Measurement of the extraocular spike potential during saccade countermanding

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**Abstract**

The stop-signal task is used to investigate motor inhibition. Several groups have reported partial electromyogram (EMG) activation when subjects successfully withhold manual responses, and have used this finding to define the nature of response inhibition properties in the spinal motor system. It is unknown whether subthreshold EMG activation from extraocular muscles can be detected in the saccadic response version of the stop-signal task. The saccadic spike potential provides a way to examine extraocular EMG activation associated with eye movements in electroencephalogram (EEG) recordings. We used several techniques to isolate extraocular EMG activation from anterior electrode locations of EEG recorded from macaque monkeys. Robust EMG activation was present when eye movements were made, but no activation was detected when saccades were deemed canceled. This work highlights a key difference between the spinal motor system and the saccade system.
**Introduction**

Rapid inhibition of prepared motor responses has been studied extensively with the stop-signal or countermanding task (reviewed by Verbruggen and Logan 2008). In this task, subjects make quick responses to target stimuli. On a subset of trials, a second stimulus follows the target, instructing subjects to withhold their responses. When subjects are successful in canceling their responses, behavioral measures cannot be recorded because no overt behavior occurs. However, using a modeling approach, the timing of the covert inhibitory process can be estimated (Logan and Cowan 1984; Colonius 1990; Logan 1994). A saccadic response version of the stop-signal task has been used to characterize properties of the ocular motor system (Hanes and Schall 1996; Hanes et al., 1998; Hanes and Carpenter 1999; Logan and Irwin 2000; Pare’ and Hanes 2003; Corneil and Eisley 2005; Walton and Gandhi 2006; Boucher et al., 2007; Emeric et al., 2007).

Several groups have reported subthreshold electromyogram (EMG) activation on canceled trials in the manual response version of the countermanding task (De Jong et al., 1990; McGarry and Franks 1997; McGarry et al., 2000; van Boxtel et al., 2001; Scangos and Stuphorn 2010). However, it is unknown if partial extraocular EMG activation is present when eye movements are deemed canceled. The possibility that extraocular muscles may contract without producing detectable eye movement seems unlikely. However, the literature is inconclusive on this point. While it is true that the inertia of the eye within the orbit is negligible, the surrounding tissue of the oculomotor plant exerts viscous and elastic forces on the eye which are significant (Porter et al., 2003). It is difficult to estimate the extent to which these forces counteract eye movement production, because research has resulted in contradictory evidence (Robinson 1964; Sklavos et al., 2005; Anderson et al., 2009; Quaia et al., 2009). In fact, very few experiments have been reported on this matter. Furthermore, most of these
studies have been conducted using anesthetized animals, but larger time constants for visco-
elastic relaxation of orbital tissues have been noted in alert animals (Anderson et al. 2009).

When considering whether or not extraocular muscles are able to generate contractions
that do not result in eye movements, it is also important to consider the muscles themselves.
The extraocular muscles are relatively poor actuators. During periods of fixation, only 23% of
muscle innervation is ultimately transferred to the tendons to result in rotation of the eyeball
(Quaia and Optican 2003). Thus, when saccades are initiated, a force of much larger
magnitude must be supplied to overcome that dissipated by the muscles themselves. This
initial burst of force can be observed in the well known "pulse-slide-step" discharge pattern of
oculomotor neurons (Fuchs and Luschei 1970; Robinson 1970). The "pulse" portion of muscle
innervation is thought to be necessary in order to overcome static viscous drag exerted by the
passive orbital tissue (Sparks 2002). These considerations leave open the possibility that small
extraocular muscle contractions may occur in the absence of detectable eye movements.

If partial EMG activation were observed in the primate ocular motor system when trials
were deemed canceled, it would provide a powerful and versatile tool for examining motor
control in saccadic tasks. This development would be particularly useful for neurophysiological
research, since most work using the stop-signal paradigm with monkeys has been carried out in
the ocular motor domain. On the other hand, there is reason to believe that partial muscle
activation should not be readily produced by the primate ocular motor system. First, saccades
are thought to be initiated in an all-or-none manner. Second, although manual responses can
be canceled by coactivating agonist and antagonist muscles, it should be nearly impossible to
perform this type of cancelation in the ocular motor domain. The contralateral inhibitory circuitry
of the brainstem saccade generator precludes this type of muscle coactivation (Hikosaka et al.,
1978; reviewed by Scudder et al., 2002; Sparks 2002).

Because of their positions in the orbit, it is difficult to record EMGs directly from the
extraocular muscles. However, an EEG effect associated with eye movements, the saccadic
spike potential (SP), has been consistently noted in humans and monkeys (Blinn 1955; Keren et al., 2010; Sander et al., 2010). Several studies provide strong evidence that the SP does not originate in cortical activity or from the corneo-retinal potential (Thickbroom and Mastaglia 1985; Moster and Goldberg 1990; Picton et al., 2000). Instead, this component is myogenic, derived from contraction of the lateral and medial recti (Blinn 1955; Thickbroom and Mastaglia 1985). The SP appears as a prominent, high-frequency component occurring just prior to or concomitant with saccade onset. It takes the form of a frontal negativity with scalp distribution ipsilateral to the direction of eye movements (Thickbroom and Mastaglia 1985; Moster and Goldberg 1990; Keren et al. 2010). With appropriate filtering techniques, SPs have been shown to reliably precede saccades as small as 0.2° in amplitude, and to predict saccades with amplitudes less than 0.2° above chance level (Keren et al. 2010). Research on the SP has lapsed over the last few decades, but interest was recently renewed with the observation that many findings of gamma-band activity in scalp EEG recordings that were attributed to cognitive processes may actually have been artifacts from the SP associated with microsaccades (Yuval-Greenberg et al., 2008). Consequently, methods for isolating and removing SP activation from EEG recordings have been described (Keren et al. 2010).

In the present study, we tested the hypothesis that partial activation of eye-movement responses are made in the stop-signal task, similar to findings from manual stop-signal studies. This hypothesis predicts that partial muscle activation can occur on canceled trials. We tested this prediction by recording EEG and isolating SPs during periods when eye movements were prepared but not detected. We found strong SPs when saccades were made, but found no evidence of SP activation when movements were deemed canceled.

**Methods**

**Animal Care**
Data were collected from one male bonnet macaque monkey (*Macaca radiata* ~8.5 kg) and one female rhesus macaque monkey (*Macaca mulatta* ~7 kg). Both animals were cared for in accordance with policies set forth by the USDA and Public Health Service Policy on Humane Care and Use of Laboratory Animals. Animal care, procedures, and experiments were also carried out with supervision and approval from the Vanderbilt Institutional Animal Use and Care Committee. Fruit juice was given as positive reinforcement for correctly completed trials. During periods of testing, *ad libitum* access to liquids was withdrawn. In consultation with attending veterinarians, each animal’s weight and food intake were monitored, and fluids were supplemented as needed.

**Surgical Procedures**

All surgical procedures were carried out under aseptic conditions. Access to food was withdrawn 12 hours prior to surgery. Animals were sedated with ketamine (10-30 mg/kg) and provided with an initial dose of buprenorphine (0.005-0.010 mg/kg) to alleviate post operative discomfort. Ophthalmic ointment was applied to prevent corneal drying. Robinul (0.004-0.008 mg/kg) was administered to minimize mucosal secretions and help prevent vagal bradycardia. Animals were intubated and catheters were inserted into saphenous veins for administration of support fluids throughout the procedure. Monkeys were anesthetized with an isoflurane/oxygen mixture (1-3% C₃H₂ClF₂O), shaved, positioned in stereotax, and scrubbed. EKG, rectal temperature, respiration, and blood pressure were monitored. Expiratory CO₂ was maintained at ~4%. After subcutaneous administration of lidocaine (~1-2 ml of 2% soln’), the subjects’ skulls were exposed and titanium headposts were firmly attached with titanium, orthopedic screws (Synthes, West Chester, PA) to immobilize the animals’ heads during testing. Solid gold surface electrodes, Teflon coated stainless steel wires, and plastic connectors were constructed and implanted following the method of Woodman et al. (2007). Surgical sutures and staples were used to close incisions in layers. In consultation with attending veterinarians, analgesics...
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(bupronorphine 0.005-0.010 mg/kg) and prophylactic antibiotics (naxcel 2.2 mg/kg) were administered for at least 3 days following surgery.

**Task**

During testing, monkeys were seated comfortably 51 cm from a cathode ray tube monitor (48 x 48°, 80Hz) in enclosed polycarbonate and stainless steel primate chairs and head restrained using surgically implanted head posts. Stimulus presentation, task contingencies related to eye position, and delivery of liquid reinforcement were all under computer control in hard real time (TEMPO, Reflective Computing, Olympia, WA). Stimuli were presented using computer-controlled raster graphics (TEMPO Videosync 1,280 x 1,040 pixel resolution, Reflective Computing, Olympia, WA). Stimuli had a luminance of 30 cd/m² (fixation point) or 10 cd/m² (targets) on a 1 cd/m² background.

Behavior and electrophysiological signals were recorded during the countermanding (i.e., stop-signal) task (Figure 1). Additional details about the behavioral training regime and task have been described previously (Hanes and Schall 1995; Hanes et al. 1998). Trials were initiated when monkeys fixated a centrally presented square which subtended 0.34° of visual angle. After a foreperiod ranging from 200 ms to 1100 ms, the central fixation point was extinguished and a target subtending 3° of visual angle simultaneously appeared at 10° to the left or right of fixation. The foreperiod was randomly sampled from a distribution described by the function:

\[ p(t) = (1 - \exp(-t/\tau_g)) \cdot (\exp(-t/\tau_d)) \]

where \( p(t) \) describes the probability of selecting a specific foreperiod, \( \tau_g \) describes the growth rate, and \( \tau_d \) describes the decay rate. We chose a growth rate of 1000 ms and a decay rate of 200 ms to approximate a non-aging foreperiod. We added 200 ms to this distribution and truncated it at 1100 ms to achieve the desired range. On no-stop trials (Figure 1 top), no further
visual stimuli were presented. Monkeys were required to make a saccade to the target within 600 ms to obtain reward. Correct trials were rewarded with several drops of juice and an audible tone. On stop trials (Figure 1 bottom), the fixation point was re-illuminated after a variable delay providing a “stop-signal” which instructed the monkeys to cancel their impending eye movements and maintain central fixation. In practice, two trial outcomes were then possible. If monkeys successfully withheld the eye movement and maintained fixation for a minimum of 600 ms, they obtained tone and juice reward. These trials were designated as "canceled”. If monkeys were unable to inhibit the movement, a 1500 ms timeout was added to the normal inter-trial interval of 200 ms, no rewards were given, and the trial was termed “noncanceled”. The stop-signal delay (SSD) or time between target and stop-signal presentation determines the probability with which movements can be successfully countermanded (Logan and Cowan 1984). An initial set of SSDs from 0 to 420 ms and separated by either 40 or 60 ms was selected for each recording session. We then manipulated SSD using an adaptive staircasing algorithm which adjusted stopping difficulty based on performance. When subjects failed to inhibit responses, the SSD was decreased by a random step of 1, 2, or 3 increasing the likelihood of success on the next stop trial. Similarly, when subjects were successful in inhibiting the eye movement, the next SSD was increased by a random step of 1, 2, or 3 decreasing the future probability of success. This procedure was used to ensure that subjects failed to inhibit action on ~50% of stop trials overall. Stop trials were 30 to 70% of all trials in a given session with a typical session consisting of several thousand trials. Reaction time data did not show any evidence that subjects slowed responses to “wait for” the stop signal (see Results). Saccade initiation and termination were detected offline using a custom algorithm implemented in the MATLAB programming environment (MathWorks, Natick, MA) which first detected instantaneous velocity elevated above 30°/s and then calculated the beginning and ending of the monotonic change in eye position.
**Data Acquisition**

Time stamps of relevant trial events were recorded at 1 kHz with analog data using a Plexon Multichannel Acquisition Processor (MAP) box (Plexon, Dallas, TX). Eye position was monitored using a video based infrared eye-tracking system (ASL, Bedford, MA) and was streamed to the Plexon MAP box parallel with trial events and EEG data using a 64 channel Plexon Breakout Board (PBOB, Plexon, Dallas, TX). We estimated the spatial resolution of our eye tracking setup by recording standard deviations while monkeys were actively fixating the central fixation point. Across all sessions, the mean standard deviations were ±0.54° and ±0.51° for monkeys F and Y respectively. The maximum standard deviations while fixating for a session were ±0.74 and ±0.67 for monkey F and Y respectively. Unfortunately, this spatial resolution was not high enough to detect microsaccades, although it was more than sufficient to detect the onsets of large task related responses. Implanted EEG surface electrodes were referenced to clip style Ag/AgCl cup electrodes (Electro-Cap International, Eaton, OH) which were filled with conductive paste and clipped to either the left ear (monkey F) or linked to both ears (monkey Y). All data are recorded from an electrode approximating Fz of the international 10-20 system for humans in monkey F, and an electrode approximating Fpz in monkey Y. Since data are reported from a single midline electrode in both subjects, the asymmetric referencing used for monkey F did not result in any significant differences. The EEG from each electrode was amplified with a high-input impedance head stage (>1 GΩ, ~2 pF of parallel input capacitance, HST/8050-G1-GR, Plexon Inc.) and filtered between 0.7 and 170 Hz with two cascaded, one-pole, low-cut, Butterworth filters and a four-pole, high-cut, Butterworth filter.

**Race model behavioral analysis**

A race model has been used with great success to account for both behavioral performance and neural activity in the countermanding paradigm (Logan and Cowan 1984; Boucher et al., 2007; Lo et al., 2009; reviewed by Verbruggen and Logan 2008).
trials, reaction times (RTs) can be observed directly. On stop-signal trials, noncanceled RTs can be recorded, along with the probability of committing an errant noncanceled saccade at each SSD. The latter measure tends to assume the form of an increasing sigmoid curve, and has traditionally been referred to as an inhibition function. By treating the inhibition function as a cumulative probability distribution and comparing it to the distribution of RTs on no-stop trials, one is able to use the logic of the race model to estimate the median time required to cancel execution of a motor response (Logan 1994; Band et al., 2003; see also Colonius 1990). This stop-signal reaction time (SSRT) provides a measure of the otherwise covert stop process.

Following the methods of Hanes et al. (1998), we first fit a Weibull function with the following form to the inhibition function for each monkey averaged across sessions.

\[ W(t) = \gamma - (\gamma - \delta) \cdot \exp\left(-\frac{t}{\alpha}\right)^\beta \]

Where \( t \) = time after target onset, \( \gamma \) = the maximum probability value, \( \delta \) = the minimum probability value, \( \alpha = 64\% \) of the maximum probability value, and \( \beta \) = slope. Next, we used the fitted inhibition functions and the combined no-stop RT data to estimate SSRTs for each monkey using two different methods. The first of these methods assumes that SSRT is a random variable, while the second method assumes that SSRT is constant across SSDs (Hanes et al. 1998; Band et al. 2003). Since there is no reason to suppose an advantage of either of these SSRT estimation methods, we averaged the two estimates together to obtain a final SSRT estimate separately for each monkey (Hanes et al. 1998; Pare` and Hanes 2003).

A robust finding in the stop-signal literature is that noncanceled RTs are significantly lower than no-stop RTs. This is a straightforward prediction of Logan and Cowan's (1984) horse race model, since trials with faster GO processes will tend to finish before the STOP process, thus escaping behavioral inhibition. It also suggests that noncanceled trials cannot be accurately compared to the entire distribution of no-stop trials when RT is a potential confounding variable. An accurate comparison can only be made between noncanceled trials...
and no-stop trials with relatively faster RTs. Specifically, noncanceled trials should only be compared to no-stop trials with RTs < SSRT + SSD. These are the trials which would have escaped behavioral inhibition and resulted in errant saccades had a stop-signal been presented. Similarly, for accurate comparisons, canceled trials must be matched to slower no-stop trials with RTs > SSRT + SSD. Thus, even though no response is generated on successfully canceled trials, RT ranges can be estimated for this trial type. The technique of matching noncanceled and canceled trials to no-stop trials with RTs from the appropriate portion of the RT distribution has been termed “latency matching” (Hanes et al. 1998). In the current study, it was especially important that we compare canceled trials to their latency matched no-stop counterparts. This allowed us to estimate times when eye movements were likely even though they were not detected. Where appropriate, we used our derived SSRT estimates to latency match at each SSD.

Event-Related Potential (ERP) and Event-Related Velocity (ERV) analyses

ERPs were time-locked to saccade initiation or target onset and baseline corrected to the interval from 150 ms to 50 ms before these events. Canceled trials did not contain saccade events. Instead, a virtual saccade event was created for trials in this condition by randomly sampling from the distribution of latency matched no-stop RTs with replacement. Canceled trials were then aligned to this virtual saccade event and baseline corrected. Trials with voltage deflections greater than ±300 µV due to artifacts were excluded from further analysis. This threshold for rejection was an order of magnitude greater than the variability in the ERPs observed across monkeys (i.e., maximum root mean square for monkey F target aligned no-stop trials = 42.2 µV, canceled trials = 39.8 µV, noncanceled trials = 41.4 µV; maximum root mean square for monkey Y target aligned no-stop trials = 42.7 µV, canceled trials = 45.2 µV, noncanceled trials = 40.7). Single trial EEG signals were truncated 50 ms before the onset of the second, non-task related saccade to eliminate “smeared” saccade related artifacts. It was
important to estimate the relative timing of saccades and to display this estimate graphically. Instead of using a traditional method such as displaying a histogram of saccade latencies, we collapsed across saccade velocity profiles. This method is essentially the same as creating an ERP from EEG data, except the data were radial eye velocity traces (Figure 2). The resulting average does not only contain information about saccade latency, but also takes into consideration saccade amplitude and duration, making it a more complete descriptor of average saccade dynamics. Since these velocity profiles have been aligned to particular events and collapsed across trials in the same way as ERPs, we will refer to them as "event-related velocities" (ERVs). ERVs were not baselined since an ERV value of 0 is not arbitrary as it is in an ERP. As a rule, the single trial velocity profiles which made up the ERVs were truncated at the onset of the second, non-task related saccade to avoid contamination of the task related saccade velocity trace.

Narrow digital band-pass filters (frequency ± 1 Hz) were employed to discriminate the SP from other saccade related components (see results). Each filter was created using a Hamming window of length \( (2 \cdot T + 0.001) \) s, where \( T = 1/f \). A zero phase-shift digital filter was applied to the data using the specified hamming window. Analytical power of the filtered data at each time \( t \) was approximated using a sliding window function of the form:

\[
P(t) = \frac{\max(A) - \min(A)}{2}
\]

where \( A \) is the time interval \([t - \frac{T}{2}, t + \frac{T}{2}]\). These methods ensured a high level of filter specificity while minimizing sacrifices in timing estimation accuracy at each band-pass frequency.

A signal to noise ratio was estimated for each applied filter to assess how well it isolated the SP from the surrounding EEG. After applying each filter to single session data and estimating analytical power, the mean value in a 41 ms time window centered on the peak of the SP was recorded. This value was termed the "signal". The mean value in a 1 s time window
centered on saccade onset and excluding the signal time window was also recorded. This value was termed "noise". (Note that in this context, noise does not just refer to variability, measurement error, or unwanted line voltage fluctuations. Noise also refers to EEG fluctuations and includes those fluctuations which are task related. Task related EEG fluctuations do not average out in ERPs, and they can obscure the SP which is our component of interest.) The filter yielding the highest signal to noise ratio was then used to isolate single trial SPs in subsequent analysis.

Results

Behavior

Reaction times, average probability of committing errors, and SSRT estimates collapsed across sessions are summarized in Table 1. Both animals exhibited noncanceled trials with probability > 50%. Since we used a staircasing algorithm to adjust SSD, this departure suggests that both animals tended to speed up, causing a reduction in SSD. This pattern of behavior has been described before in animals performing the saccade stop-signal task, and it appears to be an effective strategy for speeding up trial presentation and maximizing the rate of reward delivery (Godlove et al., 2009). In any case, our estimates of SSRT are lower than the more typical estimates of 80 to 100 ms recorded in the literature. If our estimates are artificially low due to violations of the race model, it presents a problem for latency matching, since we may have erroneously underestimated the time of probable SP activation on canceled trials. Accordingly, when results depend on latency matching, large reaction time windows have been displayed and analyzed to ensure that late SP activation was not missed in canceled trials.

Saccade Dynamics

Figure 3 plots main sequences of no-stop (blue) and noncanceled (red) saccades separately for each subject and each target. These data are summarized numerically in Table
2. We carried out 3 way ANOVAs to test the hypotheses that saccade amplitude and/or velocity differed between subjects, targets, or trial types. Both amplitude \( (p < 0.001, df = 87) \), and velocity \( (p < 0.001, df = 87) \) were found to differ between targets. Monkeys tended to make slightly larger amplitude and higher velocity saccades toward the right target. This may be an artifact induced by the monocular eye tracking procedures we employed. Since we only tracked the right eye of each subject, saccades traces to the right target reflected abduction of the tracked eye while saccade traces to the left target reflected adduction of the tracked eye. On the other hand, the difference may reflect a real bias that both monkeys developed toward the right target. Peak saccade velocity was also found to differ between subjects \( (p < 0.001, df = 87) \). Monkey F made saccades with higher peak velocities than monkey Y. However, neither amplitude \( (p = 0.701, df = 87) \) nor peak velocity \( (p = 0.380, df = 87) \) differed significantly between trial types. Since main effects of around 1° proved highly significant in the target contrast, the failures to reject null hypotheses in the trial type contrasts cannot be attributed to a deficiency of statistical power. These results replicate earlier findings by Hanes and Schall (1995).

Saccade Aligned ERPs

Figure 4 plots saccade aligned ERPs and ERVs from both subjects. On trials in which saccades were detected, we observed a high amplitude, high frequency negativity occurring concomitant with or slightly before saccade initiation. This saccade-related component has been described many times in human subjects (Evdokimidis et al., 1991; Everling et al., 1997) and at least once in non-human primates (Sander et al. 2010).

For our purposes, the most important finding is the absence of the SP on canceled trials. At least two alternatives exist to explain this finding. First, we may conclude that partial muscle activation does not occur on canceled saccade trials, so no saccadic SP is evident. Second, we may conclude that aligning EEG to a virtual saccade event obtained by random sampling from
-existing RT distributions is too coarse a method to detect the saccadic SP on canceled trials. If partial motor activation did occur on these trials, we do not know when. Therefore, aligning on virtual randomly sampled RT events and collapsing across the data may have smeared any partial SPs and rendered them difficult to detect. We note that even if small amplitude SPs had been generated on the canceled trials but were temporally smeared by averaging, they should be revealed by a low amplitude, broad negativity during the measurement epoch. As is evident in Figure 4, we did not observe a waveform on canceled trials consistent with this pattern.

However, we carried out an additional time-frequency analysis to isolate SP activation from the surrounding EEG and test for the presence of extraocular EMG activation during canceled stop trials.

**Isolated SP activation**

In our data, the SP is readily visible as a stereotyped high frequency negativity (Figure 4). Because of its unusually high frequency and its invariance across sessions, we hypothesized that SP activation could be discriminated from the surrounding EEG on a trial-by-trial basis after application of an appropriate filter (see also Keren et al. 2010). We applied narrow digital band-pass filters in steps of 10 Hz to search for a frequency which optimally discriminated SP activation from the surrounding EEG. After filtering the data and calculating power as a function of time, we constructed response aligned ERPs for no-stop trials at each band-pass frequency for each recording session. We then calculated signal-to-noise ratios for each filtered ERP. The result of this analysis is plotted in Figure 5f. A band-pass filter centered on 95 Hz was found to provide the greatest discrimination between the SP and the surrounding EEG for monkey F, while a band-pass filter centered on 35 Hz was found to be optimal for monkey Y. At first glance, this difference may seem surprising. However, our technique does not simply measure the frequencies contributing power to the SP. Instead, it isolates the frequency which optimally discriminates the SP from the surrounding EEG. Therefore, this
difference reflects variations in overall EEG frequency spectra between the two monkeys.

Differences in EEG frequency spectra are to be expected due to several factors. For example, the skulls of monkeys F and Y were observed to be of different thicknesses during surgery (Nunez and Srinivasan 2006).

Application of optimal discrimination band-pass filters allowed us to observe the SP separate from the surrounding EEG. By using this technique, we were able to search for SP activation in target aligned ERPs made up either of no-stop or canceled trials. This comparison is plotted for a sample session from monkey F in Figure 6. The SP is visible in the unfiltered data when aligned on response onset, but is impossible to resolve, even on no-stop trials, when aligned on target onset (left column). After filtering, the SP is readily apparent in the response aligned, single trial data as a vertical band of elevated power (Figure 6 top right). A diffuse band of power can also be observed in the target aligned no-stop trials during the period of time when saccades are initiated (Figure 6 middle right). But no coherent band of elevated power can be discriminated on successfully canceled trials (Figure 6 bottom right).

Our band-pass filtering technique also provided us with power measurements which were amenable to statistical testing. After filtering the data, and performing latency matching to compare canceled trials with the appropriate no-stop trials, we measured average normalized power during a discrete window around mean RTs. For our window, we chose the period from the 25th percentile RT to the 75th percentile RT. Following this method ensured that we sampled power on canceled trials during the period of time when SPs were most likely to occur. Since power was baseline corrected to the interval 150 ms to 50 ms before target onset, power measurements collected at each SSD could be subjected to t-tests allowing us to test the null hypothesis that canceled trials do not show SP activation in the absence of overt eye movements. Results from this analysis are plotted in Figure 7. Each observation represents the average power for one SSD measured during the period of time when saccades were likely. No-stop trials (left) show an increase in power above baseline when saccades were produced.
(mean = 0.26 µV). This increase is statistically significant ($p < 0.001$, $df = 167$), and demonstrates that there was a reliable increase in SP activation associated with saccades. In contrast, canceled trials (right) show slightly decreased power during the period of time when saccades were likely to occur (mean = -0.11 µV). Although this effect is small, it is statistically significant ($p < 0.001$, $df = 167$) suggesting a small but reliable decrease in SP activation during periods when saccades were canceled. Thus, no partial EMG activation is present when monkeys cancel eye movements in the saccade countermanding task.

**Discussion**

We have provided evidence indicating that partial muscle activation does not occur in the primate ocular motor system when monkeys inhibit saccades in a countermanding task. Our conclusion is supported by the following observations. First, when canceled ERPs are aligned on a virtual saccade event to create saccade aligned ERPs, no evidence of EMG activation in the form of a SP can be observed around the time of saccade initiation. Second, when the SP activation is isolated from the surrounding EEG using band-pass filters, no-stop trials show EMG power which is significantly elevated above baseline while saccades are being made. Canceled trials, on the other hand, do not show EMG power that is elevated above baseline. Instead, trials in which saccades were deemed canceled display slightly reduced EMG activation as measured by the SP. This is strong evidence against partial motor activation in the ocular-motor system on canceled saccade trials.

The saccadic countermanding paradigm is a versatile tool which has led to many key findings over the last two decades. Human psychophysics experiments using the saccadic stop-signal task have helped elucidate the nature of conjugate gaze shifts (Corneil and Elsley 2005), differences between predictive and reactive stimulus tracking (Joiner et al., 2007), the relative contributions of reflexive foveal stimulation to stopping (Cabel et al., 2000), and the influence of stimuli timing and salience on saccade inhibition (Morein-Zamir and Kingstone...
Physiological recordings from monkeys carrying out the stop-signal task have helped uncover cortical (Hanes et al. 1998; Brown et al., 2008; Ray et al., 2009; Stuphorn et al., 2009; Scangos and Stuphorn 2010) and subcortical (Pare` and Hanes 2003) mechanisms of saccade generation. The task is useful for investigating performance monitoring in both human (Curtis et al., 2005; Endrass et al., 2005) and animal subjects (Stuphorn et al., 2000; Ito et al., 2003; Stuphorn and Schall 2006; Emeric et al., 2008, 2010). In addition, the saccadic countermanding task has given rise to a strong computational modeling literature leading to breakthroughs in understanding neural saccade production and regulation (Hanes and Schall 1996; Asrress and Carpenter 2001; Boucher, Palmeri et al. 2007; Lo, Boucher et al. 2009; Wong-Lin et al., 2010). Finally, the saccadic stop-signal task has had broad clinical significance, providing insight on the action of several popular anesthetic agents (Khan et al., 1999; Nouraei et al., 2003), as well as the core dysfunctions underlying disorders such as mild traumatic brain injury (DeHaan et al., 2007), Parkinson's disease (Joti et al., 2007), and ADHD (Armstrong and Munoz 2003; Hanisch et al., 2006). Given the wide experimental significance of the saccadic stop-signal paradigm, the observation of partial muscle activation on canceled saccade trials would have provided important theoretical leverage to the study of behavioral inhibition.

Several groups have found partial motor activation on canceled trials during the manual response version of the countermanding task. Partial motor activation on canceled trials has been taken as evidence against a ballistic phase of motor execution (De Jong et al. 1990; McGarry and Franks 1997; McGarry et al. 2000). Partial motor activation has also been used to study the unity or diversity of stopping under different circumstances (De Jong et al. 1990; van Boxtel et al. 2001). In addition, partial motor activation on canceled trials has been compared to full motor activation on no-stop trials, used as a proxy measure for SSRT, and compared to neural data to assess the relative contribution of supplementary motor neurons to movement initiation (Scangos and Stuphorn 2010). Clearly, partial motor activation on canceled trials is a
useful measurement for characterizing countermanding behavior. In contrast to manual
response countermanding, partial extraocular muscle activation appears to be absent on
canceled trials in the saccade countermanding task.

Lack of partial extraocular muscle activation on canceled trials is not surprising given our
current understanding of the saccadic system. The saccadic system and the spinal motor
system differ in several important ways. Unlike manual responses and smooth pursuit eye
movements, saccade initiation is, in many ways, ballistic (reviewed by Sparks 2002; Scudder et
al. 2002). Kornylo and colleagues (2003) found that pursuit eye movements could be canceled
more quickly than saccadic eye movements, and concluded that saccade production includes a
final ballistic stage which is not observed during pursuit.

One possible criticism of this work concerns the linking proposition identifying the SP
with the extraocular EMG. Since its first observation and characterization as the external rectus
muscle potential (Blinn 1955) the SP has been almost uniformly appreciated as myogenic in
nature (Picton et al. 2000; but see also Kurtzberg and Vaughan 1982; Balaban and Weinstein
1985; Riemslag et al., 1988). This conclusion is supported by the following seven observations.
First, the corneo-retinal potential cannot contribute to the SP since the SP can still be recorded
in total darkness (Riggs et al., 1974; Moster and Goldberg 1990) and observed in patients with
ocular prosthesis and intact extraocular musculature (Thickbroom and Mastaglia 1985).
Second, the SP is not considered to be cortical in origin, since it has been obtained with normal
topography after complete hemispherectomy (Thickbroom and Mastaglia 1985). Third, the SP
is attenuated or absent in patients with lateral rectus palsy or patients in whom the intra-orbital
musculature have been surgically removed (Thickbroom and Mastaglia 1985). Fourth, the
amplitude of the SP remains constant, but its scalp distribution changes predictably with
saccades made in different directions (Thickbroom and Mastaglia 1985; Moster and Goldberg
1990; Keren et al. 2010). Fifth, both its scalp distribution (Moster and Goldberg 1990; Lins et
al., 1993a; Keren et al. 2010; Sander et al. 2010) and dipole source modeling (Thickbroom and
Mastaglia 1985; Lins et al., 1993b) suggest that the SP is maximal around the eyes. Sixth, there is a close and consistent timing correlation between the peak of the SP and saccade onset (Thickbroom and Mastaglia 1985; Keren et al. 2010). Seventh, the amplitude of the SP shows a positive correlation with saccade amplitude (Keren et al. 2010). Thus, using the strong inference method advocated by Platt (1964), an extensive body of evidence demonstrates that the SP should be viewed as an extraocular EMG. It is a natural step then, to search for the presence of extraocular EMG activation using SPs recorded in the stop-signal task.

Another possible criticism concerns the resolution of our EMG measurement. One may argue that our proxy measure of extraocular EMG was not sensitive enough to detect small muscle activations. If so, partial muscle activation may have been present on some canceled trials which was unobservable as single trial SP. Using a wide band-pass filter, Keren et al. (2010) were able to reliably isolate single SPs from the raw EEG. They then used signal detection theory to quantify the accuracy with which single SPs predict saccades. These researchers found that they could detect greater than 80% of saccades 0.5 - 1° in amplitude with close to zero false alarms, and they could detect saccades of 0.02 - 0.2°V in amplitude above chance level. They concluded that single SPs might serve as more reliable saccade indicators than the traditional method of detecting corneo-retinal dipole shifts in EEG recordings.

We refined the technique presented by Keren et al. (2010) by adopting a frequency optimization procedure which ensured that small SPs would be highly detectable. The average power traces which we were able to construct for no-stop trials containing 10° saccades suggest that we would have been able to detect SPs associated with very small amplitude movements (see Figures 5 and 6). Still, the fact remains that canceled trials may be associated with subthreshold EMG activation which is too small to detect with surface electrodes. In order to test this hypothesis further, recordings would be needed from microelectrodes inserted into the motor nuclei themselves.
It is noteworthy that we did not simply observe a lack of extraocular EMG on canceled saccade trials. Instead, we report a small but significant decrease in EMG activity when eye movements were withheld. Before baselining, a tonic increase in EMG was observed in the period of time around task related saccades. (Figure 5f and 5g.) We speculate that this tonic resting EMG activity was produced by microsaccades which occurred throughout our recordings (Yuval-Greenberg et al. 2008). On canceled trials, we observed a significant decrease in tonic EMG activity during periods when saccades were likely (Figure 6 lower right). Following this logic, we suggest that fewer microsaccades are probably made while eye movements are suppressed during canceled trials. This would be an interesting finding, useful for further characterizing the function of fixation cells during the countermanding task. Unfortunately, the spatial resolution of our current eye tracking data set does not allow us to test this hypothesis directly. Future work should measure the presence or absence of microsaccades during periods when task related saccades are canceled in the countermanding task.

In summary, we isolated EMG activation associated with eye movements from the EEGs of monkeys performing a saccade-countermanding task. We found that eye movements were reliably accompanied by EMG activation on noncanceled trials, but no subthreshold EMG activation was detectable on successfully canceled trials. This finding demonstrates the ballistic nature of saccade initiation, and highlights a basic difference between the spinal motor system and the saccadic ocular motor system.

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Neuroscience.
References


Brown JW, Hanes DP, Schall JD and Stuphorn V. Relation of frontal eye field activity to
saccade initiation during a countermanding task. *Experimental Brain Research* 190: 135-
151, 2008.

Cabel DWJ, Armstrong IT, Reingold E and Munoz DP. Control of saccade initiation in a
countermanding task using visual and auditory stop signals. *Experimental Brain

Collewijn H and Kowler E. The significance of microsaccades for vision and oculomotor

Colonius H. A note on the stop-signal paradigm, or how to observe the unobservable.

Corneil BD and Elsley JK. Countermanding eye-head gaze shifts in humans: Marching orders
are delivered to the head first. *Journal of Neurophysiology* 94: 883-895, 2005.

Curtis CE, Cole MW, Rao VY and D'Esposito M. Canceling planned action: An fMRI study of

De Jong R, Coles MGH, Logan GD and Gratton G. In search of the point of no return - the

DeHaan A, Halterman C, Langan J, Drew AS, Osternig LR, Chou LS and van Donkelaar P.
Cancelling planned actions following mild traumatic brain injury. *Neuropsychologica* 45:

Emeric EE, Brown JW, Boucher L, Carpenter RHS, Hanes DP, Harris R, Logan GD,
Mashru RN, Pare M, Pouget P, Stuphorn V, Taylor TL and Schall JD. Influence of
history on saccade countermanding performance in humans and macaque monkeys.


Sander V, Soper B and Everling S. Nonhuman primate event-related potentials associated

Scangos KW and Stuphorn V. Medial frontal cortex motivates but does not control movement

Scudder CA, Kaneko CRS and Fuchs AF. The brainstem burst generator for saccadic eye

Sklavos S, Porrill J, Kaneko CRS and Dean P. Evidence for wide range of time scales in
oculomotor plant dynamics: Implications for models of eye-movement control. *Vision

Sparks DL. The brainstem control of saccadic eye movements. *Nature Reviews Neuroscience

Stevenson SA, Elsley JK and Corneil BD. A "gap effect" on stop signal reaction times in a

Stuphorn V, Brown JW and Schall JD. Role of supplementary eye field in saccade initiation:

Stuphorn V and Schall JD. Executive control of countermanding saccades by the

Stuphorn V, Taylor TL and Schall JD. Performance monitoring by the supplementary eye

Thickbroom GW and Mastaglia FL. Presaccadic spike potential - investigation of topography

van Boxtel GJM, van der Molen MW, Jennings JR and Brunia CHM. A psychophysiological
analysis of inhibitory motor control in the stop-signal paradigm. *Biological Psychology

Verbruggen F and Logan GD. Response inhibition in the stop-signal paradigm. *Trends in


Figure Captions

Figure 1. The stop-signal (or countermanding) task in a schematic representation. No-stop trials (top) were initiated when monkeys fixated a centrally presented fixation point. After a variable time, the fixation point was extinguished and simultaneously a peripheral target was presented at one of two possible locations. Monkeys were required to fixate targets with quick saccades for juice rewards. Stop trials (bottom) were initiated in the same way. After a variable time termed stop-signal delay (SSD) the fixation point was reilluminated, instructing the monkeys to withhold movement. Successful inhibition of saccades resulted in rewarded Canceled trials, but errant saccades resulted in unrewarded Noncanceled trials. Black squares indicate stimulus locations. Dotted circles represent area of fixation. F = fixation point, T = target, RT = reaction time, SSD = stop-signal delay.

Figure 2. The timing of eye movements relative to task events was displayed using event related velocity (ERV) plots. This technique is similar to creating ERPs from raw EEG signal. Top left shows single trial radial positions for a sample session aligned on saccade onset. Bottom left shows instantaneous radial velocity for the same trials (black) along with the mean instantaneous velocity collapsed across all trials (red). Top right shows the same single trial radial positions in relation to target onset. Bottom right shows single trial instantaneous velocity in relation to target onset, as well as the average radial velocity collapsed across all trials. This target aligned ERV gives information about average saccade latency, velocity, and duration relative to target onset.

Figure 3. Saccade dynamics do not differ between no-stop and noncanceled trials. Scatter plots display saccade amplitude vs. peak saccade velocity (main sequences) across all sessions. Histograms display associated probability densities for each measurement.
Binwidths are 10 deg/s for velocity distributions and 0.25 deg for amplitude distributions. Blue dots and broken lines represent saccades on no-stop trials. Red dots and solid lines represent saccades on noncanceled trials. Rows separate data by target. Columns separate data by subject.

**Figure 4.** No SP is evident in canceled trials aligned on a virtual saccade event. Black traces show ERPs and colored traces show ERVs (see text). The thin solid traces show saccade aligned ERPs and ERVs on no-stop trials. The most prominent components in the ERPs are the sharp negative SPs, which occurs just prior to or concomitant with saccade onset and the several positive and negative deflections which follow. The first several components which follow saccade onset probably include a strong contribution from the corneo-retinal potential. The broken traces show ERPs and ERVs on errant noncanceled trials. Note the extreme similarity of the ERVs for no-stop and noncanceled trials. Note also the similarity between no-stop and noncanceled ERPs. This similarity is especially apparent in the time before saccade onset when the SP is visible. The thick solid traces depict ERPs and ERVs on canceled trials aligned to a virtual saccade event. No elevated velocity can be detected in the ERVs, and no SP can be detected around time 0 in the ERP. Data are collapsed across 15 sessions and recorded from a location approximating Fz for monkey F. Data are collapsed across 7 sessions and recorded from a location approximating Fpz for monkey Y. ERP data are baselined to the period from 150 ms to 50 ms before saccade onset. The number of trials in each ERP follows. Monkey F; no-stop n = 13,764, canceled n = 6,256, noncanceled n = 6,552. Monkey Y; no-stop n = 4,782, canceled n = 1,489, noncanceled n = 1,120.

**Figure 5.** Band-pass filters were optimized to find frequencies which allowed for the highest discrimination between the SP and non SP components. A One second example of raw EEG centered on saccade onset. Note that in this and following panels negative is plotted down so
that later power traces appear facing upward. b The same EEG signal processed with a 35 Hz band-pass filter. After filtering, the analytical power was estimated (see methods) and this estimate is depicted by the thick blue line. c Power at 35 Hz for every no-stop trial in the example session. Each horizontal line of color depicts a single trial centered on saccade onset. Warmer colors indicate more power. Note the faint band adjacent to saccade onset indicating that the 35 Hz band-pass filter was somewhat successful in isolating SP related activation. d This result is further demonstrated by collapsing across all trials and creating an ERP from the power traces at 35 Hz. A "signal" and "noise" time period was chosen based on SP peak time measured from unfiltered session ERPs. The time period highlighted in white was the signal time period, and the time period in gray was the noise time period for monkey F. Average power in both time periods was recorded and used to calculate signal to noise ratios (S:N). e S:N for each band-pass frequency was calculated for each session. These traces show the average S:N separately for monkey F (blue) and monkey Y (green) ± SEM. The highest S:N was found at a band-pass frequency of 95 Hz for monkey F (f) and 35 Hz for monkey Y (g).

Figure 6. Filtering EEG makes it possible to observe the SP independent of surrounding EEG, but no SP is observed on canceled trials. Traces at top show ERVs to display saccade timing (conventions as in Figure 4). Heat maps show individual trials (conventions as in Figure 5). Black lines show ERPs collapsed across trials. Thin lines show no-stop trial ERPs, and thick lines show canceled trial ERPs. The left column displays raw voltage. At top, data are presented from no-stop trials aligned to saccade onset. The ERV appears as a narrow component beginning at saccade onset. The heat maps display negative bands of activation at saccade onset corresponding to the SP. Collapsing across the data in the ERP makes the SP readily apparent in both the raw and filtered data. At middle, data are presented from no-stop trials aligned to target onset. The ERV reflects this change. Now saccades are smeared around 200 ms centered roughly at 210 ms after target onset. Because of this smearing, it is no
longer possible to discern negative activation associated with the SP in the raw heat map. This activation should be apparent centered around 200 ms after target onset. SP activation is also smeared in the raw ERP, rendering it invisible. However, in the filtered data, SP activation is clear around 200 ms in both the heat map and ERP. At bottom, data are presented from canceled trials aligned to target onset. The ERV never approaches 30 deg. s\(^{-1}\) (criteria for saccade initiation). No SP is apparent in the raw heat map data, or in the raw ERP. But it is impossible to tell if no SP exists, because it is also unobservable in the raw no-stop data plotted above due to overlapping components and smear. The filtered data at right allows for examination of SP activation. No SP activation can be observed in the time around saccade initiation. If anything, a small depression in high frequency SP activation is all that can be observed.

**Figure 7.** No-stop trial EEGs display significantly increased SP activation during periods when saccades are produced, but canceled trial EEGs show no increase in SP activation. After latency matching trials and filtering EEG data (see Figure 6), the average power during a discreet time window was measured on a trial by trial basis. For the time window, we chose the period between the 25th and 75th RT percentiles. Since no-stop trials were latency matched to canceled trials, this is the period of time during which SP activation was most likely to occur in both trial types. Power averages were collected from this time window at each SSD. Each SSD from each recorded session yielded a single observation for each trial type. Histograms depict the results of this analysis. The observations are gathered in 0.1 \(\mu\)V bins for display purposes. Grand average power is reported for each trial type above the appropriate histogram. Note that the sign of these averages is negative for canceled trials. Both distributions deviate significantly from 0 (students t-test, \(p < 0.001\), \(df = 167\)).
Footnote

1 Keren et al. (2010) report data from a bin that included saccade amplitudes ranging from 0.2° to 0.5°. As correctly pointed out by an anonymous reviewer, the distribution of saccade amplitudes within this bin was not reported. Strictly speaking, it is therefore impossible to say with certainty that SPs associated with saccades of 0.2° in amplitude could be reliably detected. However, it is well known that histograms displaying amplitudes of saccades recorded during a given time interval tend to take the form of decreasing exponential distributions (e.g. Collewijn & Kowler, 2008). In other words for any given distribution, saccades of smaller amplitude tend to be made with exponentially higher frequency than saccades of larger amplitude. Therefore, it is reasonable to expect that saccades with amplitudes ~ 2° made up a large proportion of the saccades used for this analysis.
Radial position (deg.)

Saccade aligned

Radial velocity (deg. s⁻¹)

Time from saccade (ms)

Target aligned

Radial velocity (deg. s⁻¹)

Mean radial velocity (deg. s⁻¹)

Time from target (ms)
Peak velocity (deg. s\(^{-1}\))

Left target

200
400
600

Right target

FY

5 10 15 5 10 15

No-stop saccades
Noncanceled saccades
Response aligned ERPs and ERVs

![Response aligned ERPs and ERVs](image)

- **Y**: 459 Deg/s
- **F**: 533 Deg/s

- ERP No-stop
- ERP Noncanceled
- ERP Canceled
- ERV

Time from saccade (ms)
**Raw EEG (single trial)**

![Raw EEG graph](image)

**Mean power (μV)**

S:N=1.31

**Optimal S:N (monkey F)**

S:N=1.84

**Optimal S:N (monkey Y)**

S:N=1.35

**Power at 35 Hz (single trial)**

![Power at 35 Hz graph](image)

**Power at 35 Hz (single session)**

![Power at 35 Hz session graph](image)

**Mean session power at 35 Hz**

S:N=1.31

**Mean power (μV)**

S:N=1.35
No-stop trials

Canceled trials

\( \mu = 0.26 \)

\( \mu = -0.11 \)
<table>
<thead>
<tr>
<th></th>
<th>no-stop RT</th>
<th>noncanceled RT</th>
<th>p(noncanceled)</th>
<th>SSRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>monkey F</td>
<td>224 ± 52</td>
<td>211 ± 57</td>
<td>0.58</td>
<td>59</td>
</tr>
<tr>
<td>monkey Y</td>
<td>243 ± 77</td>
<td>206 ± 75</td>
<td>0.53</td>
<td>59</td>
</tr>
</tbody>
</table>

Table 1. Summary statistics for stop-signal task performance

Reaction times (± 1 standard deviation), probability of committing errant noncanceled saccades, and SSRTs for each subject collapsed across sessions.
Table 2. Countermanding saccade dynamics

<table>
<thead>
<tr>
<th></th>
<th>amplitude (deg.)</th>
<th>peak velocity (deg. s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>left target</td>
<td>right target</td>
</tr>
<tr>
<td></td>
<td>no-stop noncanceled</td>
<td>no-stop noncanceled</td>
</tr>
<tr>
<td>monkey F</td>
<td>9.7 ± 0.7</td>
<td>10.7 ± 0.8 10.5 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>473 ± 92</td>
<td>463 ± 97 623 ± 66 607 ± 104</td>
</tr>
<tr>
<td>monkey Y</td>
<td>9.3 ± 0.9</td>
<td>10.5 ± 0.9 10.5 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>428 ± 97</td>
<td>469 ± 306 502 ± 52 509 ± 247</td>
</tr>
</tbody>
</table>

Mean amplitude and mean peak velocities (± 1 standard deviation) across sessions separated by subject, target location, and trial type.