## Introduction

Stimulation of some regions within the rostral portion of the NRG produces ipsilteral, horizontal head rotation. During fixation, these head movements are accompanied by eye counter rotation with a gain less than one. This counter rotation is likely due to the vestibulo-ocular reflex (VOR), which is active during fixation. Stimulation of this region during a gaze shift has been shown to alter the head's contribution to the movement. This is accompanied by changes in eye velocity several times as large as the stimulation-induced changes in head velocity. The magnitude of this change in eye velocity cannot be due to the VOR, which causes eye movements proportional to head velocity with a gain less than or equal to one. Instead, it is consistent with the hypothesized interaction between the head and eye motor commands at the level of the gaze shift burst generator.

The role of the VOR in coordinating the eyes and head during pursuit is controversial. X X or both. Testing the system's response to head perturbations has failed to resolve the issue satisfactorily. The results from experiments of this type have been found support both an active and suppressed VOR. Attempts to reconcile these results include hypotheses that allow the VOR to ignore active head movements, while maintaining the ability to cancel unexpected or passive head movements. This means that experiments employing a head brake or other method to physically perturb the head are insufficient to properly test these hypotheses.

When the NRG is stimulted during gaze shifts, the system responds as though the head motor command were altered. This suggests that electrical stimulation of the NRG can be used to probe the coordination of the eyes and head during pursuit without introducing external, passive head perturbations.

In this experiment, we stimulate the NRG of monkeys while they perform head-unrestrained gaze pursuit. We expect that the stimulation will act as an alteration of the ongoing head motor command and result in altered head movement during pursuit, and will assess how the system responds to such a perturbation. We suggest three hypotheses for this response: (1) There is no interaction between the eyes and head during pursuit. The VOR is not activated by active pursuit-related head movements. Stimulation-induced changes in the head motor command will have no effect on eye movement. (2) The head and eye movement commands interact in a manner similar to what is observed during gaze shifts. Stimulation-induced changes in the head motor command will result in changes in eye movement that do not necessarily depend on the movement of the head. (3) The head and eyes are coordinated by the VOR during pursuit. Eye movement during pursuit is a result of an active eye motor command combined with VOR signals resulting from active head movement. Stimulation-induced alterations in the head motor command will result in changes in the counter-rotation of the eyes that is proportional to the changes in head movement with a latency of 8-10ms.

## Methods

One rhesus monkey participated in this experiment. This monkey also participated in the experiments described in the previous two chapters, and was familiar with head-unrestrained gaze pursuit and gaze shift tasks. For this experiment, the recording electrode was returned to the same region of the brainstem as in chapter 2, following the methods described in Quessy and Freedman (2004). We chose locations that appeared to produce horizontal head rotation without head roll for further analysis. Stimulation tranes were 100ms of 200-300 Hz at 40 micro-amps of current. In this report, we analyzed 11 locations, each on a separate day. At each location, we stimulated during two behavior paradigms, which were interleaved with control trials that were identical except that the stimulator was not activated. We will refer to the two experimental trial types as static stimulation and stimulation during pursuit.

#### Static Stimulation

During static stimulation trials, the subject fixates on a central visual target, and must align the head with the visual target as well. This is accomplished by illuminating a head-mounted laser, aligned with the mid-sagittal plane, which the monkey was trained to align with the visual target. After fixating for a random period of 1000-1500ms, the fixation light was turned off for 50ms (the gap) and the stimulator was turned on for 100ms. After the stimulation train was completed, a second visual target was illuminated 200ms later and the subject was required to make a gaze shift to this target. This gaze shift is not analyzed in this report. Instead, we analyze the period before the second target is illuminated to determine the effect of the stimulation. For control trials, the appearance and disappearance of the visual targets is identical, but no stimulation occurs during the gap.

#### Stimulation during Pursuit

We employ a step-ramp paradigm with a step size chosen to allow gaze pursuit to begin without the initial catch-up saccade. We employed a similar paradigm in chapter 1. Subjects begin by fixating on, and aligning the head to a visual target, in a manner identical to the previous trial type. After the random fixation period, the target suddenly makes a step change in position to the left or right, and then begins moving in the opposite direction. For the majority of the trials analyzed here, the target moved at a speed of 40 deg/s, but other speeds were used as well. During this trial type, the visual target disappears 400ms after it begins moving. After 50ms, the stimulation train is executed, which lasts for 100ms. The target remains invisible for 200 ms after stimulation ends then re-appears, traveling at the same velocity. Since our visual targets are produced by moving robot-mounted lasers, the act of turning the visual target on or off does not affect its position. The effect is that the target appears to have been occluded for 350 ms, but continued to travel at the same velocity while occluded. Subjects receive a juice reward for continuing to follow the target after it reappears. To isolate the effects of stimulation from the effects of a 350 ms target disappearance, we compare these trials to control trials in which the target disappears for an identical amount of time and no stimulation occurs. These trials are interleaved, so the subject cannot guess whether a given trial will contain stimulation or not.

#### Calculating Latency to Stimulation

We define the latency to stimulation of the eyes or head as the amount of time it takes for stimulation to produce eye or head movements. When the eyes and head are stationary, we can use standard methods for detecting movements. However, in our experiment we stimulate while the eyes and head are already moving at a significant speed, so another method must be used. For consistency, we will use the same method to assess both static and pursuit stimulation.

The figure below shows the following method diagrammatically. We will use an acceleration threshold and regression method to determine when the movement begins. First, we fit a regression line to the acceleration of each trial for the 200ms period before stimulation begins. Next, we average the acceleration for all stimulation trials at a location, and identify the peak acceleration (positive or negative) over the 20ms time period beginning when the stimulation starts. To avoid the effect of outliers on the peak acceleration, we will set a threshold of 2/3 the peak velocity and identify the time when the acceleration of each trial exceeds this value. We will then fit a regression line to the 25ms period preceding this time period. We define the latency for that trial as the time when these two regression lines intersect. If the calculation yields a negative latency, or a latency greater than 150ms, we count that as a missing value (NaN or NA) and do not include it in later analysis. This most often occurs when there is an extraneous movement, such as a gaze shift, that overlaps with the stimulation period.

#### Calculating VOR gain

We use a method similar to Quessy and Freedman (2004) to calculate the gain of the VOR on each trial. In their study, they calculate the gain of the VOR by computing a linear regression to the eye velocity as a function of head velocity. The slope of the resulting fit is used as a measure of the VOR. They use a subset of the eye and head data for this calculation, either from the beginning to end of the head movement, the beginning of head movement to the end of stimulation, or the time of peak head velocity to the end of the head movement. None of these methods can be adopted in our study, since the head is moving before stimulation, and continues to move long after stimulation ends. Instead, we use the 150ms time period beginning from when the stimulation-induced head movement is detected (see above section for details of how this is calculated). Example below:

## Discussion

In this study, we have assessed the effect of stimulation of the nucleus reticularis gigantocellularis (NRG) during head-unrestrained gaze pursuit. We designed these experiments to test critical predictions of the hypothesis that the eyes and head are coordinated by an active vestibulo-ocular reflex (VOR). Alternative hypotheses are that the eyes and head move independently during pursuit, or that the eyes and head are coordinated through a mechanism other than the VOR. Evidence that the eyes and head are coordinated through a non-VOR interaction during gaze shifts has been accumulating.