# Relative contribution of lateral vestibular neuron and abducens internuclear neuron inputs to the discharge activity of medial rectus motoneurons

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## ABSTRACT

Medial rectus motoneurons mediate nasally-directed horizontal eye movements. These motoneurons receive two major excitatory inputs, the abducens internuclear neurons (ABD Ints) and neurons of the lateral vestibular nucleus whose axons course through the ascending tract of Deiters (ATD). In the present work, we have recorded in the alert chronic cat preparation the discharge activity of these two premotor neurons simultaneously with eye movements. ABD Int discharge was accurately correlated with eye movements, displaying high eye position and velocity sensitivities. ATD neurons also discharged in relation to spontaneous and vestibular eye movements but showed significantly lower eye position and velocity sensitivities during both types of eye movements. An outstanding result of the present study was that ATD neurons presented a significantly lower eye position threshold for recruitment in comparison with both ABD Ints and medial rectus motoneurons. Therefore, ATD neurons exhibited eye position and velocity signals during spontaneous and vestibular eye movements, which were of lower magnitude than those of ABD Ints, but due to their low recruitment threshold, they could play an important role in facilitating ABD Int signal transmission onto medial rectus motoneurons.

**Keywords:** eye movements, vestibulo-ocular reflex, oculomotor system, medial longitudinal fascicle, syndrome of internuclear ophthalmoplegia, extracellular single-unit recordings, ascending tract of Deiters

# INTRODUCTION

Medial rectus motoneurons are located in the mesencephalic oculomotor nucleus and are responsible for generating the horizontal adducting movement of the ipsilateral eye, i.e., towards the nasal direction. These motoneurons receive two principal inputs from pontine structures, both of excitatory nature (Büttner-Ennever 2006; Carpenter and Carleton 1983). First, neurons of the ventrolateral vestibular nucleus project ipsilaterally to medial rectus motoneurons through the ascending tract of Deiters (ATD). ATD axons course through the pontine reticular formation between the brachium conjunctivum and the medial longitudinal fascicle (MLF) (Reisine and Highstein 1979; Reisine et al. 1981; Markham et al. 1986). They are second-order vestibular neurons that receive disynaptic input from the horizontal semicircular canals (Baker and Highstein 1978). The second source of pontine afferents to medial rectus motoneurons corresponds to the abducens internuclear neurons (ABD Ints), which lay intermingled with the motoneurons within the abducens nucleus. Their axons cross the midline and course through the MLF, terminating on the contralateral medial rectus motoneurons (Bienfang 1978; Delgado-Garcia et al. 1986). By means of recording (Delgado-Garcia et al. 1986; Fuchs et al. 1988) and lesion experiments (de la Cruz et al. 2000; Evinger et al. 1977; Gamlin et al. 1989; Lee et al. 2022), ABD Ints have been demonstrated to mediate conjugate eye movements in the horizontal plane. Unilateral MLF lesion leads to the inability of the ipsilateral eye to adduct across the midline, and thereby the incapacity of this eye to cross past the primary position towards the contralateral oculomotor hemifield (de la Cruz et al. 2000). These oculomotor deficits caused by MLF lesion are known in clinics as the syndrome of internuclear ophthalmoplegia (Carpenter and McMasters 1963; Christoff et al. 1960; Fiester et al. 2020; Pola and Robinson 1976;

Virgo and Plant 2017), which disturbs the performance of versional horizontal eye movements.

ABD Ints have been previously recorded in the alert behaving animal and their discharge pattern characterized as tonic-phasic (de la Cruz et al. 2000; Delgado-García et al. 1986; Fuchs et al. 1988). Their firing rate is related to both eye position (tonic component) and eye velocity (phasic component), thus displaying eye position and eye velocity sensitivities, for both spontaneous and vestibularly-induced eye movements. Medial rectus motoneurons also show a tonic-phasic firing pattern similar to that of their afferent ABD Ints (de la Cruz et al. 1989). By contrast, little is known about the discharge of ATD neurons, and only one work carried out under alert conditions describes that these neurons encode head velocity and a weak eye position signal, but a quantification of the signals displayed by these afferents is missing (Reisine et al. 1981). The fact that ABD Ints provide medial rectus motoneurons with all eye-related signals except vergence makes it difficult to understand the role of the ATD input, which has even been suggested to be superfluous (Pola and Robinson 1978).

On the other hand, intracellular electrophysiological experiments have revealed that VIIIth nerve stimulation generates in medial rectus motoneurons excitatory postsynaptic potentials (EPSPs) of large amplitude, that reverse at lower levels of injected currents than those evoked (monosynaptically) by the ABD Int stimulation, indicating a more proximal location of ATD synaptic boutons (Baker and Highstein 1978; Highstein and Baker 1978). Electron microscopy studies have confirmed that indeed ATD terminals contact medial rectus motoneurons preferentially on the soma and proximal dendrites, whereas ABD Int synaptic endings terminate mainly on distal dendrites (Markham et al. 1986; Nguyen et al. 1999).

The present work aims to investigate and compare the signals displayed by these two major inputs converging on medial rectus motoneurons to shed light on the distinctive role they play in the information processing carried out by the medial rectus motoneurons, as the final common pathway for oculomotor behavior. For this purpose, we have used the chronic alert animal preparation, which is the only approach that allows the direct correlation between discharge activity and eye movement. Therefore, we have analyzed and compared the firing rate of ATD neurons, ABD Ints, and that of their postsynaptic medial rectus motoneurons, with respect to eye position and velocity, during spontaneous and vestibular eye movements.

# MATERIALS AND METHODS

## Animals and surgical procedures

Experiments were performed on adult female cats weighing 2.0-2.5 kg obtained from authorized suppliers (Universidad de Córdoba, Spain). All procedures were performed in accordance with the guidelines of the European Union (2010/63/EU) and the Spanish legislation (R.D. 53/2013, BOE 34/11370-421) for the use and care of laboratory animals and approved by the ethics committee (P10-CVI-6053). All efforts were made to reduce the number of animals used and their suffering during experiments.

Four animals were used for the recordings of ATD and ABD Ints under alert conditions. Medial rectus motoneuron recordings were obtained from our previous publication (Hernández et al. 2017).

Animals were prepared for chronic recordings as previously described (Calvo et al. 2022; Davis-López de Carrizosa et al. 2011). Briefly, after a protective injection of atropine sulfate (0.5 mg/kg, i.m.) to reduce vagal reflexes, animals were anesthetized with ketamine hydrochloride (20 mg/kg, i.m.) mixed with xylazine (1 mg/kg, i.m.) and placed in a stereotaxic frame. Surgery was then performed under sterile conditions to implant stimulating electrodes, scleral coils, and the recording chamber. Two bipolar stimulating electrodes were implanted intracranially on the left and right VIth nerves at their exit from the brainstem (Fig. 1, St.; only the left side electrode is shown for clarity). The abducens nucleus was located by stereotaxic approach followed by the recording of its antidromic field potential evoked by the electrical stimulation to the ipsilateral (left) VIth nerve (Fig. 1, St.). Units that were isolated in the abducens nucleus but that were neither antidromically activated nor collided after electrical stimulation to the VIth nerve were considered as ABD Ints. Thus, ABD Ints were recorded in the left abducens nucleus (Fig. 1, Rec., in purple). Recordings of ATD neurons were carried out in the right ventrolateral vestibular nucleus (Fig. 1, Rec., in green), using the abducens nucleus as a reference (see below). Medial rectus motoneurons from our previous publication (Hernández et al. 2017) were recorded in the right side (Fig. 1, Rec., in orange) and were antidromically activated from the ipsilateral IIIrd nerve (Fig. 1, St.). For more details about medial rectus motoneuron recordings see Hernández et al. (2017). Thus, recordings were carried out reproducing the anatomical connections: left ABD Ints and right ATD neurons project onto right medial rectus motoneurons (Fig. 1). Coils, made up of two turns of Teflon-isolated stainless-steel wire, were implanted in the sclera of both eyes for the recording of eye movements. A square window (5 x 5 mm) was drilled in the occipital bone to allow transcerebellar access to the brainstem for recordings. A restraining system was also constructed to immobilize the head during the recordings. Pre- and postoperative care was provided daily, as needed.

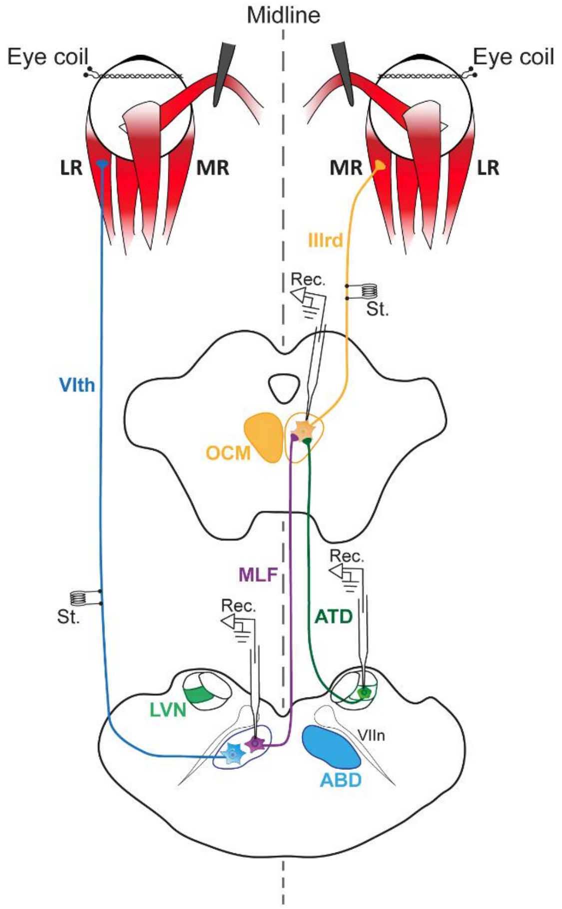


**Fig. 1** Schematic diagram of the experimental design. Extracellular single-unit recordings (Rec.) were carried out in medial rectus (MR) motoneurons (in orange), located in the oculomotor nucleus (OCM), or in their two main pontine excitatory inputs, the contralateral abducens (ABD) internuclear neurons (in purple), and the ipsilateral lateral vestibular neurons (LVN, in green). The axons of these two afferents travel through the medial longitudinal fascicle (MLF) or the ascending tract of Deiters (ATD), respectively. Coils were implanted in the sclera of both eyes to record ocular movements. A bipolar stimulating electrode (St.) was implanted in the IIIrd or VIth nerve for the electrophysiological activation of motoneurons. LR stands for lateral rectus muscle, which is innervated by the abducens motoneurons (in blue). VIIn represents the facial nerve, which forms the facial genu dorsal to the abducens nucleus.

## Chronic extracellular recordings

Recording sessions started after 10 days of postoperative recovery. The animal was gently restrained inside a fabric bag, wrapped with elastic bandages, and placed in a Perspex box (with its head immobilized), which was located inside the magnetic field for eye movement recordings (Fuchs and Robinson 1966). Single-unit extracellular recordings were carried out with glass micropipettes filled with 2 M NaCl, and fixed to a three-axis micromanipulator, which descended through the intact cerebellum to reach the brainstem.

Once the abducens nucleus was located by its antidromic field potential at its maximum amplitude (~ 5 mV, assuming this location represented the center of the nucleus), the recording micropipette was then moved using the micromanipulator between 2-2.5 mm lateral and 1-1.5 mm dorsal with respect to the abducens nucleus, for the recording of ATD neurons (lateral vestibular nucleus, ventral division), according to the stereotaxic coordinates of Berman´s atlas (1968). When a unit was isolated within these coordinates and responded as a type I neuron during horizontal head rotation (Gernandt 1949), that is, its discharge increased for head rotations towards the ipsilateral side of the recording, then we recorded the extracellular action potentials of this cell simultaneously with eye movements under alert conditions. These neurons were thus considered as ATD neurons. Single-unit extracellular action potentials were recorded during both spontaneous and vestibularly-induced eye movements. Vestibular stimulation in the horizontal plane was achieved by a servo-controlled motor attached to the turntable to produce sinusoidal rotations of the table at a frequency of 0.125 Hz and amplitude range between ±20 and ±30 degrees zero to peak. Neuronal activity was amplified and filtered at a bandwidth of 10 Hz - 10 kHz for display and digitalization purposes.

### Data storage and analysis

The recording of horizontal eye position of both eyes and the simultaneous neuronal activity were digitally stored for off-line analysis (Power 1401, Cambridge Electronic Design, Cambridge, UK). Computer programs written in Matlab 7.5 were used for selecting the data of instantaneous firing frequency (calculated as the reciprocal of the interspike intervals) along with the corresponding position and velocity of both eyes.

The firing rate of extraocular motoneurons has been described as having a static component related to eye position and a dynamic component related to eye velocity.

Thus, the equation described for the discharge of these cells corresponds to $FR = F_0 + k \cdot EP + r \cdot EV$ (Davis-López de Carrizosa et al. 2011; Robinson 1970), where FR is the firing rate of the neuron (in spikes/s), $F_0$ is the firing rate when the eye is centered in the orbit (i.e., straight-ahead gaze), k is the neuronal eye position sensitivity (in spikes/s/degree), EP is eye position (in degrees), r is the neuronal eye velocity sensitivity (in spikes/s/degree/s) and EV is eye velocity (in degrees/s). For spontaneous eye movements, neuronal eye position and velocity sensitivities were named $k_s$ and $r_s$, and for vestibular eye movements, they were named as $k_v$ and $r_v$. Eye-related parameters for ATD neurons, ABD Ints, and medial rectus motoneurons were calculated with respect to their respective ipsilateral eye.

During eye fixations, since eye velocity is zero, the equation simplifies to $FR = F_0 + k_s \cdot EP$. Therefore, we calculated the $k_s$ coefficient by linear regression fitting, so that the slope of the regression line thus obtained represents the neuronal eye position sensitivity during fixations (i.e., $k_s$). We also calculated the eye position threshold for neuronal recruitment into activity as the eye position value at which $FR = 0$, therefore, from the above equation, threshold was obtained for each neuron as $-F_0/k_s$ (in degrees). During spontaneous rapid eye movements or saccades, we correlated firing rate, and previous subtraction of the eye position component ($k_s \cdot EP$), with eye velocity, so that the above equation changes to $FR - k_s \cdot EP = F_0 + r_s \cdot EV$. Therefore, we used the linear regression analysis, and the slope obtained from the regression line represents the neuronal eye velocity sensitivity during spontaneous saccades ($r_s$).

During vestibularly-induced eye movements, the equation used was $FR = F_0 + k_v \cdot EP + r_v \cdot EV$. We selected between cursors the slow phases of the nystagmus and used the multiple regression analysis to obtain the neuronal sensitivities $k_v$ and $r_v$.

### Statistics

Comparisons between groups were performed using the one-way ANOVA test, in all cases at an overall level of significance of $p < 0.05$. ANOVA tests were followed by *post hoc* pairwise multiple comparisons, by means of the program SigmaPlot version 11 (Systat Software, Inc., San Jose, CA, USA). All regression equations obtained from the fit of firing rate with eye position and eye velocity were significant ($p < 0.05$). The effect size was indicated by Cohen´s d (d). Quantitative data are represented with box-and-whisker plots showing the median, 25th (Q1), and 75th (Q3) quartiles, with 90th and 10th percentiles as the error bars. All data points are superimposed in the graphs.

## RESULTS

### Discharge pattern of medial rectus motoneurons and their afferents ATD neurons and ABD Ints during spontaneous eye movements

Medial rectus motoneurons showed a tonic-phasic firing profile that was proportional to eye position and eye velocity. During fixations, they increased their firing rate for eye positions located more in the on direction, which was that contralateral to the recording side (i.e., the nasal direction of the ipsilateral eye). Since recordings were carried out in the right oculomotor nucleus, this means that discharge frequency was higher when gaze was directed toward the left side (Fig. 2A). In addition, medial rectus motoneurons displayed a phasic component which was present for rapid eye movements or saccades.

They exhibited a burst of spikes for on-directed saccades (Fig. 2A, solid dots) and an abrupt decay in firing or a pause for off-directed saccades (Fig. 2A, asterisks).

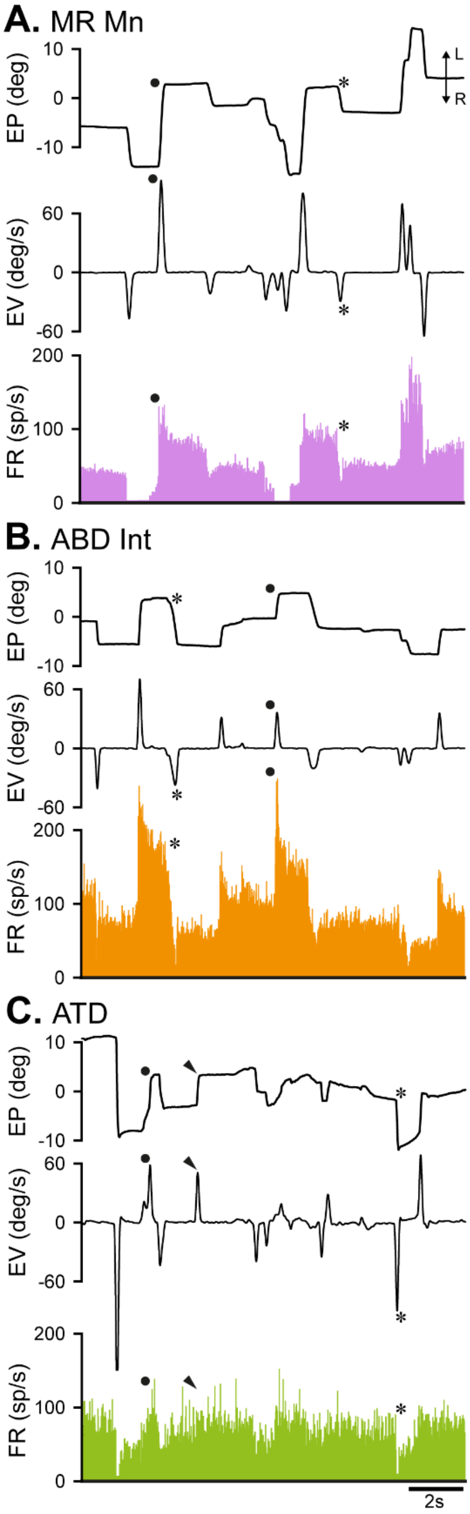


**Fig. 2** Discharge activity of MR motoneurons, ABD Ints, and ATD neurons, during spontaneous eye movements. The figure shows examples of the firing rate (FR, in spikes/s) during spontaneous eye movements of the three neuronal types studied: **A** MR motoneurons (Mns), **B** internuclear neurons (Ints) of the ABD, and **C** vestibular neurons of the ATD. L and R next to the double arrow in A stands for leftward and rightward eye movements, respectively (for **A**-**C**). MR motoneurons and ATD neurons were recorded in the right side, and ABD Ints in the left side. The horizontal eye position (EP, eye position, in degrees) and velocity (EV, eye velocity, in degrees/s) are shown for the eye ipsilateral to the recording site in **A**-**C**. Note that the discharge of the three neurons increases with eye movements toward the left side, although the correlation between FR and EP and EV can be better appreciated in the ABD Int (**B**) and the MR Mn (**A**) as compared to the ATD neuron (**C**). Solid dots point to burst-like increases in FR during on-directed saccades, asterisks indicate an abrupt decay in FR for off-directed saccades, and arrowheads show an example of absence of FR response during saccades in the ATD neuron (C).

ABD Ints exhibited a tonic-phasic discharge pattern that was similar to that of medial rectus motoneurons, except that their on direction was the opposite, i.e., towards the ipsilateral side of the recording (i.e., the temporal direction of the ipsilateral eye). In particular, as recordings were performed in the left abducens nucleus, their discharge increased for eye movements towards the left side. Nevertheless, ABD Ints carry appropriate information to medial rectus motoneurons, since their axons cross the midline and terminate excitatorily on these motoneurons located in the contralateral oculomotor nucleus (Fig. 1; Highstein and Baker 1978). Thus, given that medial rectus motoneurons were recorded in the right oculomotor nucleus and ABD Ints in the left abducens nucleus, the on-direction of both cell types was the same, i.e., toward the left (Fig. 2B). In general, it was observed that ABD Ints fired at higher tonic-phasic rates than medial rectus motoneurons, in particular, the bursts of spikes for on-directed saccades reached higher frequencies (Fig. 2B, solid dots). Similar to medial rectus motoneurons, ABD Ints rapidly decreased or ceased their firing for off-directed saccades (Fig. 2B, asterisks).

During spontaneous eye movements, the discharge of ATD neurons differed somewhat from that of motoneurons and ABD Ints. Thus, as can be appreciated in Fig. 2C, although they also modulated in relation to eye movements, their degree of correlation with eye position was less conspicuous. They increased their discharge rate as eye positions were fixed more to the contralateral side (left) of the recording (right) site, as medial rectus motoneurons did. Since ATD neurons project excitatorily and ipsilaterally onto these motoneurons (Baker and Highstein 1978), the signals conveyed by these premotor cells were appropriate for driving medial rectus motoneurons. Therefore, the three neuronal types recorded as illustrated in Fig. 1 presented eye movements to the left as the on-direction. The behavior of ATD neurons also showed a phasic component. During on-directed saccades, they discharged a burst of spikes, which was however of low frequency (Fig. 2C, solid dots), and on occasions, it was not present (Fig. 2C, arrowheads). For off-saccades, ATD neurons usually decreased their firing rate (Fig. 2C, asterisks), and rarely they showed no change in their discharge activity or this was not consistent. Thus, their phasic activity was present (Fig. 2C), but it was not as precise as that of medial rectus motoneurons (Fig. 2A) and ABD Ints (Fig. 2B).

### Quantitative comparison of the signals displayed during spontaneous eye movements in ATD neurons, ABD Ints and their target medial rectus motoneurons

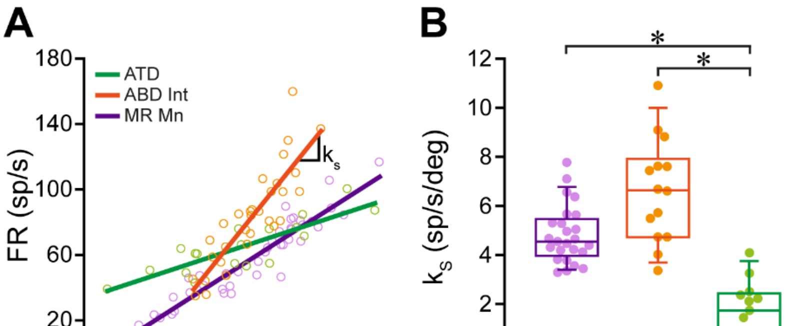
A comparison of eye-related parameters between the three neuronal types was carried out during spontaneous eye movements. Although our principal aim was the comparison between ATD neurons and ABD Ints, we also included the discharge properties of medial rectus motoneurons to discern the degree of similarity in the firing activity of both inputs with respect to their target motoneurons. The number of neurons analyzed during spontaneous eye movements was: $n = 25$ medial rectus motoneurons, $n = 14$ ABD Ints, and $n = 13$ ATD neurons.

During fixations, neuronal eye position sensitivity ($k_s$) was calculated as the slope of the line obtained from the linear regression analysis between firing rate and eye position. The result of this analysis for the three neurons illustrated in Fig. 2 is shown in Fig. 3A. It can be appreciated that the slopes, and therefore $k_s$ values, of the ABD Int and the medial rectus motoneuron, were higher than that of the ATD neuron.

When neuronal eye position sensitivities ($k_s$) were compared between the three neuronal populations, it was found that $k_s$ values of ATD neurons were significantly lower than those of both ABD Ints and medial rectus motoneurons (Fig. 3B; one-way ANOVA on ranks followed by Dunn´s method, $p \leq 0.001$, H = 31.470, Cohen´s $d$ =

2.287). Mean ± SEM $k_s$ values were 4.81 ± 0.24, 6.62 ± 0.57, and 1.81 ± 0.3 spikes/s/degree, for medial rectus motoneurons, ABD Ints, and ATD neurons, respectively. Moreover, $k_s$ values of ABD Ints were similar to those of motoneurons ($p > 0.05$) whereas those of ATD neurons

**Fig. 3** Quantitative comparison of eye-related parameters obtained during spontaneous fixations and saccades between MR motoneurons and their two major pontine inputs. **A** Correlation between firing rate (FR, in spikes/s) and eye position (EP, in degrees) was carried out by linear regression analysis. The slope of the regression line thus obtained represents the neuronal eye position sensitivity ($k_s$, in spikes/s/degree). The three lines correspond to the neurons shown in Fig. **2**, whose $k_s$ values were 4.1, 7.9, and 2.1 spikes/s/degree, for the MR Mn of Fig. **2A**, the ABD Int of Fig. **2B**, and the ATD neuron of Fig. **2C**, respectively. **B** Box-and-whisker plot showing eye position sensitivity during eye fixations ($k_s$, in spikes/s/degree) between MR Mns, ABD Ints, and ATD neurons. One-way ANOVA test revealed significant differences ($p \leq 0.001$) between groups. Pairwise multiple comparisons (Dunn´s method) demonstrated that ABD Ints and MR Mns showed significantly higher $k_s$ values than ATD neurons ($p < 0.05$ for both cases; asterisks). **C** Same as **B** but for the eye position threshold at which the neuron was recruited into activity (Th, in degrees). One-way ANOVA test revealed significant differences ($p \leq 0.001$) between groups. All pairwise multiple comparisons (Dunn´s method) were significantly different ($p < 0.05$ for the three cases; asterisks). ATD neurons showed the lowest threshold and MR Mns the highest threshold. **E** Same as **B** but for eye velocity sensitivity during saccades ($r_s$, in spikes/s/degree/s). Significant differences were present between the three groups (one-way ANOVA, $p \leq 0.001$). When pairwise multiple comparisons were carried out (Dunn´s method) all of them resulted in significant differences ($p < 0.05$). ABD Ints showed the highest $r_s$ values, while ATD neurons presented the lowest. For **B**-**D**, $n$ = 25 MR Mns, 14 ABD Ints, and 13 ATD neurons.



were significantly lower ($p < 0.05$) (Fig. 3B). It should be noted that, however, the statistical comparison of the coefficients of determination ($R^2$) for the rate-position regression equations between the three populations showed no statistical difference (ANOVA on ranks, $p = 0.158$, H = 3.696), indicating that the $k_s$ signal was present in the two premotor inputs, as well as in the motoneurons.

The eye position threshold for recruitment into activity was also calculated from the regression equations and compared between the three groups. An interesting result was that, in this case, there was a significant difference between all neuronal populations, so that ATD neurons showed the lowest threshold and medial rectus motoneurons the highest (Fig. 3C; one-way ANOVA on ranks followed by Dunn´s method, $p \leq 0.001$, H = 28.013, $d = 2.001$). Therefore, the recruitment threshold of the two afferent populations (ATD neurons and ABD Ints) was lower than that of the motoneurons they terminate on. In turn, the threshold of ATD neurons was significantly ($p < 0.05$) lower than that of ABD Ints, that is, they were recruited into activity at more negative (off-directed) eye positions in the orbit. We would like to highlight that the results in threshold were quite striking, particularly due to the extremely low threshold of ATD neurons as compared to ABD Ints and medial rectus motoneurons (mean ± SEM were: -36.37 ± 5.11 degrees, -13.71 ± 1.46, and -8.17 ± 0.54, respectively).

During saccades, the neuronal eye velocity sensitivities ($r_s$) obtained for the three populations reflected the qualitative observations described above from their respective firing pattern. Thus, ABD Ints showed the highest $r_s$ values (1.26 ± 0.06 spikes/s/degree/s), and ATD neurons had the lowest $r_s$ values (0.19 ± 0.04 spikes/s/degree/s). Medial rectus motoneurons data were between both (0.50 ± 0.02 spikes/s/degree/s). The differences between the three groups reached statistical significance (Fig. 3D; one-way ANOVA on ranks followed by Dunn´s method, $p \leq 0.001$, H = 42.109, $d = 3.673$). The saccadic signal of ATD neurons was weak and not always consistent. This yielded low correlations of determination of the rate-velocity regression equations for ATD neurons, which were significantly lower than those obtained for both ABD Ints and motoneurons (one-way ANOVA followed by Holm-Sidak method, F(2,49) = 60.517, $p \leq 0.001$). It should be highlighted that with respect to all eye-related parameters calculated in the present work, only eye velocity sensitivity for spontaneous saccades ($r_s$) produced significantly lower coefficients of determination in ATD neurons compared to ABD Ints and medial rectus motoneurons. In all other parameters, the three groups of neurons showed similar coefficients of determination. Therefore, the saccadic signal in ATD neurons was not as accurate as that of the other main input to the medial rectus motoneurons, the ABD Ints.

## Discharge pattern of medial rectus motoneurons, ABD Ints, and ATD neurons during vestibular eye movements

During vestibularly-induced eye movements the firing profile of medial rectus motoneurons and that of ABD Ints was similar (Fig. 4A, B). They modulated during both the slow and the fast phases of the nystagmus. However, whereas the discharge of medial rectus motoneurons increased for head rotations towards the same side of the recording (type I response; Gernandt 1949), ABD Ints increased their firing for head rotations towards the opposite side (type II response; Gernandt 1949). As mentioned above, as ABD Ints were recorded in the left side and medial rectus motoneurons in the right side, then both neuronal types increased their discharge for head rotations toward the right. As can be observed in Fig. 4A, B, although they showed the same discharge pattern, ABD

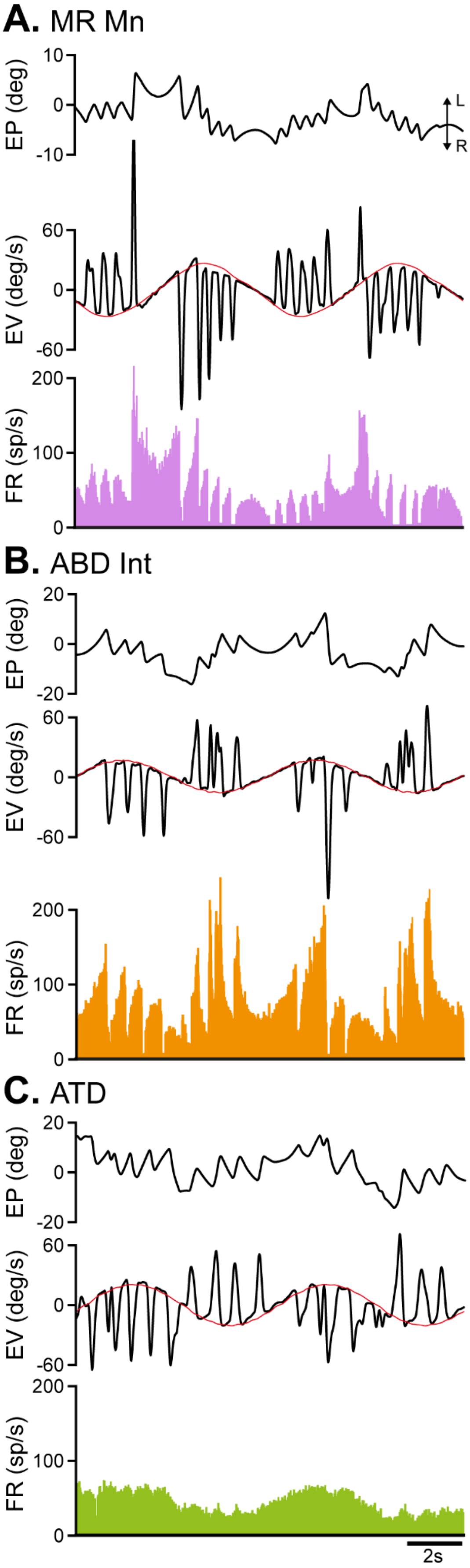


**Fig. 4** Behavior of MR Mns, ABD Ints, and ATD neurons during vestibular eye movements. Traces in **A**-**C** illustrate from top to bottom: eye position (EP, in degrees), eye velocity (EV, in degrees/s), and firing rate (FR, in spikes/s). The red trace in EV represents head velocity, which is shown inverted for clarity. L and R next to the double arrow in **A** indicate leftward and rightward eye movements, respectively (for **A**-**C**). MR motoneurons and ATD neurons were recorded in the right side, and ABD Ints in the left side. Note that in the three neuronal types (**A**-**C**), on-directed eye movements were those toward the left. The firing pattern of MR Mns (**A**) and ABD Ints (**B**) during vestibularly-induced eye movements was similar. Thus, their discharge increased for slow and fast phases of the nystagmus in the on direction and decreased for slow and fast phases in the opposite direction. However, ATD neurons clearly modulated during the slow phases of the vestibulo-ocular reflex but lacked any signal related to fast phases (**C**). Note also that whereas ATD neurons and MR Mns showed type I response during vestibular eye movements (i.e., their discharge increased for head rotations toward the ipsilateral side of the recording), ABD Ints showed type II response (i.e., firing increased for head rotations toward the contralateral recording side).

Ints reached, in most cases, higher firing frequencies than medial rectus motoneurons during vestibular eye movements.

The response of ATD neurons during vestibularly-induced eye movements was type I since their firing increased for head rotations towards the ipsilateral side of the recording (Gernandt 1949; Markham et al. 1986). During vestibular eye movements, their firing pattern differed from that of motoneurons and ABD Ints (Fig. 4C). They modulated sinusoidally (as the stimulus) without showing bursts or decreases in activity for the on- and off-directed fast phases of the nystagmus, respectively, in marked contrast to motoneurons and ABD Ints (Fig. 4A, B). This behavior likely corresponds to the head velocity signal described by Reisine and Highstein (1979).

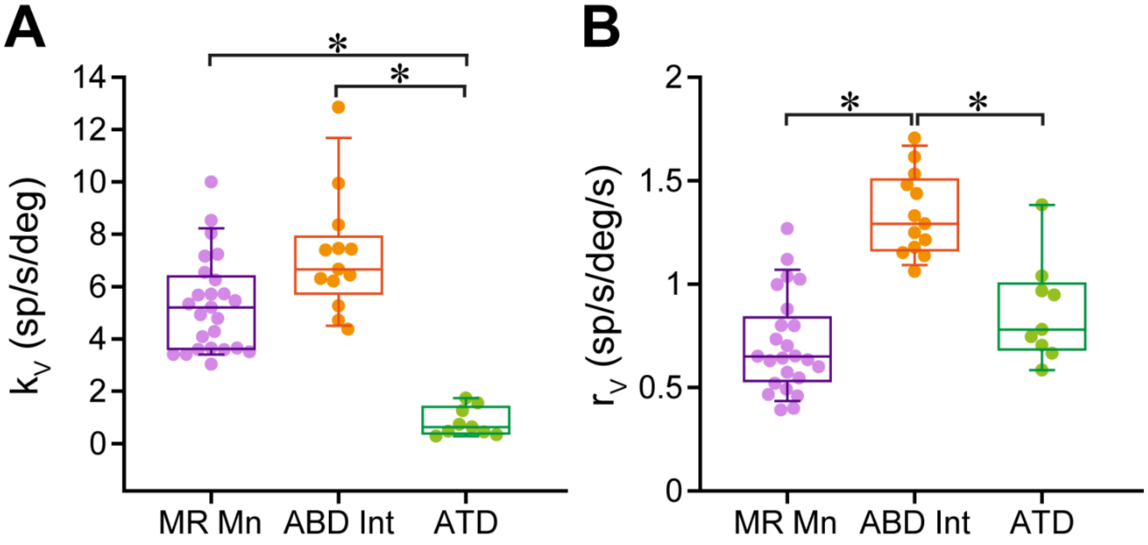
## Quantitative comparison of ATD neurons and ABD Ints signals during vestibular eye movements in relation to medial rectus motoneurons

Eye position and eye velocity sensitivities were calculated during vestibular eye movements ($k_v$ and $r_v$, respectively) selecting the slow phases of the nystagmus and using multiple regression analysis. ABD Ints showed higher $k_v$ values than ATD neurons (Fig. 5A; one-way ANOVA on ranks followed by Dunn´s method, $p \leq 0.001$, H = 25.816, $d$ = 1.838). Mean ± SEM data were 7.18 ± 0.63 and 0.83 ± 0.18 spikes/s/degree, respectively. When compared with medial rectus motoneurons (5.31 ± 0.37 spikes/s/degree), ABD Ints showed similar $k_v$ ($p > 0.05$), whereas $k_v$ in ATD neurons was significantly lower than in the motoneurons ($p < 0.05$) (Fig. 5A).

Regarding eye velocity sensitivity ($r_v$), ABD Ints also exhibited higher values (1.37 ± 0.06 spikes/s/degree/s) than ATD neurons (0.87 ± 0.08 spikes/s/degree/s) (Fig. 5B; one-way ANOVA on ranks followed by Dunn´s method, $p \leq 0.001$, H = 26.054, $d$ =

1.856). However, in this case, eye velocity sensitivity of ATD neurons during vestibular eye movements ($r_v$) was similar ($p > 0.05$) to that of motoneurons (0.71 ± 0.05 spikes/s/degree/s), whereas ABD Ints showed significantly higher $r_v$ values than motoneurons and ATD neurons ($p < 0.05$) (Fig. 5B).

**Fig. 5** Eye-related parameters of MR Mns, ABD Ints, and ATD neurons during vestibular eye movements. **A** Box-and-whisker plot illustrating the values of neuronal eye position sensitivity during the slow phases of the vestibular nystagmus ($k_v$, in spikes/s/degree) for MR Mns, ABD Ints, and ATD neurons. One-way ANOVA on ranks detected significant differences between groups ($p \leq 0.001$). *Post-hoc* pairwise multiple comparisons (Dunn´s method) revealed that MR Mns and ABD Ints showed significantly ($p < 0.05$, asterisks) higher $k_v$ values than ATD neurons. **B** Box-and-whisker plot showing the values of neuronal eye velocity sensitivity during the slow phases of the vestibulo-ocular reflex $r_v$ (in spikes/s/degree/s). One-way ANOVA on ranks revealed that there were significant differences between groups ($p \leq 0.001$). When pairwise multiple comparisons were performed (Dunn´s method), we obtained that ABD Ints showed significantly higher $r_v$ values than both ATD neurons ($p < 0.05$) and MR Mns ($p < 0.05$). The number of neurons analyzed in **A** and **B** was 25 MR Mns, 13 ABD Ints, and 9 ATD neurons.



# DISCUSSION

The results of the present work have demonstrated that ATD neurons exhibit eye position and eye velocity sensitivities during both spontaneous and vestibular eye movements. Nevertheless, these sensitivities were significantly lower than those of ABD Ints. During spontaneous eye movements, the eye position signal of ATD neurons was weak, and their response during saccades was not as consistent as that of ABD Ints. On the other hand, during vestibular eye movements, ATD neurons clearly modulated with eye movements, increasing their firing for head rotation towards the ipsilateral direction (type I response) but lacked the phasic component during the fast phases of the vestibular nystagmus. ABD Ints exhibited a type II response and modulated during both slow and fast phases of the nystagmus. Eye position and eye velocity sensitivities for both spontaneous and vestibular eye movements ($k_s$, $r_s$, $k_v$, and $r_v$) were significantly higher in ABD Ints as compared to ATD neurons, likely indicating that ABD Ints constitute the major input driving medial rectus motoneuron discharge (Delgado-Garcia et al. 1986; Hernández et al. 2017; Highstein and Baker 1978).

However, it should be emphasized that the quantitative analysis between ATD neuron firing rate and eye position and velocity, during both spontaneous and vestibular eye movements, demonstrated that these vestibular neurons encoded eye-related signals with coefficients of determination that were similar to those of ABD Ints, except for eye velocity sensitivity during spontaneous saccades ($r_s$), where coefficients of determination for ATD neurons were significantly lower. This was evident in the firing activity of ATD neurons during saccades, which was not as accurate as that of ABD Ints, and on occasions these vestibular neurons lacked any response or displayed an inappropriate signal during saccades.

As stated above, previous recordings of ATD neurons revealed that these neurons convey a head velocity and a weak eye position signal to medial rectus motoneurons (Reisine and Highstein 1979; Reisine et al. 1981). Since ABD Ints discharge in a tonic-phasic fashion during spontaneous and vestibular eye movements (Delgado-Garcia et al. 1986; Fuchs et al. 1988), this implies that an integrated head velocity signal is already contained in this projection and thus, information coming from ATD neurons has been suggested as redundant (Furuya and Markham 1981). Other authors have claimed that, by analogy with the other five types of extraocular motoneurons, a direct head velocity signal may provide an additional feed-forward boost necessary to overcome the sluggish oculomotor plant, so that motoneuron response may effectively compensate head velocity with appropriate eye velocity (Baker and Highstein 1978; Reisine and Highstein 1979).

Intracellular recordings of medial rectus motoneurons carried out in acute cats following the electrical stimulation to the ipsilateral vestibular nerve have shown high amplitude disynaptic EPSPs originating from ATD neurons, which reverse with the injection of small depolarizing currents, indicating a somatic and/or proximal dendritic location (Baker and Highstein 1978). The electrical stimulation of ABD Ints produces also large (monosynaptic) EPSPs on medial rectus motoneurons, but the reversal of these synaptic potentials requires higher currents, indicating a distal location of abducens terminals on the motoneuron dendritic tree (Highstein and Baker 1978). These results were later confirmed at the electron microscopy level by the anterograde labeling of each afferent population and the observation of their respective labeled axons on medial rectus motoneurons (Nguyen et al. 1999). That study demonstrates that the two populations of excitatory inputs differ in their soma-dendritic distribution: the majority of ATD synaptic endings contact proximal dendrites or somata, whereas most abducens synaptic boutons terminate on distal dendrites. In contrast, by means of anterograde labeling, the vestibular projection has been shown to be much less abundant on the medial rectus motoneuron subgroup in the oculomotor nucleus, as compared with the massive projection of afferent terminals arising from the ABD Ints (Carpenter and Carleton 1983; Hernández et al. 2017).

An outstanding result of the present work was the very markedly low eye position recruitment threshold present in ATD neurons compared with ABD Ints (as well as with medial rectus motoneurons). This finding indicates that ATD neurons may maintain medial rectus motoneurons at a low level of tonic excitatory influence. Therefore, the ATD input may facilitate the recruitment of motoneuron firing by the more distal projection from the abducens internuclear pathway. In this respect, we suggest that the very low threshold of ATD neurons might play a relevant role by facilitating the transmission of signals from ABD Ints onto medial rectus motoneurons.

According to the present data, the ATD signals, compared to those found in the ABD Int population, appear not to be intense enough to produce the maximal firing in the motoneuron pool which is necessary to move the adducting eye across the midline. Indeed, lesions of the MLF rostral to the abducens nucleus produce specific disturbances of conjugate eye movements, with paralysis of ocular adduction on attempted lateral gaze but preservation of convergence (the convergent signal arises from a different afferent, likely the midbrain near response cells, Zhang et al. 1992). Thus, after MLF lesion, the ipsilateral eye shows an incapacity to adduct across the midline, and eye movements are restricted to the ipsilateral oculomotor hemifield. These oculomotor deficits are identified clinically as the syndrome of internuclear ophthalmoplegia (Carpenter and McMasters 1963; Christoff et al. 1960; Lee et al. 2022; Pola and Robinson 1976) and have also been experimentally induced in cats and monkeys by the lesion or inactivation of the MLF (de la Cruz et al. 2000; Evinger et al. 1977; Gamlin et al. 1989). The fact that the lesion of the MLF, through which ABD Int axons course, leads to the incapacity of the ipsilateral eye to cross midline towards the contralateral hemifield, even though the ATD pathway is intact, implies that ATD neurons are not able to activate medial rectus motoneurons enough to cause the eye to cross the midline. In patients suffering from unilateral or bilateral MLF lesions, there is partial preservation of the horizontal vestibulo-ocular reflex, indicating a role for ATD neurons in mediating, at least in part, horizontal vestibular eye movements (Aw et al. 2017). However, those patients are unable to perform adducting horizontal saccades during the vestibulo-ocular reflex (Aw et al. 2017), in congruence with the present data showing absence of saccadic responses in ATD neurons during the fast phases of the vestibular nystagmus.

The selective unilateral section of the ATD in cats produces oculomotor deficits in the ipsilateral eye, such as a reduction of range during spontaneous eye movements and a decrease in the gain of the vestibulo-ocular reflex, although all those alterations recover over time, due to compensation from the intact MLF pathway. In addition, the section of the ATD leads to a reduction in eye position and eye velocity sensitivities of medial rectus motoneurons recorded under alert conditions, during both spontaneous and vestibular eye movements (Hernández et al. 2017). Those results imply that vestibular neurons projecting to medial rectus motoneurons contribute by their synaptic action to generate eye-related signals in the discharge pattern of these motoneurons. However, the reduction in oculomotor signals displayed by medial rectus motoneurons after ATD transection are short-lasting (5-7 days), due to axonal sprouting of the intact ABD Int pathway that compensates for all the alterations induced by the ATD section in a short time. In contrast, when the MLF is sectioned leaving intact the ATD, changes observed in eye movements and medial rectus motoneuron discharge are of higher magnitude and of longer duration. In this case, compensation for the loss of MLF input by intact ATD axons is only partial (Hernández et al. 2017). Accordingly, ABD Ints play a more powerful synaptic influence on the firing activity of medial rectus motoneurons under alert conditions than ATD neurons, and are endowed with a larger degree of plastic mechanisms such as axonal sprouting after lesion. On the other hand, ATD neurons are not a redundant input onto medial rectus motoneurons, as their loss disturbs eye movements and motoneuron discharge.

Comparison of eye-related parameters of both inputs with those of medial rectus motoneurons yielded different results. ABD Ints showed similar eye position sensitivity to medial rectus motoneurons during both spontaneous and vestibular eye movements (i.e., $k_s$ and $k_v$). However, eye velocity sensitivities for spontaneous saccades ($r_s$) and vestibular-induced eye movements ($r_v$) were higher in ABD Ints than in motoneurons. This likely reflects a greater synaptic influence of ABD Ints when the medial rectus muscle requires a stronger force. On the other hand, ATD neurons showed lower eye position ($k_s$) and velocity ($r_s$) sensitivities than medial rectus motoneurons during spontaneous movements. However, during vestibular eye movements, although ATD neurons presented a lower eye position sensitivity ($k_v$), the eye velocity sensitivity ($r_v$) was similar to that of motoneurons. Thus, this may be the major signal conveyed by ATD neurons on medial rectus motoneurons, and is in consonance with the head velocity signal previously reported for ATD neurons (Reisine and Highstein 1979; Reisine et al. 1981).

In summary, it might be concluded that the signals encoded by ATD neurons and ABD Ints interact at the level of the medial rectus motoneurons to produce the horizontal vestibulo-ocular reflex and conjugate horizontal gaze. The lower threshold of

ATD neurons might favor the activation of motoneurons by the ABD Int pathway. Nevertheless, both inputs are required for the normal operation mode of medial rectus motoneurons under physiological conditions.

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## Competing interests

The authors have no relevant financial or non-financial interests to disclose.

## Author contributions

A.M.P., B.B.T. and R.R.C. designed the experiments. R.G.H. and A.M.P. carried out the recordings. R.R.C. designed and supervised the analysis and figures. R.G.H. performed the analysis and figures. R.R.C. and A.M.P. wrote the manuscript. All authors have approved the final article and agree to the submission of the final manuscript.

## Data availability

Data are available upon kind request.

## Ethical approval

All procedures were performed in accordance with the guidelines of the European Union (2010/63/EU) and the Spanish legislation (R.D. 53/2013, BOE 34/11370-421) for the use and care of laboratory animals and approved by the ethics committee (P10-CVI-6053).