Afferent signals from pigeon extraocular muscles modify the vestibular responses of units in the abducens nucleus

I. M. L. DONALDSON AND P. C. KNOX

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, U.K.

SUMMARY

Although the extraocular muscles contain stretch receptors it is generally believed that their afferents exert no influence on the control of eye movement. However, we have shown previously that these afferent signals reach various brainstem centres concerned with eye movement, notably the vestibular nuclei, and that the decerebrate pigeon is a favourable preparation in which to study their effects. If the extraocular muscle afferents do influence oculomotor control from moment-to-moment they should exert a demonstrable effect on the oculomotor nuclei.

We now present evidence that extraocular muscle afferent signals do, indeed, alter the responses of units in an oculomotor nucleus (the abducens, VI nerve nucleus, which supplies the lateral rectus muscle) to horizontal, vestibular stimulation induced by sinusoidal oscillation of the bird. Such stimuli evoke a vestibulo-ocular reflex in the intact bird. The extraocular stretch receptors were activated by passive eye movement within the pigeon's saccadic range; such movements modified the vestibular responses of all 19 units studied which were all, histologically, in the abducens nucleus. The magnitude of the effects, purely inhibitory in 15 units, depended both on the amplitude and the velocity of the eye movement and most units showed selectivity for particular combinations of plane (e.g. horizontal versus vertical) and direction (e.g. rostral versus caudal) of eye movement.

The results show that an afferent signal from the extraocular muscles influences vestibularly driven activity in the abducens nucleus to which it carries information related to amplitude, velocity, plane and direction of eye movement in the saccadic range. They thus strongly support the view that extraocular afferent signals are involved in the control of eye movement.

1. INTRODUCTION

When the head moves in space compensatory eye movements ensure that images remain reasonably stable on the retina. In part this is achieved by the vestibulo-ocular reflex (VOR); head rotations cause excitation of semicircular canal afferents which drive neurons in the vestibular nuclei which in turn drive motoneurons to the six extraocular muscles (EOM) which move the eye in the orbit. This reflex has been extensively studied and modelled (see Carpenter 1988). However, one feature of other reflexes not generally thought to play any role at all in the VOR is proprioceptive feedback from the relevant effector muscles. Indeed, it is often claimed that EOM proprioception plays no part in the control of any type of eye movement.

However, it is known that the EOM of many species of vertebrate contain proprioceptors of various types (Maier et al. 1974; see Spencer & Porter (1988) for discussion). Proprioceptive feedback from the EOM has been shown to be important for the development of the visual system (Gary-Bobo et al. 1986; Hein & Diamond 1983) and for orienting behaviour in the cat (Fiorentini et al. 1982) and removal of EOM proprioception disrupts the VOR in rabbits (Kashii et al. 1989; Kimura et al. 1981). We have shown that, in a number of different species with different oculomotor behaviour, stimulation of EOM afferents by passive eye movement (PEM) modifies the responses of cells in the vestibular nuclear complex and reticular formation to vestibular stimulation (Ashton et al. 1984a, 1988, 1989; Donaldson & Knox 1990b). Furthermore, the results show that EOM afferents are able to signal the plane in which the eye moves, and even the direction of eye movement within that plane, both to units in the brainstem (Donaldson & Knox 1990b) and in the visual cortex (Ashton et al. 1984b). The vestibular nuclei project both monosynaptically and through polysynaptic pathways to the oculomotor nuclei. Thus it is of considerable interest to know whether the vestibular responses of cells in the oculomotor nuclei are modified by PEM, and, if so, to compare the type of modifications with those observed in other brainstem centres. Indeed, demonstration of an effect on the oculomotor nuclei is an essential prerequisite to any claim that EOM proprioception is involved in the control of eye movement from moment-to-moment (see Ashton et al. 1989 for discussion).

In experiments in the trout (Ashton et al. 1989) we showed that cells located in the oculomotor (III) and trochlear (IV) nuclei do carry both a vestibular and an
EOM afferent signal. There were also indications that the interactions between the vestibular and EOM afferent signals are directionally specific. However, because cell packing in these nuclei in the fish is relatively loose it was difficult to isolate many units.

Pigeons have stretch receptors in their EOM (Eden et al. 1982) and a well-developed VOR (Anastasio & Correia 1988). We have shown previously that the decerebrate pigeon is an excellent preparation for studying interactions between vestibular and EOM afferent signals (Donaldson & Knox 1990b). Having studied the effects of PEM on the vestibular responses of cells in the vestibular nuclei and surrounding reticular formation of the pigeon, we moved on to record the responses of cells in the VI cranial nerve nucleus, the abducens nucleus. Of the three oculomotor nuclei, the abducens has the advantages that it is reasonably accessible and is easy to identify histologically. Furthermore, it supplies only one eye muscle, the lateral rectus; the lateral and medial recti are the principal muscles involved in moving the eye during the horizontal VOR.

We have given a preliminary account of some of the results (Donaldson & Knox 1990a).

2. METHODS

All the units described here were recorded in decerebrate, paralysed, artificially ventilated pigeons and the recording sites of all were found, by histological reconstruction of the electrode tracks, to lie in the VI nerve (abducens) nucleus. The preparation and techniques of stimulating and recording were identical to those which we have described previously (Donaldson & Knox 1990b).

Glass-coated tungsten microelectrodes (Merrill & Ainsworth 1972) were directed vertically downward through the anterior folia of the cerebellum. Recordings were usually made from the left brain (ipsilateral to the eye which was moved) but, on some occasions, the electrode was inserted in the right brainstem. Extracellular recordings of single units were made from units which responded to vestibular stimulation.

The left eye was moved passively during vestibular stimulation by an electromagnetic servo-controlled device that acted upon a stalk carried by an opaque contact lens held in position on the cornea by suction. The device was made from the left brain (ipsilateral to the eye which was paralysed, artificially ventilated pigeons and the recording sites of all were found, by histological reconstruction of the vestibular nuclei and surrounding reticular formation of the pigeon, we moved on to record the responses of cells in the VI cranial nerve nucleus, the abducens nucleus. Of the three oculomotor nuclei, the abducens has the advantages that it is reasonably accessible and is easy to identify histologically. Furthermore, it supplies only one eye muscle, the lateral rectus; the lateral and medial recti are the principal muscles involved in moving the eye during the horizontal VOR.

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24 cycles, bin width 10 ms, cycle duration 2.5s

Figure 1. Set of four interleaved PSTHs showing the effect of passive eye movement (PEM) on the response of a unit in the abducens nucleus to horizontal, sinusoidal vestibular stimulation (±8° at 0.4 Hz); upward deflection corresponds to movement to the side contralateral to recording site. Each PSTH represents exactly one cycle of vestibular oscillation. (a) Upper traces: vestibular table position (solid line) and table velocity (dotted line). Lower panel: response to vestibular stimulation only. (b–d) Upper traces: vestibular table position and eye position, with the phases of eye movement (S1 to S3) at 90°, 180° and 270° respectively as indicated by drawings of pigeon’s head. See text for further details.

Note: (a) vestibular response during half-cycle of contralateral table movement, (b–d) Very marked inhibition of vestibular response by added PEM during phases S1 and S2 but no effect during S3. Maximum inhibitory effect at 90° towards tail (b).

greater bin-width. The critical value of $p$ for rejection of the null hypothesis was 0.025; values of $p$ greater than this led to the conclusion that the responses were not to be judged to be different.

3. RESULTS

(a) Responses to vestibular stimulation

All the nineteen units located in the abducens nuclei of seventeen pigeons were excited by contralateral rotation of the vestibular table. Thus the two units recorded from the right abducens nucleus responded to table rotation to the left and the other seventeen units, which were in the left nucleus, were excited by rightward rotation.

Units were initially tested with sinusoidal oscillation at an amplitude of ±8° at 0.4 Hz. Figure 1a shows a typical example of the vestibular response of twelve (63%) of the nineteen units investigated which all had this type of ‘cut-off’ response in which the unit responded only during a part of the vestibular cycle and was silent for the other (larger) part. The vestibular responses of all the units showed a phase lead relative to table velocity which ranged from 4° to 31° at 0.4 Hz. The unit of figure 1 had a phase lead of 4°.

We investigated the characteristics of the vestibular responses further in four units by testing them at a range of table frequencies. In these tests the table amplitude was held constant at ±5° and peak table velocity altered with frequency. The results for phase and gain of responses are shown in figure 2 plotted as mean ± s.e.m. ($n = 4$). As table frequency increased there was a clear decrease in phase lead on velocity so that, at the highest frequency, the phase approached zero or occasionally lagged by a few degrees. Over the same range of frequencies the gain of the responses increased with frequency so that smaller phase leads were accompanied by larger gains.

(b) Effect of PEM

Passive eye movement (amplitude 15° at 115° s⁻¹) modified the vestibular response of all the units investigated. In 15 units (79%) the modification was inhibitory, in three there were combinations of excitatory and inhibitory effects and in one unit the effect was purely excitatory. The extent of the commonest effect – inhibitory modulation – varied from minimal to very marked and depended on several factors described below. A typical effect is illustrated in figure...
1. In which PEM produced a profound inhibition of the vestibular response. PEM in the horizontal plane directed towards the tail (S1, 90°, figure 1b) produced the largest effect (the greatest inhibition) but PEMs directed downwards (S1, 180°, figure 1c) or towards the beak (S1, 270°, figure 1d) were also effective. Only the first (S1) PEM, an eccentrically directed eye movement starting at the central position, produced modification of the vestibular response in this unit. The return PEM (S3), a centrally directed movement from an eccentric starting position, had no effect and this was the common finding: clear effects of the return movement (R3) were observed in only two units. The directional ‘tuning’ of PEM effects is described below.

The amplitude of PEM was found to be important (figure 3). A small PEM of 5° in the horizontal plane directed towards the tail produced a slight enhancement of the vestibular response but, as the amplitude of eye movement was increased, the vestibular response was progressively reduced below control levels and a PEM of 20° was sufficient to abolish the vestibular response almost completely (figure 3e). Four units were tested in this way and all showed amplitude dependency of the effects of PEM.

Figure 4 shows the effect of altering the velocity of PEM while holding the amplitude constant at 15°. Two effects emerged from these experiments: (i) at low velocity there was very little modification of the vestibular response during or shortly after the movement of the eye (figure 4b, c and R1 on graph); (ii) while the eye was held at the eccentric position the vestibular response was clearly reduced to a significant extent (figure 4b-c and R2 on graph). At higher velocities, however, there was an R1 inhibition (figure 4c-e). Therefore, R2 values were significantly different from control values at all velocities (figure 4, graph), with some increase in effect with increasing velocity; the R1 value for the lowest velocity was not significantly different from the control but R1 showed considerably increased inhibition with increase in the velocity of PEM. Figure 4 also shows that increasing the velocity of PEM above the upper value for naturally occurring saccadic eye movements (arrow) did not produce a significantly greater inhibition.

(c) Tuning of responses

To discover whether the plane in which the eye was rotated had any influence on the type of effect which PEM produced on the vestibular response, we first of all examined the set of four PSTHS constructed for each unit; see description of figure 1. Then, if the unit could be held long enough, a set of eight PSTHS was collected to allow the effects of PEM in other planes to be observed.

PEM in either the vertical or the horizontal plane often produced an effect; see figure 1 in which PEM at 90° (in the horizontal plane directed towards the tail) clearly had the greatest effect relative both to the control histogram and also to the effect at 180° (R1, p < 0.005 for both). Of the 19 units examined in this way, 14 (74%) preferred PEM in the horizontal plane (that is, PEM in the horizontal plane produced the largest effect) and in eight of these the differences between the effects of horizontal and vertical PEM were statistically significant; 11 units (59%) preferred 90° and three (16%) preferred 270°. The remaining five units showed no clear preference. Units were investigated further by collecting sets of eight PSTHS and constructing tuning plots. Examples are shown in figure 5 which illustrates that the effectiveness of PEM in modifying the vestibular response varied with the plane in which the eye was moved. In the unit of figure 5a, although there was an effect at all angles, PEM near the horizontal plane was the most effective with the largest effect, at 60°, significantly different both from the control (p < 0.005) and from the effect in the vertical plane (0°, p < 0.025).

Tuning plots of this type were collected for ten units in which the effect of PEM was inhibitory. In each case the plots were consistent with the initial data collected for the unit. Three units were greatly influenced by PEM at all orientations while, of the remaining seven, six showed preference for PEM at or near the horizontal plane and directed towards the tail. One unit showed a preference for horizontal PEM towards the beak. As in the example in figure 5b combinations of vertical and horizontal effects were found on several occasions.

(d) Controls

As in our previous experiments (Donaldson & Knox 1990b), various standard control tests were done to...
Figures 3 and 4 illustrate the effect of changing the amplitude and velocity of horizontal passive eye movements (PEM) on the vestibular response of a unit in the abducens nucleus. The vesitibular response is measured in impulses per second (impulses s\(^{-1}\)).

- **Figure 3**: Shows the effect of changing amplitude of horizontal PEM on the vestibular response of a unit. The graph displays the magnitude of response (% control) against amplitude of passive eye movement (deg.). The lower part of the figure shows (a) vestibular response without PEM; (b-e) effects of adding PEM of 5° to 20° amplitude.

- **Figure 4**: Illustrates the effect of changing velocity of horizontal PEM on the vestibular response. The amplitude is held constant at 15°. The graph shows (R1) effect of initial eye movement (SI, see figure 1) and (R2) holding-phase (S2). The lower part of the figure shows: (a) vestibular response without PEM; (b-e) effects of adding PEM of 28 to 170° s\(^{-1}\).

In contrast to our recordings in other brainstem centres, no units were found in the abducens nucleus which responded to scraping of the beak or the head, to stretch of the eyelids, to vibration or to auditory stimuli.
4. DISCUSSION

We have shown previously that EOM afferent signals, stimulated by PM modify the vestibular responses of cells in the vestibular nuclei and surrounding reticular formation in the toad, cat and pigeon. Furthermore, early experiments had suggested similar effects in the oculomotor nucleus of the trout. Here we have extended these findings to the sixth, abducens, nucleus of the pigeon.

We have no independent evidence that the cells from which we recorded in the abducens nucleus were motoneurons. However, the contralateral vestibular responses that were found are consistent with those of lateral rectus motoneurons. Thus, when the head rotates to the right, the left lateral rectus contracts producing eye movement to the left; therefore the motoneurons of the left abducens nucleus would be expected to fire in response to contralateral rotation. This is the way in which the units which we have recorded behave. The shapes of the plots of phase and gain of the vestibular responses against frequency are very similar to those of the overall transfer function for the VOR (see Carpenter 1988). Because there is also a linear relation between motoneuron firing-rate and eye position (see Carpenter 1988) the shape of our frequency plots is that to be expected for abducens motoneurons. These observations strongly suggest that we have recorded either from motoneurons or from cells carrying similar information very close to the output of the neural drive to the eye muscles.

We have shown that the modification of the vestibular response of these cells is dependent on such parameters of the stimulus as the amplitude and velocity of PEM. The previous lack of such evidence has been used (for example, see Nelson et al. (1989)) as an argument against a functional role for EOM afferent signals in parts of the oculomotor control system. However, our present results clearly show that increasing the amplitude of PEM and thus, presumably, the magnitude of the afferent signal, increases the inhibitory effect on units in the abducens nucleus. It is clear that velocity of PEM is also important since PEMs of similar amplitudes but different velocities produce different effects. Thus it seems that for the dynamic part of the PEM stimulus (S1) to produce clear effects, the velocity must be at or above a certain magnitude, while even at the lowest velocities, during the static part of the stimulus (S2) clear effects are observed. Thus the EOM afferent signal apparently conveys both static and dynamic information to the abducens nucleus.

Our present experiments do not provide information about the details of the peripheral afferent signal from the muscle receptors. It must also be remembered that the eye muscles were paralysed and that the sensitivity of the receptors in unparalysed muscles to a given stretch may well be different; it may well be greater as the muscles will be stiffer.

The effects of the EOM signal on the vestibular responses of cells in the abducens nucleus are also dependent on the plane in which the eye is rotated. Horizontal PEM produced the largest effects in the 14 units that showed a preference for plane of rotation; most of these were also selective for direction of eye movement and preferred PEM towards the tail. Although all eye movements involve all the EOMs to some extent, the lateral rectus muscle is predominantly involved in horizontal eye movement. In these experiments we have examined the interaction between horizontal vestibular stimulation and PEM. Thus the preference of cells in the abducens nucleus for horizontal PEM suggests specific interactions based on the combination of plane and direction of PEM and plane of vestibular stimulation.

In the vestibular nuclei and the reticular formation several degrees of tuning were observed including some units which were very sharply tuned to particular directions of PEM (Donaldson & Knox 1990b). We found no such sharply tuned units in the abducens

Figure 5. Polar plots of 'tuning' data from two units showing effect of PEM in various planes and directions on the response to sinusoidal vestibular stimulation. The polar plots were constructed by counting the number of impulses falling in a time window in each PSTH. The large circles indicate the number of impulses in this window in the control PSTH. The responses are plotted as vectors in which the distance of a point from the origin represents the number of impulses and the angle of the vector shows the direction of PEM. The distances of the dots from the surrounding circle indicate the strength of the inhibitory effect produced by PEM along each vector. Circle: no eye-movement. Scale divisions: 5 impulses, i.e. 3.0 impulses s\(^{-1}\), 24 cycles, bin width 10 ms, cycle duration 2.5 s. (a) Response window bins 140-146 (70 ms); (b) response window bins 163-169 (70 ms). See text for comparison of (a) and (b).
nucleus. This may indicate that lateral rectus motor-neurons participate in eye movement in many directions. It also suggests convergence of the output of 'earlier' proprioceptive units on to the abducens nucleus.

There are other differences between interactions observed in the abducens nucleus and those which we have observed elsewhere in the brainstem. In the vestibular nuclei and reticular formation 59% of units with vestibular responses were affected by PEM, and 45% of those showed phasic excitatory interactions (Donaldson & Knox 1990b); in the abducens nucleus the responses of all units were modified by PEM and 79% were modified in an inhibitory manner. The fact that in cells of the abducens nucleus there is little evidence of R3 effects (responses to centrally directed PEM) shows that these units have a high degree of arcing selectivity (see Ashton et al. (1984b) for definitions), when compared with units in other brainstem centres (Donaldson & Knox 1990b).

These results confirm and extend our earlier reports that extraocular muscle afferent signals convey specific information about eye movement to oculomotor control centres. They show that this information is available at the output of the system, in the motor nuclei. It would seem that, since the signals produce their effects on a timescale of milliseconds and seconds, they could be involved in the moment-to-moment control of eye movement as well as possibly being concerned in longer-term oculomotor adjustment. Whether this is indeed so requires further investigation.

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