caudal dc receives its major GABAergic input from the contralateral and ipsilateral nucleus prepositus hypoglossi (NPH), which is probably one of the main neural integrators for horizontal eye movements (e.g., Kaneko 1997, 1999). The terminals of this projection innervate both the olivary cell bodies and the dendrites, including those that are directly coupled by gap junctions (De Zeeuw et al. 1993, 1994a).

To investigate the influence of this inhibitory input on the spontaneous firing frequency of caudal dc neurons and on their modulation during optokinetic stimulation (OKS), we investigated the CS responses of Purkinje cells in the VA zones of the flocculus in anesthetized rabbits before and after lesions of the ipsilateral and/or contralateral NPH. In addition, to assess the potential impact of the commissural connection between the two NPHs on the firing frequency of neurons in the caudal dc, we studied the density of this projection at the light microscopic level and the nature of the neurotransmitter in its terminals at the electron microscopic level.

**METHODS**

**Electrophysiology**

**ANIMAL PREPARATION AND RECORDING.** The electrophysiological experiments were performed on 15 Dutch-belted rabbits anesthetized with a mixture of ketamine (32 mg/kg im), acepromazine (0.32 mg/kg im), and xylazine (5.0 mg/kg im); supplemental doses (9 mg/ml ketamine, 0.09 mg/kg acepromazine, 2 mg/kg xylazine) were given every 30–45 min. The head was fixed in a holder with the nasal bone at an angle of 55° to the horizontal. The dorsal neck muscles were retracted, and a craniotomy was performed over the left paramedian lobule of the cerebellum to permit access to the flocculus. A craniotomy was also made over part of the posterior vermis, and the nodulus and part of the uvula were aspirated to view the floor of the fourth ventricle and permit later lesion of the NPH (see following text). After removing the dura over the paramedian lobule, we advanced a glass-microelectrode (2–5 MΩ) filled with 2 M NaCl into the flocculus with the use of a microdrive angled at 27° to the vertical. The CS firing frequency of individual Purkinje cells was recorded extracellularly, discriminated with the use of a level detector and analyzed on-line with the use of a CED 1401 signal capture device (Cambridge Electronics Design) and the Spike2 program. The Purkinje cells were characterized by their response to OKS provided by a planetarium projector rotating about a particular axis in space (for details, see De Zeeuw et al. 1994b). Purkinje cells whose CS activity was optimally modulated by rotation about the VA were studied further.

**VISUAL STIMULATION.** Monocular stimulation was presented by covering the contralateral eye with a patch. The CS firing frequency of each VA Purkinje cell was recorded for 15 stimulus cycles. One stimulus cycle of 20 s consisted of four 5-s periods: a stationary period, an inhibitory (nasal-to-temporal movement) period, a second stationary period, and an excitatory (temporal-to-nasal movement) period. During the inhibitory and excitatory periods, the planetarium projecting the optokinetic stimulus rotated at a constant speed of 0.5°/s, which is close to the optimal speed for modulating the floccular visual CFs in rabbits (Alley et al. 1975; Barmack and Hess 1980; Kusunoki et al. 1990; Simpson and Alley 1974). In addition, the spontaneous CS firing frequency of VA Purkinje cells was recorded in darkness.

**NPH LESIONS.** After initial baseline recordings were obtained from VA Purkinje cells, the ipsilateral and/or contralateral NPH was aspirated under visual guidance using a 22-gauge needle. In 12 animals the NPH was lesioned on the ipsilateral side with respect to the floccular recording site (Fig. 1), and in 8 of them the NPH was subsequently lesioned on the contralateral side (Fig. 2). In three animals, only the contralateral NPH was lesioned. At the end of each experiment, the rabbit was killed with an overdose of pentobarbital sodium (Nembutal, 200 mg/kg), and its head was placed in 10% formalin. After a few days the brain stem was removed and stored in a 30% sucrose solution. The brain stem was cut transversely in 50-µm sections on a freezing microtome, and every other section was collected and mounted on gelatin-coated slides. The next day the sections were stained with cresyl violet, cleared in xylene, coverslipped with Permount, and used to reconstruct the lesion. Previous retrograde and anterograde tracing studies revealed that the caudal half of the NPH contains the neurons that project to the inferior olive (Barmack et al. 1993a; De Zeeuw et al. 1993). In each animal 80–100% of this part of the NPH was aspirated.

**DATA ANALYSIS.** The average CS firing frequency was determined for spontaneous Purkinje cell activity in the dark as well as for the excitatory, the inhibitory, and the stationary periods of the optokinetic stimulus cycle. We quantified the depth of modulation by calculating a modulation index (MI), defined as the average CS firing frequency during the excitatory period of the OKS divided by the average CS firing frequency during the inhibitory period (see Kusunoki et al. 1990). Both the excitatory and inhibitory CS modulation during constant velocity OKS transiently continued (carried over) into the subsequent stationary period (see Fig. 3). Therefore the first two seconds of each 5-s stationary period were excluded when the spontaneous CS firing frequency was calculated for the stationary periods. The strength of the carryover effect was determined by calculating the CS firing frequency during the first second of the stationary period and comparing it with the CS firing frequency during the last three seconds of the same 5-s stationary period.

Four different conditions were studied: prelesion, postipsilateral lesion (after lesion of the NPH ipsilateral to the floccular recording site), postcontralateral lesion (after lesion of both NPHs), and postcontralateral lesion (after lesion of the NPH contralateral to the floccular recording). Statistical comparisons were performed using Student’s t-test for the firing frequencies, which were normally distributed, and the Mann-Whitney U test for the MIs. Significant statistical comparisons (P < 0.05 or better) between the prelesion condition and each of the three lesion conditions include the Bonferroni correction to minimize Type I errors. This method increases the stringency of the significance level when making multiple comparisons of means.
FIG. 1. Reconstruction of the rostrocaudal extent of an ipsilateral nucleus prepositus hypoglossi (NPH) lesion. The distances between the sections are indicated in mm from the most caudal (bottom left) section. The black area indicates the lesion. The arrowhead indicates the cut marking the side of the floccular recording, i.e., the ipsilateral side. CN, cochlear nucleus; DVN, descending vestibular nucleus; dc, dorsal cap of Kooy; EC, external cuneate nucleus; ICP, inferior cerebellar peduncle; IO, inferior olive; LRN, lateral reticular nucleus; MVN, medial vestibular nucleus; NPH, nucleus prepositus hypoglossi; NTS, nucleus of tractus solitarius; R, nucleus of Roller; rV, ramus descendens of trigeminal nucleus; VII, facial nucleus; gVII, genu of facial nerve; dX, dorsal motor nucleus of vagus; XII, hypoglossal nucleus. Scale bar = 1 mm.
Tracing studies

LIGHT MICROSCOPY. To investigate the density of the commissural connection of the NPH, we made small unilateral injections with Phaseolus vulgaris-leucoagglutinin (PHA-L) in the NPH of two adult Dutch belted rabbits, as described by De Zeeuw et al. (1993). The animals were anesthetized with Nembutal (120 mg/kg ip) and mounted in a stereotaxic apparatus. The occipital bone was freed of neck muscles and a stereotaxic injection was made in the NPH with a glass micropipette filled with a 2.5% PHA-L (Vector) solution in 0.05 M Tris-buffered saline. The tracer was injected by means of a positive current (4–8 μA), which pulsed 7 s on, 7 s off for a total of 30 min. After a survival time of 10 days, the animals were anesthetized with Nembutal (200 mg/kg) and perfused. Blood was rinsed out with 200 ml 0.05 M phosphate buffer (pH 7.4) containing 0.8% NaCl, 0.8%
sucrose, and 0.4% d-glucose. The rinse was followed by 1:1 of a fixative consisting of 0.5% depolymerized paraformaldehyde, 2.5% glutaraldehyde, and 4% sucrose in the same buffer. The dissected brains were transferred to a 10% sucrose solution until they sank and embedded in 10% gelatin dissolved in the same sucrose solution. The gelatin was hardened in 4% paraformaldehyde for 3 h. Finally the embedded brains were transferred to a 30% sucrose solution in phosphate buffer (pH 7.4, 4°C) in which they were stored until they sank. Serial coronal sections (40 μm) of the brain stem were cut on a freezing stage microtome and processed to reveal the PHA-L. The sections were collected in Tris-buffered saline (TBS: 0.9% NaCl in 0.05 M Tris-HCl, pH 8.6). Subsequently, the sections were rinsed in TBS+, again rinsed in TBS+, incubated for 2 h in rabbit anti-goat IgG (Sigma, 1:200 in TBS+), again rinsed in TBS+, and incubated for 2 h in goat PAP (Nordic, 1/400 in TBS+). Finally, the sections were rinsed in Tris-HCl (0.05 M, pH 7.6) and incubated with 0.05% 3,3’-diaminobenzidine-tetrahydrochloride (DAB, Sigma) and 0.01% H2O2 in Tris-HCl. Following a thorough rinsing in Tris-HCl, the sections were mounted, counterstained with either cresyl violet or neutral red, and coverslipped.

**RESULTS**

**Electrophysiology**

**PRE-NPH LESION.** We recorded the spontaneous CS firing frequency and the CS modulation to OKS of 57 VA Purkinje cells in the flocculus of 15 anesthetized rabbits before the NPH was lesioned (Table 1). In the dark, the average spontaneous CS firing frequency was 0.99 ± 0.37 (SD) spikes/s, while for the NPH third through the fifth seconds of the combined postexcitatory and postinhibitory stationary periods, it was 1.02 ± 0.35 spikes/s. During the inhibitory and excitatory periods of the OKS, the average CS firing frequency was 0.26 ± 0.18 and 1.71 ± 0.56 spikes/s, respectively; the average MI was 10.2 ± 8.8. These spontaneous firing frequencies and MIs were not substantially different from those found in earlier studies in which the nodulus and uvula were not aspirated (Graf et al. 1988; Kusunoki et al. 1990). Figure 3, B1 and C1, exemplifies the peristimulus time histograms (PSTHs) of the CS responses to OKS in the prelesion condition, and it also illustrates the carryover from both the inhibitory and excitatory periods into the ensuing stationary periods. The average CS firing frequency during the first second after the stimulus movement stopped was significantly different from that during the rest of the stationary period. During the first second of the stationary period after the inhibitory period, the average CS firing frequency was 0.52 ± 0.45 spikes/s, whereas during the last 3 s of the same stationary period, it was significantly higher (1.16 ± 0.46 spikes/s; P < 0.001). After excitation, the average CS firing frequency during the first second of the stationary period was 1.21 ± 0.53 spikes/s, whereas during the last 3 s, it was significantly lower (0.87 ± 0.3 spikes/s; P < 0.001). The significance levels of these differences did not change when the last 4 s (seconds 2–5) instead of the last 3 s of the stationary periods were considered, indicating that the carryover effect was not prominent during the 2nd second of the stationary period.

**TABLE 1. Spontaneous and optokinetically modulated floccular CS activity before and after NPH lesions**

<table>
<thead>
<tr>
<th>No. of Purkinje Cells</th>
<th>Darkness</th>
<th>Excitatory</th>
<th>Inhibitory</th>
<th>MI</th>
<th>After Inhibition</th>
<th>Excitatory</th>
<th>After Inhibition</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-NPH lesion</td>
<td>57</td>
<td>0.99 ± 0.37</td>
<td>0.26 ± 0.18</td>
<td>0.52 ± 0.45</td>
<td>1.16 ± 0.46</td>
<td>1.71 ± 0.56</td>
<td>1.21 ± 0.53</td>
<td>0.87 ± 0.30</td>
</tr>
<tr>
<td>Post-ipsilateral lesion</td>
<td>36</td>
<td>1.07 ± 0.39</td>
<td>0.36 ± 0.21</td>
<td>0.71 ± 0.54</td>
<td>1.19 ± 0.37</td>
<td>1.85 ± 0.62</td>
<td>1.34 ± 0.51</td>
<td>1.04 ± 0.34</td>
</tr>
<tr>
<td>Post-bilateral lesion</td>
<td>31</td>
<td>1.21 ± 0.36</td>
<td>0.32 ± 0.18</td>
<td>0.65 ± 0.48</td>
<td>1.35 ± 0.41</td>
<td>1.98 ± 0.55</td>
<td>1.49 ± 0.50</td>
<td>1.17 ± 0.38</td>
</tr>
<tr>
<td>Post-contralateral lesion</td>
<td>15</td>
<td>0.58 ± 0.21</td>
<td>0.20 ± 0.14</td>
<td>0.24 ± 0.21</td>
<td>0.66 ± 0.24</td>
<td>1.16 ± 0.31</td>
<td>0.86 ± 0.27</td>
<td>0.55 ± 0.24</td>
</tr>
</tbody>
</table>

Values are means ± SD. CS, complex spike; NPH, nucleus prepositus hypoglossus; MI, modulation index.
During the inhibitory and excitatory periods of the OKS, the average CS firing frequency was 0.21 ± 0.18 and 1.08 ± 0.55 spikes/s, respectively; the average MI was 8.8 ± 6.7, which was not significantly different from that found with the NPH intact. As described in the preceding text for both the intact NPH condition and for the ipsilateral NPH lesion condition, the carryover effect was present both after the inhibition (P < 0.001) and after the excitation (P < 0.01).

**POST-BILATERAL LESION.** After bilateral NPH lesions (exemplified in Fig. 3C2), the average spontaneous CS firing frequency of 31 VA Purkinje cells was 1.21 ± 0.36 spikes/s in the dark (Table 1), while the average CS firing frequency during the third–fifth seconds of the stationary periods was 1.26 ± 0.39 spikes/s. Both firing frequencies were significantly higher than those found when the NPH was intact (P < 0.03 for darkness; P < 0.015 for the stationary periods). During the inhibitory and excitatory periods of the OKS, the average CS firing frequency was 0.32 ± 0.18 and 1.98 ± 0.55 spikes/s, respectively; the average MI was 8.6 ± 6.4, which was not significantly different from that found with the NPH intact. Thus the carryover effect remained both after the inhibition (P < 0.001) and after the excitation (P < 0.01).

**POST-CONTRALATERAL LESION.** After contralateral NPH lesions, the average spontaneous CS firing frequency of 15 VA Purkinje cells was 0.35 ± 0.21 spikes/s in the dark (Table 1), while their average CS firing frequency during the third–fifth seconds of the stationary periods was 0.60 ± 0.23 spikes/s. Both firing frequencies were significantly lower than those found when the NPH was intact (P < 0.015 for both cases). This change in firing frequency for the stationary periods is illustrated in Fig. 4 along with those for the ipsilateral and bilateral lesion conditions. During the inhibitory and excitatory period of the OKS, the average CS firing frequency was 0.20 ± 0.14 and 1.16 ± 0.31 spikes/s, respectively; the average MI was 9.3 ± 8.4, which was not significantly different from that with the NPH intact. Thus the carryover effect was present in all of the lesion conditions as well as with the NPH intact (Fig. 5).

**Tracing studies**

**LIGHT MICROSCOPY.** The PHA-L injection sites were centered in the caudal NPH, and they were restricted to one side (Fig. 6A). These unilateral injections revealed a dense projection to the contralateral caudal NPH (Fig. 6B) and, as described earlier (De Zeeuw et al. 1993), also to the ipsilateral and contralateral caudal dc. The densities of varicosities in the contralateral NPH and the ipsilateral and contralateral caudal dc were in the ratio of 11:7:21 (total number of terminals counted, n = 1078).

**ELECTRON MICROSCOPY.** Ultrastructural analysis of the ultrathin sections of the NPH that were processed for GABA-immunocytochemistry following injection of WGA-HRP in the contralateral NPH revealed numerous single- and double-
Dfiring frequency, both the bilateral inhibitory projection from comprehending the impact of NPH lesions on the spontaneous CS of the carryover of CS modulation from the inhibitory or the optokinetically elicited CS modulation or on the presence ipsilaterally. NPH lesions do not have a significant influence on lesioned bilaterally or contralaterally but not when lesioned as a decrease in the spontaneous CS activity.

**DISCUSSION**

We investigated the effect of removing the NPH input to the causal dc and the inhibitory commissural connection between the NPHs must be taken into consideration.

**Effects of NPH lesions on spontaneous CS firing frequency**

Bilateral ablation of the NPH led to an increase of the spontaneous CS firing frequency both in darkness and in the presence of a stationary pattern. This observation is compatible with the fact that the causal dc receives a GABAergic projection from both the contralateral and ipsilateral NPH (De Zeeuw et al. 1993). However, ablation of only the contralateral NPH led to a decrease of the spontaneous CS firing frequency, whereas ablation of only the ipsilateral NPH did not lead to a significant change in the spontaneous CS firing frequency. These latter two observations cannot be explained by considering simply the direct projections from the NPH to the causal dc. We propose, therefore that the pronounced commissural connection of the NPH also plays a role in determining the firing frequency of causal dc neurons.

In the present study, the commissural connection between the two NPHs was found to be predominantly GABAergic and to contact, in part, GABAergic NPH neurons. These observations raise the possibility that this connection inhibits those GABAergic NPH neurons that provide the inhibitory input to the ipsilateral and contralateral causal dc. Assuming that this presumption is valid and that the physiological strengths of the several inhibitory NPH projections are appropriately weighted, then the changes in the spontaneous CS firing frequency of floccular VA Purkinje cells after the different NPH lesions have a plausible qualitative explanation.

Three different lesion conditions (i.e., bilateral, contralateral, and ipsilateral) have to be compared with the normal intact (prelesion) condition (see Fig. 7).

First, when both the ipsilateral and contralateral NPH are lesioned, the inhibitory inputs to the causal dc are virtually absent so that the firing frequency of the CFs is expected to increase, as it did.

Second, when only the NPH contralateral to the recorded flocculus is lesioned, the net inhibitory input to the causal dc on the lesioned side can, in fact, increase because removal of the inhibitory commissural connection leads to a disinhibition of the inhibitory neurons of the intact NPH that project to that causal dc. In other words, while a contralateral NPH lesion results in removal of its direct inhibitory projection to the causal dc on the lesioned side, it also indirectly enhances the inhibitory projection to that causal dc from the NPH on the unlesioned side. Because the density of the direct crossed NPH projection to the causal dc is approximately three times higher than the direct uncrossed projection, a contralateral NPH lesion is expected to result in an increase in the net inhibitory input to the causal dc on the lesioned side. This increase was manifest as a decrease in the spontaneous CS activity.

Third, when only the NPH ipsilateral to the recorded flocculus is lesioned, the stronger of the two direct inhibitory NPH projections to the causal dc on the unlesioned side is removed, but this loss of inhibition will be opposed through disinhibition of the inhibitory neurons of the intact NPH that project to that causal dc. Thus while an ipsilateral NPH lesion can lead to an increase in CS activity, the effect should be small compared...
with the increase observed after a bilateral lesion, and, as found, it need not be significant.

The arguments presented in the preceding text are based on the fact that the commissural NPH connections as well as the NPH projections to the caudal dc are predominantly GABAergic and therefore inhibitory (De Zeeuw et al. 1993). It should be noted, however, that a minor part of the commissural connection of the NPH is excitatory (present study) and that the projection from the NPH to the dc also contains a purely cholinergic, presumably excitatory component as well as a combined cholinergic and GABAergic component (Barmack et al. 1993a; Caffé et al. 1996). Nonetheless, the model proposed above allows us to conclude that the changes in spontaneous CS firing frequency of floccular VA Purkinje cells after lesions of the ipsilateral and/or contralateral NPH are compatible with the anatomical circuitry and the inhibitory nature of its major neurotransmitter (GABA).

In a previous study in rat, Lang et al. (1996) found a substantial (87%) increase in the spontaneous CS firing frequency in crus 2a following removal of the GABAergic input to the relevant part of the IO with unilateral chemical lesions of the dentate nucleus. While a unilateral lesion of the cerebellar nuclei will remove most, if not all, of the GABAergic terminals in the associated contralateral olivary subdivisions (Fredette and Muguinani 1991), a bilateral NPH lesion is required to achieve a comparable reduction of GABAergic terminals in the caudal dc. With bilateral lesions of the NPH, we found a comparatively small (22%) increase in the spontaneous CS firing frequency. While this difference may be partly due to differences between chemical and mechanical lesioning methods, it more likely reflects in large measure two substantial anatomical differences between the projections of the lesioned structures. First, the cerebellar nuclear projection to the IO is almost exclusively contralateral, whereas the NPH projection to the caudal dc has a relatively strong ipsilateral component in addition to the dominant contralateral component (De Zeeuw et al. 1993). Second, the commissural connection of the cerebellar nuclei is weak or nonexistent whereas that of the NPH is very strong. As noted in the preceding text, the relatively strong commissural GABAergic projection of the NPH may

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**FIG. 5.** Quantification of the carryover of the CS modulation for the postinhibitory stationary period (left) and the postexcitatory stationary period (right) before and after ipsilateral, bilateral, and contralateral NPH lesions. The initial part of each stationary period (1st second) is compared with the later part of that stationary period (3rd–5th seconds). During the initial part of the postinhibitory period, the CS firing frequency was significantly less than during the later part of the postinhibitory period, both prelesion and after each of the 3 types of lesions (for all 4 conditions, \( P < 0.001 \)). During the initial part of the postexcitatory period, the CS firing frequency was significantly greater than during the later part of the postexcitatory period, both prelesion and after each of the 3 types of lesions (for all 4 conditions, \( P < 0.01 \) or better). Error bars indicate SE.
well inhibit the GABAergic neurons in the NPH that project to the caudal dc. Therefore the baseline level of inhibition provided by the NPH to the caudal dc may be low compared with that provided by the cerebellar nuclei to other parts of the IO. Consequently, when the NPH or the cerebellar nuclei are removed, the loss of inhibition will be relatively low or high, respectively, and the level of CS activity will increase accordingly. The difference between our findings and those of Lang et al. (1996) may, in addition, be partly due to the fact that the projection of the NPH to the caudal dc also contains an excitatory component, whereas the projection of the cerebellar nuclei to the IO is purely GABAergic (Caffé et al. 1996; De Zeeuw et al. 1989). Considering the preceding findings, we conclude that removal of different parts of the cerebellar and vestibular nuclear complexes in general leads to an increase in the spontaneous firing frequency of IO neurons but that the strength of this effect depends on the presence of bilateral, commissural, and/or excitatory components of the projection of the lesioned nuclei.

**Effects of NPH lesions on CS modulation and the poststimulus carryover**

Like all other inferior olivary subnuclei, the caudal dc receives one predominant excitatory input and one predominant inhibitory input (De Zeeuw et al. 1998b). The excitatory input to the caudal dc originates from the pretectal nucleus of the optic tract (NOT) and the dorsal terminal nucleus (DTN) of the accessory optic system (Giolli et al. 1984; Maekawa and Takeda 1977; Simpson 1984; Simpson et al. 1988), while the inhibitory input originates from the caudal part of the NPH (Barmack et al. 1993a; De Zeeuw et al. 1993). In rabbit the response properties of many neurons in the NOT and DTN are similar to those of caudal dc neurons (Collewijn et al. 1975; Graf et al. 1988; Leonard et al. 1988; Simpson et al. 1979; Soodak et al. 1988). Therefore the NOT and DTN neurons are presumably responsible for the increase in CS activity during the excitatory period of OKS. The inhibitory projection from the NPH could conceivably be partly responsible for the decrease...
The behavioral consequences of the inhibitory feedback from the cerebellum to the IO have not been resolved. In the process of classical eye-blink conditioning, the GABAergic input from the cerebellar nuclei may block the transmission of excitatory unconditioned stimulus signals from the IO during continuation of the training (Hesslow and Ivarsson 1996; Kim et al. 1998). For the processes of compensatory eye movements, the functional role of the inhibitory feedback from the NPH to the IO is even less clear. One of the possibilities is that it inhibits the transmission of the retinal slip signals that are conveyed by the CFs from the caudal dc to the Purkinje cells for the induction of long term depression during VOR adaptation (De Zeeuw et al. 1998a; Ito 1982). A second possibility is that the inhibitory projection from the NPH could be involved in preventing the optokinetic reflex during voluntary eye/head movements (see also McCrea 1988). During this suppression, the NPH may send an efference copy of eye velocity to the caudal dc that sums with the retinal slip signals conveyed by the excitatory input from the accessory optic system and NOT. From an anatomical point of view, such a summation is quite possible because all dendritic spines in the caudal dc receive both an inhibitory GABAergic input from the NPH and an excitatory input from the NOT and DTN (De Zeeuw et al. 1993, 1994a, 1998b). A third, not exclusionary, possible function of the inhibitory NPH projection is that it influences the temporal and spatial firing pattern of ensembles of coupled caudal dc neurons and thereby the activation of sets of oculo-
motor neurons. This possibility follows from the observation that the olivary dendritic spines in the caudal dc that are coupled by gap junctions are directly innervated by GABAergic terminals derived from the NPH (De Zeeuw et al. 1993) and the suggestion that the cerebellar GABAergic input to coupled dendrites in other parts of the IO controls motor domains for specific movements (Lang et al. 1996; Welsh et al. 1995). By modulating the coupling among neurons in the caudal dc, the NPH could determine which clusters of oculo-motor neurons are called into play at various times. Different levels of CS synchrony have, indeed, been found for pairs of floccular Purkinje cells in both the anesthetized and awake rabbit, but robust modulation of this synchrony by natural stimulation has, as yet, not been seen (De Zeeuw et al. 1997; Wylie et al. 1995). Finally, the NPH could play a role in the CS modulation found for some floccular VA Purkinje cells in the awake rabbit with head rotation in the dark (De Zeeuw et al. 1995; Simpson et al. 1999; see also Leonard and Simpson 1986). Typically, the CS activity increased with contralateral head movement and thus was opposite in polarity to the CS modulation present when the animal was afforded vision. Since many NPH neurons respond well to vestibular stimulation (e.g., McFarland and Fuchs 1992), the projection from the NPH to the caudal dc is a good candidate to underlie the CS modulation that occurs during vestibular stimulation in the dark. However, recordings from NPH neurons shown to project specifically to the caudal dc have to be made to determine the extent to which they carry vestibular signals or are dominated by other signals such as those mediated by floccular Purkinje cells (Yingcharoen and Rinvik 1983). Moreover it should also be noted that particular signals may not be apparent in the anesthetized preparation; in fact, the NPH neurons involved in the neural velocity-to-position integrator have been shown to be sensitive to anesthesia (see Kaneko 1997).

In sum, we conclude that in the anesthetized rabbit the projection from the NPH to caudal dc neurons is involved in control of their absolute firing frequency rather than their response to retinal image motion. Further research needs to be done to determine the function of this projection in the alert, behaving animal in particular in relation to the firing patterns of ensembles of neurons and in relation to the modification of reflexes during voluntary movements.

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