

## Neuronal evidence for individual eye control in the primate cMRF

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**Abstract:** Previous single unit recordings and electrical stimulation have suggested that separate regions of the MRF participate in the control of vergence and conjugate eye movements. Neurons in the supraoculomotor area (SOA) have been found to encode symmetric vergence [Zhang, Y. et al. (1992). *J. Neurophysiol.*, 67: 944–960] while neurons in the central MRF, the cMRF, located ventral to the SOA and lateral to the oculomotor nucleus are associated with conjugate eye movements [Waitzman, D.M. et al. (1996). *J. Neurophysiol.*, 75(4): 1546–1572]. However, it remains unknown if cMRF neurons are strictly associated with conjugate movements since eye movements were recorded with a single eye coil in monkeys viewing visual stimuli at a distance of at least 50 cm. In the current study we addressed whether neurons in the cMRF might also encode vergence-related information. Interestingly, electrical stimulation elicited disconjugate saccades (contralateral eye moved more than the ipsilateral eye) from locations previously thought to elicit only conjugate saccades. Single unit recordings in this same area made in two rhesus monkeys trained to follow visual stimuli moved rapidly in depth along the axis of sight of an individual eye demonstrate that cMRF neurons *do not* simply encode conjugate information during disconjugate saccades; in fact our findings provide evidence that cMRF neurons are most closely associated with the movement of an individual eye. These results support the hypothesis that the midbrain shapes the activity of the pre-motor saccadic neurons by encoding integrated conjugate and vergence commands.

**Keywords:** saccade; disconjugate eye movement; mesencephalic reticular formation (MRF); superior colliculus (SC); vergence; saccade–vergence interaction; supraoculomotor area (SOA); oculomotor system; disparity

### Introduction

The classic assumption that neuronal circuitries generating saccadic and vergence eye movements are largely separate has been challenged by a

number of studies that have shown that during disconjugate saccades (i.e., when a vergence movement is combined with a saccade) vergence velocity is sped up and saccade velocities are reduced (Maxwell and King, 1992; Zee et al., 1992; Busettoni and Mays, 2005; Kumar et al., 2005). Furthermore, recent work has revealed that a number of commonly assumed “conjugate” saccadic structures in the oculomotor brainstem in fact

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have monocular tuning (i.e., a combination of conjugate and vergence signals) (McConville et al., 1994; Zhou and King, 1998; Sylvestre and Cullen, 2002; Sylvestre et al., 2003; Van Horn et al., 2008).

The source of vergence-related signals to the pre-motor saccadic neurons remains unknown. Since the bulk of the input to the paramedian portion of the pontine reticular formation (PPRF) originates in the midbrain, specifically the superior colliculus (SC) and the mesencephalic reticular formation (MRF) (Horn, 2005), and the midbrain receives inputs from cortical structures with disparity information [e.g., lateral intraparietal area (LIP) and frontal eye fields (FEF)], we have now begun to re-examine neurons in MRF. The goal in the current study was to establish whether cMRF neurons contribute to the development of neural signals that are suitable for controlling an individual eye or if their discharge is strictly for conjugate control.

## Methods

Two rhesus monkeys (*Macaca mulatta*) were prepared for chronic extracellular recording using aseptic surgical procedures described elsewhere (Sylvestre and Cullen, 1999b). The primary difference in the current experiments was the placement of the stainless steel recording chambers which were oriented stereotaxically on the skull towards the oculomotor nucleus. An eye coil was implanted in each eye to allow recordings of binocular eye movements. All procedures were approved by the University of Connecticut Health Center and McGill University Animal Care Committees and were in compliance with the guidelines of the NIH and the Canadian Council on Animal Care.

### *Behavioural paradigms, data acquisition, and analysis of cMRF neuronal discharges*

Data acquisition and behavioural paradigms are identical to those utilized previously and are not repeated for sake of brevity (see Sylvestre and Cullen, 2002). Activity of cMRF neurons was identified as previously described (Waitzman et al., 1996). Horizontal and vertical conjugate saccades

were elicited by stepping the target between horizontal and vertical positions, respectively ( $\pm 5$  to 30 deg). To elicit different types of vergence eye movements an array of 16 computer-controlled red light-emitting diodes (LEDs) was displayed on a board tilted slightly from the horizontal in front of the monkey. To elicit disconjugate saccades LEDs were positioned in a configuration similar to the Müller paradigm (see Ramat et al., 1999) to minimize the movement of one eye. Notably, the eyes are referred to as either ipsilateral or contralateral based on their location relative to the recording site. We also describe eyes movements in terms of conjugate [conjugate=(left eye+right eye)/2] and vergence (vergence=left eye–right eye) coordinates (see Sylvestre and Cullen, 1999b for more details). Analysis of cMRF neuron discharges is precisely the same as those used previously for abducens motoneurons and burst-tonic neurons in the PPRF (Cullen et al., 1996; Cullen and Guitton, 1997; Sylvestre and Cullen, 1999a, b). The dynamic eye position and velocity sensitivities of a neuron during saccades were estimated using linear optimization techniques that have been described in detail elsewhere (Sylvestre and Cullen, 1999b). The dynamic lead time of individual neurons ( $t_d$ ) was determined during conjugate saccades as described in Sylvestre and Cullen (1999b). For each model parameter in the analysis of disconjugate saccades, we computed 95% confidence intervals using a non-parametric bootstrap approach (Carpenter and Bithell, 2000), and used these confidence intervals to identify non-significant or identical model parameters.

## Results

### *Electrical stimulation*

The primary result of electrical stimulation in the cMRF is that both conjugate and disconjugate saccades can be elicited. Examples of the results of stimulating two sites in the cMRF separated by 1 mm in the same monkey are shown in Fig. 1. At the initial cMRF site located approximately 2 mm lateral to the oculomotor nucleus, electrical

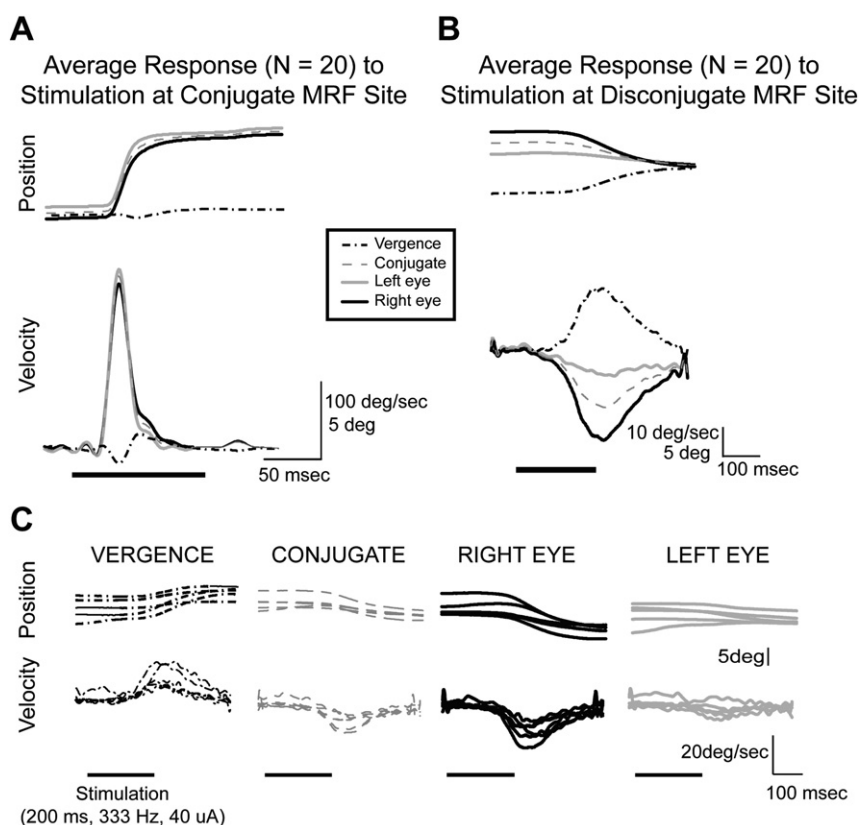


Fig. 1. Generation of conjugate/convergence eye movements following stimulation in the right cMRF of a monkey implanted with two eye coils. (A) Stimulation site in the MRF, 2 mm lateral to the oculomotor nucleus that produced conjugate saccades to the right. (B and C) A MRF site that was 1 mm more *lateral* to the site in A that elicited classic conjugate contralateral saccades. The conjugate signal (thin, dashed grey traces) is primarily the result of leftward movement of the *right* eye (thick, solid black traces) since the left eye moved only slightly (thick, solid grey traces). Average eye position and velocity traces are shown in A and B and individual eye position and velocity traces are shown in C. Note that positive vergence velocities indicate convergence and negative vergence velocities indicate divergence.

stimulation with 30–50  $\mu$ A produced conjugate saccades to the contralateral side at short latency as has been demonstrated previously (Fig. 1A). Note that both eyes moved exactly the same amount. However in contradiction to previous results (Waitzman et al., 2002) stimulation at a site located 1 mm further *lateral* to this conjugate saccade site (and therefore not in the supraoculomotor area, SOA) elicited disconjugate movements, where the right eye (contralateral to the side of stimulation) moved more than the left (Fig 1B). We have now stimulated more than 40 sites throughout the cMRF and have demonstrated that more than 50% elicit disconjugate saccades.

### Single unit recording

One potential criticism of electrical stimulation in the reticular formation is that we could have activated both conjugate and vergence mechanisms via electrical stimulation of axons in passage or antidromically activating regions that might be associated with the generation of vergence movements. Therefore we proceeded to record from single neurons located in the cMRF. An example of one such neuron is shown in Fig. 2. As has been demonstrated previously (e.g., Cromer and Waitzman, 2006), this cMRF neuron fired for contralateral conjugate movements of the eyes.

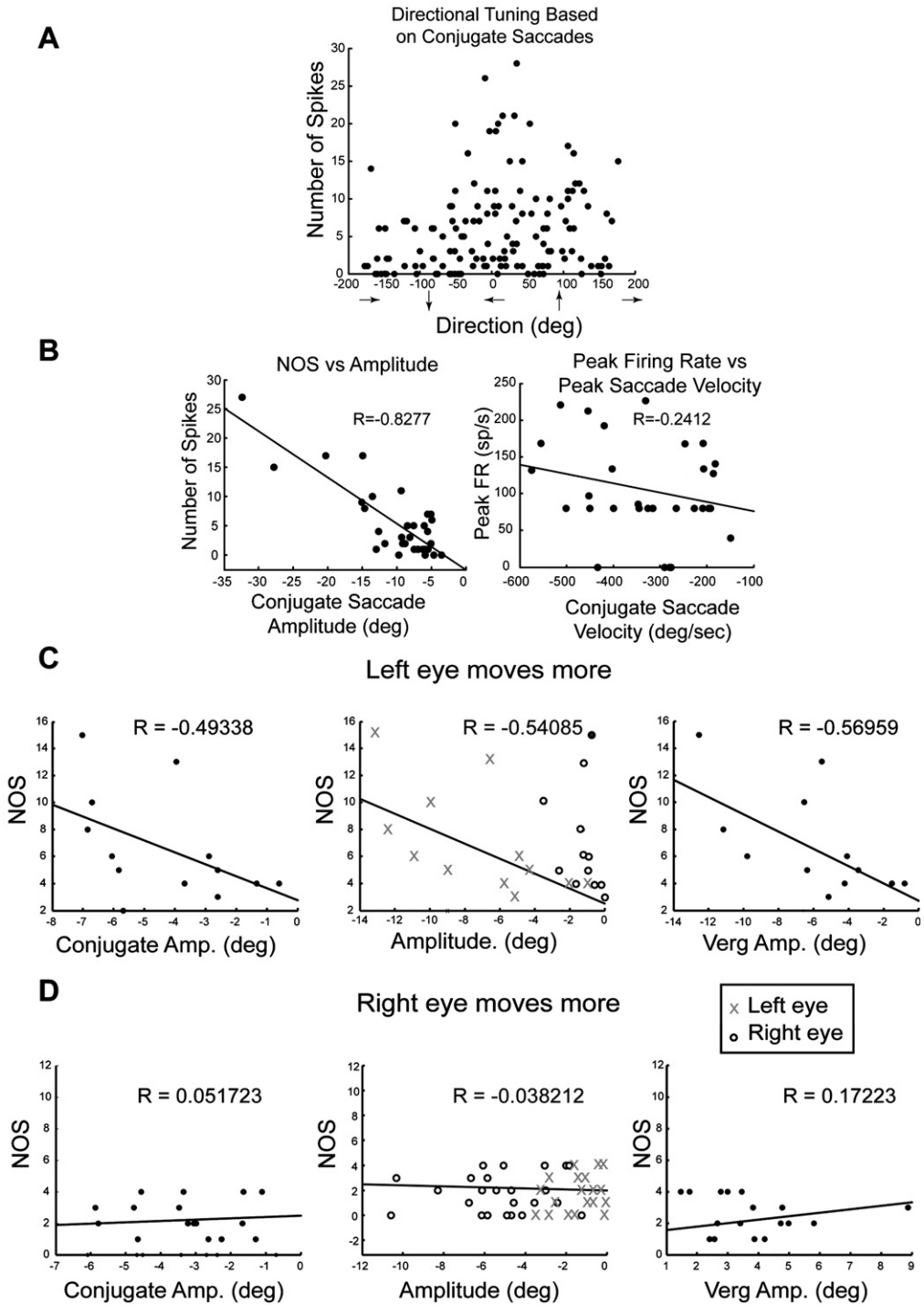


Fig. 2. Comparison of the relationships between number of spikes (NOS) and conjugate or individual eye amplitude and velocity. (A and B) Analysis of conjugate saccades. Note that tuning was best for saccades to the contralateral side. (C and D) Analysis of disconjugate saccades during which the left (C) or right (D) eye moved more. Note the marked improvement in correlation when left eye amplitude (C) was used as opposed to the right eye amplitude (D). Filled circles represent conjugate amplitude, and x's and open circles represent left and right eye amplitude, respectively.

Therefore its movement field consisted of all saccade vectors to the contralateral side (Fig. 2A). In addition there was a monotonic relationship between contralateral saccade amplitude (Fig. 2B, left) and saccade associated spike number, but a weak relationship between peak discharge and peak velocity (Fig. 2B, right).

However, we then analysed the disconjugate saccade trials and segregated them into trials when the left eye moved more (Fig. 2C) or when the right eye moved more (Fig. 2D). It was clear that the neuron fired primarily when the left eye moved to the left (Fig. 2C). The number of spikes in the burst during vergence eye movements was closely associated with vergence amplitude, while movements of the right eye were poorly correlated with the number of spikes regardless of the amplitude measured: conjugate, vergence, or right eye (Fig. 2D). Our sample of cMRF neurons yielded similar results such that during disconjugate saccades the number of spikes was better correlated with individual eye amplitude rather than the amplitude of the conjugate component of the movement.

The number of spikes approach, however, encompasses an inherent assumption that spike number is proportional to amplitude and thus firing rate is proportional to eye velocity. This may not be the case especially for neurons that project directly to the motoneurons and have a direct relationship between eye position and discharge rate (see the Appendix of Sylvestre and Cullen, 2002). Therefore, we used a dynamic method that made no such assumption. The dynamic approach has the added advantage that we can test the prediction of a specific model under a variety of conditions. For instance, we could compare the prediction of the conjugate model with a model that uses the position and velocity of an individual eye. We tested this prediction directly for a neuron located in the cMRF for which we obtained the response during conjugate saccades and hypothesized that the neuronal discharge would be similar during disconjugate saccades. We used the conjugate gaze model:

$$FR(t) = b_{CS} + k_{CS}CJ(t-t_d) + r_{CS}\dot{C}J(t-t_d) \quad (1)$$

where  $FR(t)$  is the neuron's instantaneous firing rate,  $b_{CS}$ ,  $k_{CS}$ , and  $r_{CS}$  constants and represent the

neuron's firing rate at eye position zero, the neuron's conjugate eye position, and eye velocity sensitivities, respectively. However, it was clear that during disconjugate saccades the conjugate gaze model could account for no more 3% of the variance (Fig. 3, top row). We then tested a binocular model that included terms for each individual eye position and velocity:

$$FR(t) = b_{DS} + k_{i-DS}IE(t-t_d) + k_{c-DS}CE(t-t_d) + r_{i-DS}I\dot{E}(t-t_d) + r_{c-DS}C\dot{E}(t-t_d) \quad (2)$$

where  $FR(t)$  is the neuron's instantaneous firing rate,  $b_{DS}$ ,  $k_{i-DS}$ ,  $k_{c-DS}$ ,  $r_{i-DS}$ , and  $r_{c-DS}$  are constants and represent the neuron's bias, ipsilateral and contralateral eye position, and ipsilateral and contralateral eye velocity sensitivity, respectively. The terms  $IE(t)$ ,  $CE(t)$ ,  $I\dot{E}(t)$ , and  $C\dot{E}(t)$  represent the instantaneous ipsilateral and contralateral eye position and eye velocity, respectively. Note that this model follows a similar form to that of the conjugate equation, but now each term is assigned to each eye (i.e., binocular model). The results of the fit to the data with this binocular model were much better and could account for 48% of the variance (Fig. 3A, second row).

Although this observation strongly supported the idea that this neuron did not encode conjugate eye movements, it did not provide enough information to determine if it solely encoded the movements of one eye or a weighted mixture of both eyes' movements. To address this limitation we estimated the 95% confidence intervals in the *binocular* model Eq. (2) using the bootstrap technique (see Sylvestre and Cullen, 2002). Figure 3B shows the bootstrap distributions and the 95% confidence intervals (thick horizontal bars) for the estimated eye position sensitivities. Two important observations can be made from the 95% confidence intervals. First, the parameter values estimated for the ipsilateral ( $k_{i-DS}$ ) and contralateral ( $k_{c-DS}$ ) eye position were statistically different (i.e., the confidence intervals did not overlap). Second, neither position parameters overlapped with zero indicating both position terms were significant. We called such neurons "binocular", since they had unequal sensitivity to the position (or the velocity in some cases) of the

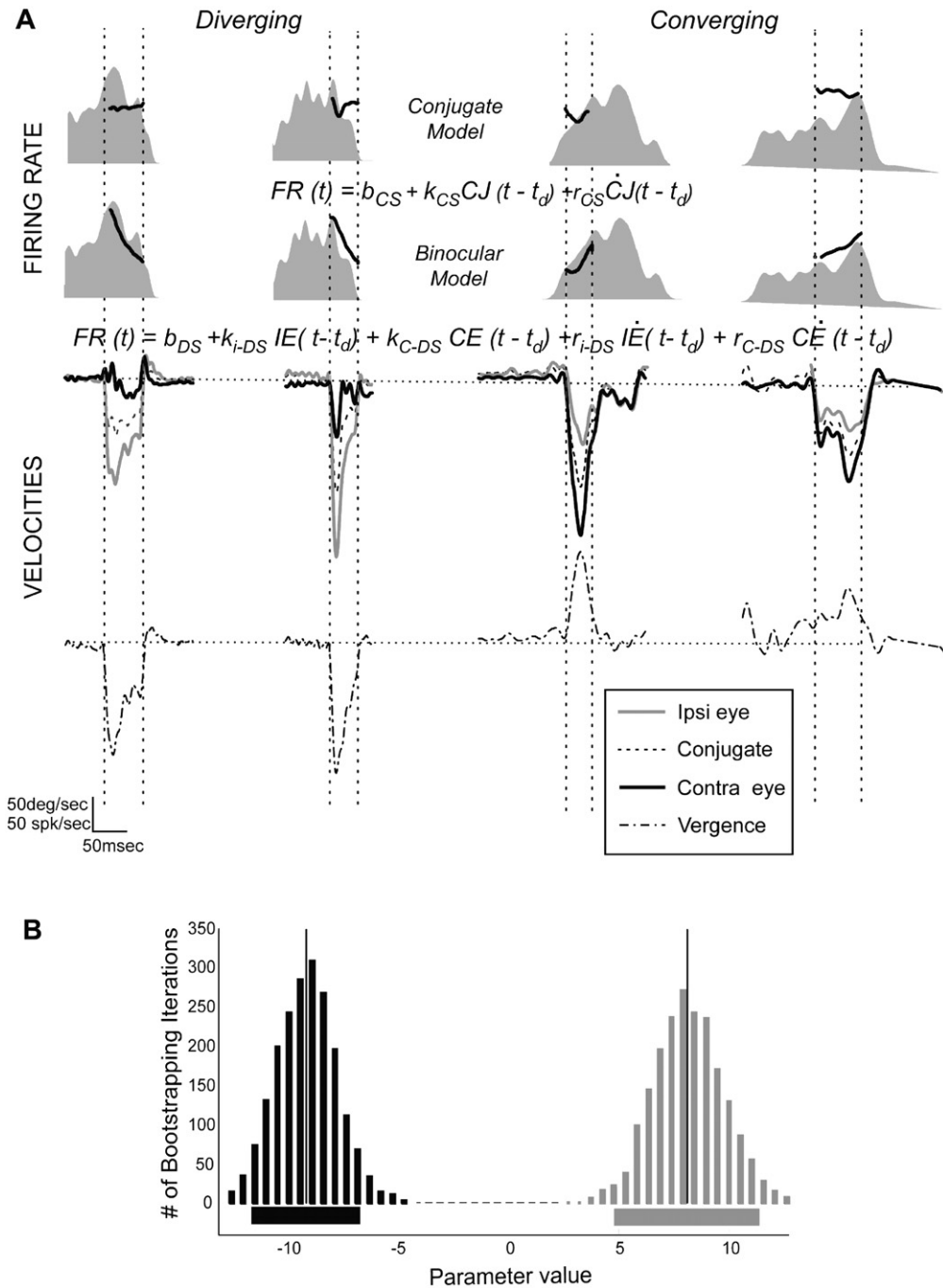


Fig. 3. (A) Model fits for cell ML41\_3 during disconjugate saccades (two left columns are diverging saccades and the two right columns are converging saccades). The thick black lines on the firing rates are the model fits using conjugate model parameters (top) and the binocular model (second row). In velocity traces (third row) solid black lines are the contralateral eye and solid grey lines are the ipsilateral eye. Vergence velocity is shown in the fourth row. (B) The histograms resulting from application of the bootstrapping method to the cell shown in A for the position parameters. The 95% confidence intervals for the estimates of position relationship are shown by the heavy horizontal bars below. The black distribution is the contralateral eye and the grey distribution is the ipsilateral eye. Thin vertical black lines indicate the mean for each distribution.

two eyes (ratio of the means of the two position distributions was not 1). Both velocity terms ( $r_{c-DS}$  and  $r_{i-DS}$ ) overlapped with zero indicating the velocity parameters were not important (data not shown). The majority of the neurons in our sample were comparable in that the discharges of the cMRF neurons were best described using individual eye position and/or velocity. Overall, this suggests that cMRF neurons encode the movement of an individual eye rather than solely encoding the conjugate component of a given saccade. Thus, when characterizing the discharge of cMRF neurons it is critical to consider the movement of *both* the ipsilateral and contralateral eye.

## Discussion

The findings described in the present study lead to two main conclusions. First, electrical stimulation of the MRF elicits both disconjugate and conjugate saccades. Second, the results of single unit recording from neurons in the cMRF of monkeys have demonstrated that the discharge of MRF neurons can dynamically encode the movement of an individual eye, rather than conjugate eye motion. Taken together, these results support the hypothesis that the midbrain shapes the activity of the pre-motor saccadic neurons by encoding integrated conjugate and vergence commands.

### *Role of neurons of the MRF in the control of saccades and vergence*

We and others have hypothesized that cMRF neurons could provide a feedback signal from the PPRF or omnipause neurons about the current progress of the saccade to the SC (Waitzman et al., 1996; Soetedjo et al., 2002; Cromer and Waitzman, 2006). It is also likely that neurons in the MRF provide a parallel pathway for descending information to the omnipause region. The projections from the MRF to the omnipause region (Horn, 2005) could inhibit the omnipause neurons and thereby indirectly activate PPRF burst neurons. Notably, our results further suggest that these ascending and descending projections convey not only conjugate but also vergence related information.

Coordinated inputs from the cMRF and intermediate and deep layers of the SC (dSC) to the saccadic pre-motor neurons could serve as an alternative to the previously described cortico-pontine-cerebellar-midbrain loop for the control of vergence (Gamlin et al., 1996). A number of pieces of evidence support this idea. Both the dSC and cMRF receive inputs from disparity sensitive cortical (FEF: Ferraina et al., 2000; LIP: Gnadt and Mays, 1995; Gnadt and Beyer, 1998; Genovesio and Ferraina, 2004) and subcortical regions [e.g., superficial layers of the SC (Mimeault et al., 2004)]. Furthermore, consistent with this idea, are the findings that stimulation of the cMRF (goldfish: Luque et al., 2006; monkey: present study) and SC (Chaturvedi and Van Gisbergen, 1999) have clear effects on vergence. Moreover, neurons in the SC (cat SC: Jiang et al., 1996; monkey SC: Walton and Mays, 2003) and the cMRF are modulated during vergence eye movements. Because the modulation of primate SC neurons was observed to be more robust for purely conjugate than disconjugate saccades, it has been suggested that the SC is not tuned in three dimensions (Walton and Mays, 2003). However, the present results combined with other recent findings (Van Horn et al., 2008) that showed neurons in the PPRF were associated with individual eye movement (Zhou and King, 1998; Sylvestre et al., 2003), suggest neurons in the dSC should be re-examined for evidence of an individual eye signal.

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