**INTRO**

Humans and other primates use combinations of eye and head movements to move the line of sight. Depending on the behavioral task, different types of movements may be employed. Gaze shifts are used to quickly acquire a new target using a rapid head rotation combined with a saccadic eye movement. Gaze pursuit can be used to follow a moving target and combines head rotation with smooth pursuit eye movements. These behaviors are often used in combination to efficiently view objects of interest within the natural world.

Investigations of the neural correlates of these behaviors reveal that separate neural mechanisms are employed. The superior colliculus (SC) is a key structure in the control of gaze shifts. Experimental evidence demonstrates that the SC contains an organized motor map that represents a desired gaze displacement signal used to generate gaze shifts. No analogous organized structure has been identified for pursuit movements. Instead, pursuit seems to be controlled by a reciprocal cerebro-ponto-cerebellar circuit. This circuit includes areas of visual motion processing and the frontal eye fields in the cortex, pontine nuclei that relay these signals to the cerebellum and follicular neurons, which are likely to be responsible for generating smooth pursuit eye movements. Although there is evidence of gaze-related signals at each stage in this circuit, it has not been demonstrated that these commands are used to generate head movements during pursuit.

The identification of brain regions responsible for decomposing gaze signals into the appropriate eye and head motor commands is an ongoing scientific pursuit. The technique of restraining the head has allowed researchers to understand the pathways driving eye and gaze movements, but does not distinguish between the two. When the head is free to move, behavioral paradigms can be employed to dissociate gaze from eye-related signals. This, combined with head-restrained studies, has allowed for significant progress in the mapping of the oculomotor premotor circuits. A similar method can be used to map the premotor circuits responsible for driving head movements.

Anatomic evidence exists for the neurophysiologic basis of head control in gaze shifts. In particular, some neurons in the reticular formation receive inputs from the SC and project to motor neurons in the cervical spinal cord. This places them in the ideal location to transform gaze displacement signals from the SC into appropriate head motor commands, though the activity of these neurons has not been described in primates performing head-unrestrained movements.

Recordings from the medullary and pontine reticular formation in cats have identified some neurons with activity correlated with certain dynamics of head movement (Isa and Naito 1995). Micro-stimulation of analogous structures in monkeys has been shown to produce movements of the eyes, head, ears, mouth and produce other movements, depending on the region stimulated (Quessy and Freedman 2004). Quessy and Freedman (2004) investigated a region of NRG that produces ipsilateral horizontal head rotation when stimulated, with kinematics similar to those observed during horizontal gaze shifts. They further demonstrated that while stimulating these regions does not produce eye movement directly, stimulation does alter ongoing eye movements initiated as part of a gaze shift (Freedman and Quessy 2004), implying that NRG is part of the circuit used to produce gaze shifts.

In addition to likely gaze-shift-related inputs from the SC, the NRG also receives input from many other areas, including motor and prefrontal cortex, the cerebellum and basal ganglia. This diversity of inputs suggests the potential for a greater role for NRG, including the potential for involvement in producing the head movements associated with gaze pursuit. Cats do not employ smooth pursuit movements like humans and monkeys do, so the neurophysiology of this region will require recording in the primate.

In this study, we return to the portion of NRG stimulated by Quessy and Freedman to record the activity of neurons that may be responsible for producing the head movement observed during stimulation. We use established behavioral paradigms to dissociate gaze, eye and head movement during gaze shifts that allow us to identify neurons whose activity is associated with head movement apart from gaze or eye movements. New techniques for dissociating the gaze, eye and head movements associated with gaze pursuit are also employed, enabling us to identify any neurons involved in producing the head movements associated with pursuit and to determine whether these are a separate population from those involved in producing head movements during gaze shifts. Our behavioral paradigms also allow us to assess neurons for activity related to eye position in the orbits. This is information required to produce a head-specific motor command from gaze-related signals.

## Methods

The two resus monkey subjects from chapter 1 also served as subjects in this experiment. The neurophysiologic recordings described in this chapter were made concurrently with the behavior described in the previous chapter.

### Neurophysiology

A tungsten microelectrode (microprobes) was inserted into the brainstem via a supporting canula through the trephine craniotomy. The depth of the canula was chosen to reach the bottom of the 4th ventrical. The electrode was then lowered further using a microdrive (koph). The anterior/posterior position of the electrode in the chamber was chosen using the characteristic firing pattern of the abducens motor nucleus as a landmark. We close electrode tracts that traveled posterior to the nucleus to avoid damaging motor neurons, and continued deeper. On most tracts, the characteristic population bursting for horizontal gaze shifts of PPRF was noted, as well as occasional MLBs and LLBNs. On many tracts, once we were below the level of population gaze-shift-related activity, we also characterized the location's response to microstimulation. We sought regions that produced horizontal head rotation on stimulation. Superficial to this region, we observed evoked ear movements as well as head movements with vertical or roll components. Any neurons isolated deep to the level of population gaze activity was recorded as a candidate for inclusion in this study.

### Modeling

We are attempting to find a function of the recorded eye and head movements that will predict the firing rate of the neuron during the trial. We convert the recorded spike times into a continuous function by convolving them with a Gaussian with a 15ms standard deviation to create a spike density function. We scale the spike density function so that it approximates the firing rate in spikes per second. For this analysis, we separate leftward and rightward movements to produce 12 possible predictor variables: (right/left)(eye/head)(position/velocity/acceleration), represented by the abreviations: *rhp, lhp, rep, lep, rhv, lhv, rev, lev, rha, lha, rea* and *lea*.

We use Matlab’s **stepwiselm** function, beginning with a constant model. The function evaluates the set of available terms, which includes the predictor variables described above, as well as pairwise interactions. If any of these terms improve the R2 of the model by 0.05 or more, the threshold criterion we chose, it includes the best term and then repeates the evaluation to see if any other terms could improve the model further. If these neurons were involved in generating the observed eye and head behavior, we assume that there would be a time delay between neural activity and movement. We repeated this stepwise model fitting to shifted data, in 10ms increments up to 200ms. We employed stepwise fitting method to find the best fit at each location independently. We then chose the delay that gave the best fit, determined by the R2 weighted by the number of terms in the model. Each additional term must improve the fit by at least 0.05. For example, if the best fit at a 50ms delay was a model with two terms an R2 of 0.29, and the best fit at 60ms was a model with three terms and an R2 of 0.30, we chose the simpler model.

**DISCUSSION**

In this experiment, we examined the activity of neurons in a head movement-related area of the brainstem: the nucleus reticularis gigantocellularis (NRG). We recorded the firing patterns of individual neurons in this region while subjects performed head-unrestrained gaze movements, which included gaze shifts and gaze pursuit. Subjects performed these movements using two behavioral tasks, a standard delayed gaze shift task (See: Freedman and sparks 1997, Walton and Freedman 2014), and a visual tracking task that subjects accomplished using a combination of smooth gaze pursuit and catch-up saccades (gaze shifts). We used established behavioral paradigms to dissociate head, eye and gaze velocity by controlling the initial positions of the eyes in the orbits. This provided us with a data set that includes gaze shifts and pursuit movements with a range of contributing head movements.

Our interest in the activity of the NRG is derived largely from the effort to identify neural correlates of eye-head coordination, particularly the regions involved in transforming gaze signals into individual eye and head motor commands. The activity of the superior colliculus (SC) has been well documented during head unrestrained gaze shifts. The deeper layers of the SC have been shown to encode a gaze displacement signal, corresponding to the amplitude of the gaze shift without regard for the combination of eye and head movements that are used to execute it (Freedman and Sparks 1997). This implies that regions downstream from the SC transform this gaze amplitude information into appropriate eye and head motor commands.

Consistent with the predictions from anatomy, microstimulation and recordings in cats, we find strong evidence of activity related head movement in the neurons we recorded. The peak firing rate of the majority of neurons was significantly correlated with peak head velocity for movements in at least one direction. Although head and eye movements may be correlated, we show through stepwise multiple linear regression that head velocity is the most important factor for predicting firing rate in most of our neurons. Eye velocity is not an important factor in any of the neurons in our data set, but we intentionally attempted to record from neurons with head -related activity, so this should not be taken as a random sample of neurons within NRG. Consistent with the hypothesis that activity in this region could be driving head movements, we observe head-velocity-related activity that precedes the observed movements, although there is significant variability of this latency between neurons. It is possible that these differences latency could be due to recruitment of motor neurons in different phases of the head movement.

The head-movement-related activity that we observed was not restricted to head movements made during gaze shifts. We observe similar head-movement-related activity during head movements made as part of gaze pursuit. This suggests that the NRG is not dependent on the SC to participate in generating head movement. The NRG could represent a region shared by the saccadic gaze shift and smooth gaze pursuit pathways, which otherwise depend on largely segregated circuits. For the majority of neurons we recorded, we did not detect any significant influence of task type on the relationship between the firing rate and velocity of the head, but such differences were apparent in a minority of cells. We show this in figure \ref{gs/pscomparison} where we compare the peak firing rate to peak velocity. For some cells, the statistical differences may be due to an uneven distribution of head velocities, but others show quite dramatic differences which warrant further investigation. Multiple regression analysis indicated that these differences could not be explained by sensitivity to head acceleration or any parameters of eye movement, which are known to differ between gaze shifts and pursuit. It is possible that this subset of neurons is sensitive to another aspect of the movement that we did not record, such as head roll, or the activity could be related to something other than movement. Another possibility is that the difference in activity is really due to differences in how the circuits involved in generating each movement type incorporates the NRG in the generation of head movements. For example, the NRG may not be the only input to the neck motor neurons active during pursuit or gaze shifts. If an additional premotor areas are recruited by one behavior type, that could account for the differences observed, although this does not explain why this effect would only appear in a minority of neurons.

In addition to head velocity, we also found activity related to eye and head position in our neurons. Neurons with head position related activity could participate in maintaining the head in an eccentric position by activating the same target neurons involved in generating head movements. Our data set does not include neurons with eye velocity related activity, but we do find some cells with eye position related activity, often together with head velocity related activity. Even though this region of the NRG does not seem to be involved in generating eye movements, information about the positions of the eyes in the orbits is essential for generating the appropriate head movement in response to a particular gaze displacement signal from the SC. We have also recently shown that this information is also essential to generate appropriate head movements in response to moving visual targets as part of gaze pursuit. The existence of this information encoded by neurons in the NRG provides further support for the hypothesis that the NRG is responsible for generating head movement commands as part of coordinated eye-head movements.