COUNTERMANDING IN RATS AS AN ANIMAL MODEL OF INHIBITION OF ACTION: VALIDATION OF THE RACE MODEL

by

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Abstract

Executive function, the cognitive processes that allow the voluntary control of goal-directed behaviour, can be studied through the examination of inhibition of action. The countermanding paradigm has been shown to be a powerful tool to examine a subject’s ability to withhold responses to a go stimulus when a stop signal is presented occasionally. Logan and Cowan (1984) developed a race model to account for countermanding performance in humans, proposing that independent go and stop process initiated by the go and stop signals respectively, race toward a finish line whereby the first process to cross its finish line determines the behavioural outcome. The model allows estimation of the stop signal response time, a variable that is not directly observable. The race model has yet to be validated for countermanding performance in rats.

Using a new rodent countermanding task inspired directly from human studies, male Wistar rats were trained to respond to a visual stimulus (go signal) by pressing a lever below an illuminated light for food reward, but to countermand lever the press (25% of trials) subsequent to an auditory tone (stop signal) presented after a variable delay. The ability to cancel a response decreased as stop signal delay increased. The stop signal response time for rats was estimated to be 157 ms, a value within the range of human estimates. Predictions of countermanding performance made by the race model were generally respected. Response times of movements that escape inhibition: 1) were shorter than those of movements made in the absence of a stop signal; 2) gradually lengthened with increasing stop signal delay; and most importantly, 3) were predicted by the race model. These findings demonstrate that the countermanding performance of rodents can be accounted for by a simple race model, which has been applied successfully in human studies and nonhuman primate models. This new animal model will permit complementary invasive investigations of brain mechanisms underlying inhibitory control and refine the existing rodent models of neurological disease and impulsivity.
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Chapter 1

Introduction

As will be discussed below, previous research has examined behavioural inhibition with the countermanding task as a measure of executive function, particularly in human and non-human primates. Recently, rodent countermanding tasks have been developed, providing a new tool for evaluating behavioural inhibition. An introduction to executive function and the study of behavioural inhibition will be presented. A brief overview of the countermanding task will follow, with a description of the race model of neural processing that has been established to account for performance in the task. This will be followed by a review of human and non-human primate countermanding task findings. Finally, rodent stop task studies will be discussed. This will be followed by a statement of the hypothesis examined in this thesis.

1.1 Executive Function

A primary goal of neuroscience is to determine how the brain exerts control over behaviours. It can be argued that production of movement is the major function of the central nervous system, as movement is the primary means by which an organism can interact with the environment in order to obtain survival necessities (Cotterill, 2001; Wolpert et al., 2001). A fundamental aspect of movement control pertains to decision processes that lead to optimal behaviour. Voluntary control of behaviour allows organisms to integrate perception, cognition and action by combining encoded stimulus information with a behavioural intention to produce goal-directed responding (Smith & Ratcliff, 2004).

Executive function is the set of cognitive abilities that allow the voluntary control of goal-directed behaviour. In a dynamically changing environment, executive processes are internally generated acts of control that allow an organism to adapt to changing situations and
bring courses of thought and action in line with current goal sets (Logan, 1994). Thus, behaviour produced by executive function processes in the brain result from a series of acts of control that can be conceptualized as interactions between an executive system that forms intentions and issues commands to an interpreting subordinate system that performs the instructions (Logan & Cowan, 1984).

One basic function of the executive system is the ability to inhibit a course of thought or action when it is no longer appropriate in light of new goals. In order to initiate a new course of action, one must immediately stop current, inappropriate behaviour (Logan, 1994). Moreover, deficits in the ability to inhibit inappropriate behaviour are thought to underlie the symptoms of a number of disorders including attention deficit/hyperactivity disorder (ADHD), obsessive compulsive disorder, Parkinson’s disease and schizophrenia (Aron et al., 2003a; Badcock et al., 2002; Royall et al., 2002). Thus, behavioural inhibition is a simple, singular act of control that permits the investigation of an important executive function. Furthermore, insights into inhibitory processes are necessary to provide a better understanding of executive dysfunction in specific neurological illnesses and impulsive behaviour (Solanto et al., 2001).

Behavioural inhibition is theoretically difficult to study because it is only manifested through the absence of an overt behaviour (Band & van Boxtel, 1999). Nevertheless, previous researchers have examined various aspects of inhibitory control of action using a variety of tasks. For example, in the go/no-go task, subjects are expected to produce a behaviour in response to the presentation of a particular stimulus but are to withhold response when an alternative stimulus is presented (Pare & Wurtz, 2001). In well trained subjects, performance on no-go trials in the task is highly successful and consequently there is potentially valuable information left unexplored regarding failed inhibition (Akerfelt et al., 2006). Similarly, inhibitory control has been examined in humans and non-human primates in the Stroop and change tasks; however these different
approaches likely draw upon distinct executive functions (Rubia et al., 2007). Moreover, there are no well defined models that resolve why mistakes are made in these tasks.

Several tests of behavioural inhibition have been developed for rodents (Dalley et al., 2008). Along with the go/no-go task, two of the most regularly employed are the 5-choice serial reaction time and the differential reinforcement of low rate of responding tasks. In the 5-choice serial reaction time task, rats are required to wait for a go stimulus indicating which of 5 recessed holes require a nose-poke response to receive reinforcement. Similarly, in the differential reinforcement of low rate of responding task, subjects must delay operant responses for reward as reinforcement is only available for the first response after a specified time interval. While these tasks tend to measure inhibition of response initiation, they do not examine stopping of processes in progress. Alternatively, the countermanding or stop task offers a better approach to the study of control of inhibition after response initiation, as it requires a deliberate stopping response (Logan & Cowan, 1984).

1.2 The Countermanding Task

Stopping as the intentional prevention of a response is produced quickly, without much thought about required mechanics and can easily and precisely be studied experimentally with the countermanding paradigm (Aron et al., 2007b). The task was originally developed to investigate voluntary control of action (Lappin & Eriksen, 1966). In the countermanding task, subjects are given a primary task to perform when a go signal is presented. To correctly complete go trials, a primary response is required. The time interval beginning at go signal onset and ending with go response completion is recorded as the response time. On a small subset of trials, a stop signal is presented concurrently or following the go signal. In these stop trials inhibition of the primary go response is required. Stop signal onset can occur after a variable delay following the go signal;
this delay is termed the stop signal delay. The amount of time required to inhibit the response after stop signal onset is termed the stop signal response time (Logan & Cowan, 1984). Fast responding in the stop task is encouraged by limiting the amount of time that subjects are given to make the go response and by limiting the number of stop trials in a session to ensure that subjects do not strategically slow their response times (Band et al., 2003; Eagle & Robbins, 2003b). Thus, this paradigm provides information about failed as well as successful inhibition and allows comparisons to be made with go trial responding (Akerfelt et al., 2006). Furthermore, a model has been developed to account for performance in the countermanding task.

1.3 The Race Model

Logan and Cowan (1984) developed a race model to account for stop task behaviour in human and non-human primates. The model proposes that two sets of neural processes are involved in stopping performance. A go process is initiated by go signal onset. Stop signal presentation results in the initiation of an independent stop process. The two processes race toward a finish line, whereby the first process to cross its finish line wins the race and determines the behavioural outcome. If the go process crosses first, the response is executed. If the stop process wins the race, the response is countermanded (Figure 1). These two independent processes are suggested to build stochastically by accumulating noisy stimulus information until sufficient information is accumulated (Smith & Ratcliff, 2004).

The model predicts that the stop signal delay biases the relative finishing time of the go and stop processes, changing the likelihood of which process will finish first (Logan & Cowan, 1984). If the stop signal delay is short, the stop process is more likely to win the race. If the stop signal delay is long, the stop process has less time to cross the finish line and the go process is more likely to win the race. Thus, the probability of incorrectly responding in a stop trial
increases as the stop signal delay increases. If the stop signal delay is long enough, the response should always occur, while if the stop signal delay is short enough, the response should always be inhibited. By varying the stop signal delay, it is possible to produce a response inhibition function displaying the differing probabilities of making a non-canceled response based on the length of the stop signal delay. Thus, the outcome of the race is determined by variability in go process duration, variability in stop process duration and variability in the probability of triggering the stop process.

**Figure 1:** Race Model of countermanding performance. Two sets of processes, one initiated by a go signal and one by a stop signal after a variable stop signal delay (SSD), race toward a threshold. If the go process wins its race a non-canceled response is made. If the stop process wins its race the response is inhibited. The stop signal response time (SSRT) can be estimated as the time between stop signal onset and the point where the stop process crosses the threshold to countermand the response (Adapted from Paré & Hanes, 2003).
The strength of the race model is that it predicts a way to estimate stop signal response
time, a variable that is not directly observable. The model assumes that the go and stop processes
act independently. As a result of this assumption, the response time distribution is the same for go
trials and non-canceled stop trials (i.e., when a response is made the response time should be the
same with or without stop signal presentation). Moreover, the stop signal response time is
assumed to be constant. Thus, the stop signal response time is calculated as the time between the
stop signal onset (i.e., the start of the stop process) and the last possible response time in the
response time distribution that could escape inhibition (i.e., the finishing time of the stop process)
(Band et al., 2003; Logan & Cowan, 1984). The simplest way to find the finishing time of the
stop process is to find the proportion of non-canceled responses made at a given stop signal delay,
and infer that this proportion reflects the proportion of response times in the response time
distribution that are fast enough to escape inhibition (Boucher et al., 2007a; Logan, 1994). The
frequency distribution of response times can be integrated until the integral is equal to the
probability of making a non-canceled response at that stop signal delay. The stop signal response
time is estimated by subtracting the stop signal delay from this time. For example, 4 go trial
response times are ordered 100-, 200-, 300-, and 400 ms, and the proportion of non-canceled
responses on stop trials is 0.5 at a stop signal delay of 100 ms. The calculated stop signal response
time in this example would be the response time where the proportion of the response time
distribution equals 0.5 (i.e., 200 ms) minus the stop signal delay (i.e., 100 ms), which equals 100
ms (See Methods and Figure 7 for further details).

The race model of countermanding performance has been tested numerous times in
humans and non-human primates. For example, other methods of estimating stop signal response
time have scrutinized whether this variable is constant; however mathematical analysis has shown
that assuming stop signal response time is constant does not substantially change the estimation
Moreover, the go and stop processes are not completely independent, as physiological recordings have shown interactions between movement initiation and inhibition neurons (See below). A recent report by Boucher and colleagues (2007) has addressed this issue by showing how it is likely the two processes only interact briefly just before cancellation, but the estimations provided by the original race model fully account for countermanding task behaviour.

1.4 Human Performance in the Countermanding Task

The stop-signal paradigm was formalized by Logan and Cowan (1984). In their task, human participants responded to a visual go signal with a key press and inhibited the response when an auditory stop stimulus was presented. They reported that the probability of making a non-canceled response on stop trials increased as stop signal delay increased. Mean go trial response time was approximately 400 ms. Non-canceled response time in stop trials was faster and lengthened with stop signal delay. Stop signal response time averaged 200 ms. Generally, go trial response time and stop signal response time become faster from childhood into adulthood and then slow with old age (Bedard et al., 2002).

Several researchers have since elaborated on inhibitory processing mechanisms with variations on the stop task. Hanes and Carpenter (1999) developed a saccadic countermanding task, whereby subjects had to fixate on a central stimulus. When this stimulus disappeared, a target stimulus appeared in the peripheral visual field indicating that subjects had to make a saccadic eye movement toward the target stimulus. The fixation stimulus reappeared on some trials, acting as the stop signal, instructing that the saccade should be inhibited and the subject should hold their gaze on the fixation stimulus (Figure 2). In their version of the task, mean saccadic latencies on go trials ranged from 200-300 ms and were faster with a higher luminance contrast visual stimulus. Stop signal response times ranged from 125-145 ms, but did not vary
with target luminance. Eye movements were chosen because they are simple movements that are easy to measure with high precision and the anatomy and physiology of the primate saccade system is well understood (Curtis et al., 2005; Schall, 2001).

![Diagram of saccade countermanding task]

**Figure 2: Saccade Countermanding Task.** In the task, participants fixate on a central stimulus and are instructed to make an eye-movement toward the target stimulus when it appears. On a small subset of trials (stop trials), the fixation stimulus reappears after a variable delay from target stimulus presentation, instructing the participant to remain fixated on the central stimulus (From Hanes & Carpenter, 1999).

Coxon and colleagues (2006) studied motor evoked potentials in hand muscle after transcranial magnetic stimulation, a technique where magnetic fields are rapidly altered non-invasively around the head to excite neurons, in a stop task requiring a key lift with the hand.
They showed a reduction of excitability for stop trials relative to go trials and suggested that the reduction was due to increased intra-cortical inhibition. De Jong and colleagues (1995) looked at lateralized readiness potentials when inhibiting a response and observed comparable changes in a stop-all, stop-change and selective stopping task; however, they found that distinct mechanisms controlled inhibition in the stop task when compared to a change task and concluded that motor cortical regions were not solely involved in different kinds of inhibitory control. Alternatively, van Boxtel and associates (2001) recorded event-related potentials in a stop task and go/no-go task and noted that the timing relation between the brain potentials was sufficient for determining movement production in both tasks suggesting a common, single inhibitory mechanism. Their findings imply that motor commands are quelled by a central inhibitory signal.

In further experiments, Asrress and Carpenter (2001) discovered that stop signal response time was similar with a central or peripheral visual stop cue in the saccade task; however combining the cues resulted in faster stop signal response times, suggesting independent inputs to a centralized stop process. Numerous experiments have reported that stop signal response times were slightly longer when an auditory stimulus acted as the stop signal as opposed to a visual stimulus (Armstrong & Munoz, 2003; Cabel et al., 2000; Morein-Zamir & Kingstone, 2006). Furthermore, Mirabella and colleagues (2006) examined inhibition of arm reaching toward a visual target with an infrequent visual stop signal. They noted that the stop signal response time for inhibiting reaching movements was slightly longer (206 ms) than saccadic movements and that inhibition was faster for target positions that were on the ipsilateral side of the reaching arm. Moreover, Boucher and associates (2007b) reported shorter stop signal response time for eye movements than hand movements and that an auditory stop signal resulted in a greater lengthening of saccadic inhibition than hand movement inhibition. Further research has suggested that similar brain regions are activated during inhibition of both manual and saccadic responses
Together these results suggest that while different motor behaviours are controlled by the coordination of distinct motor systems, they may all be influenced by a centralized executive system.

Several experiments have questioned the independence of the go and stop processes proposed in the race model. For example, mean non-canceled response times in stop trials with short stop signal delays have been reported to be longer than the race model predicts (Boucher et al., 2007b; Hanes & Carpenter, 1999; Ozyurt et al., 2003). Similarly, mean go trial response time in the stop task is generally longer than mean response time in tasks with no stop trials (Akerfelt et al., 2006; Mirabella et al., 2006; Stuphorn & Schall, 2006). Moreover, physiological evidence suggests that behavioural inhibition depends on neural inhibition, which theoretically should involve interacting networks (Aron et al., 2007b). Recently, the race model has been revised to account for a possible interaction between go and stop processes, whereby the two processes are independent for most of the stop process duration, but interact briefly in the last few milliseconds before the response is inhibited as the stop unit interrupts the go unit (Boucher et al., 2007a).

Neural correlates of stopping have been studied with imaging. Band and van Boxtel (1999) suggested the frontal cortex, thalamus and basal ganglia were likely candidates for inhibitory systems based on previous response inhibition literature. This hypothesis was supported by studies showing increased blood-oxygen-level-dependent (BOLD) response in frontal cortical areas (e.g., frontal eye fields, anterior cingulate cortex, supplementary eye fields and ventrolateral prefrontal cortex) in response to stop trials in comparison to go trials and on non-canceled stop trials compared to canceled stop trials with functional magnetic resonance imaging (fMRI) (Curtis et al., 2005; Leung & Cai, 2007). Further support for cortical involvement comes from finding that increased stop signal response time was correlated with damage to the right inferior frontal gyrus (Aron et al., 2003b; Aron et al., 2004). Moreover,
inhibition of limb as well as verbal responses has been shown to activate the right inferior frontal cortex and presupplementary motor area (Xue et al., 2008). Support for the role of the basal ganglia in inhibitory processes comes from fMRI findings that stopping in the countermanding task activated the subthalamic nucleus, a subsection of the basal ganglia system (Aron & Poldrack, 2006; Li et al., 2008). Through diffusion weighted-imaging tractography, Aron and colleagues (2007a) found evidence of a white matter tract connecting the inferior frontal gyrus, subthalamic nucleus and presupplementary motor area, suggesting a putative “hyperdirect” pathway for inhibitory signaling.

Numerous experiments have investigated inhibitory deficits of ADHD patients in the countermanding paradigm. Stop signal response time has been shown to be slower in children diagnosed with ADHD (Hanisch et al., 2006; Oosterlaan et al., 1998; Schachar et al., 2007; Schachar et al., 2000). Stop signal response time of adult ADHD patients has also been reported to be longer in a saccadic countermanding task, particularly with an auditory or peripheral visual stop signal (Armstrong & Munoz, 2003). Clark and colleagues (2007) discovered that longer stop signal response time in adults diagnosed with ADHD was correlated with working memory impairments in a search task where subjects had to search boxes for hidden tokens and remember that once a token had been found in a box, that box would not yield another token for the rest of the trial. Moreover, these correlated impairments were shown by right frontal lobe damage patients, particularly with damage to the right inferior frontal gyrus, implicating dysfunction of this cortical area in ADHD. Methylphenidate, a norepinephrine and dopamine reuptake inhibitor and central nervous system stimulant used in the treatment of ADHD, normalized stop signal response time in both children and adults diagnosed with ADHD (Aron et al., 2003a; Bedard et al., 2003; Tannock et al., 1989).
Impairments in stop task performance have also been witnessed in patients diagnosed with schizophrenia. Badcock and colleagues (2002) noted that while stop signal response time was not significantly different between patients and control subjects, patients displayed slower response execution and an impaired ability to trigger the inhibitory response. Alternatively, Enticott and colleagues (2008) reported longer stop signal response times in schizophrenic patients. Similarly, Bellgrove and associates (2006) found significantly longer stop signal response times for the left hand in early-onset schizophrenia patients displaying a high level of negative symptomology. Research has also shown that as the percentage of stop trials in a session increases, schizophrenic patients display faster go trial response time, more non-canceled responses, and lower striatal activation when compared to control participants as the likelihood of having to inhibit a response increased. This striatal dysfunction was also apparent in unaffected siblings of patients with schizophrenia (Vink et al., 2006). Further research has shown no difference in task performance between patients with schizophrenia and controls; however patients displayed decreased BOLD response in the anterior cingulate cortex and increased subcortical activation during stop trials (Rubia et al., 2001). Differential antipsychotic treatment of patients with schizophrenia produces differences in cognitive task performance (Savina & Beninger, 2007). This may help explain inconsistencies in stop task studies with schizophrenic patients. The variety of stop task performance findings in schizophrenic patients is also consistent with the notion that schizophrenia is a heterogeneous disorder with substantially differing symptomologies.

Further research has noted stop task performance impairments in a number of other behavioural disorders. For example, Gauggel and associates (2004) discovered significantly longer stop signal response times in patients with Parkinson’s disease compared to controls in the stop task. Furthermore, abstinent chronic methamphetamine abusers displayed increased stop
signal response time but no difference in go trial response time when compared to control participants, indicating that methamphetamine use is associated with a decrease in inhibitory control (Monterosso et al., 2005). Similarly, Chen and colleagues (2008) have recently discovered that stop signal response time is longer for violent offenders in comparison to control participants when the maximum amount of time given to make a go response is greatly limited and may be correlated with increased impulsivity.

1.5 Non-Human Primate Performance in the Countermanding Task

Neural correlates of inhibition in the countermanding task have come from single-neuron recording studies of non-human primates. Hanes and Schall (1995) developed a saccadic countermanding task for monkeys similar to the task that was later developed for humans. They found that the proportion of non-canceled responses on stop trials increased as stop signal delay increased. Stop signal response time was estimated to be approximately 100 ms. Physiological recordings have demonstrated that saccades are made when movement-related neurons in the frontal eye fields and superior colliculus reach a threshold of activation, while stop signal presentation results in a decay in activation of these cells (Hanes et al., 1998; Paré & Hanes, 2003). These results are in agreement with finding in human studies showing that stop signal presentation produces an attenuation of motor evoked potentials (Coxon et al., 2006). Concomitantly, fixation-related neurons showed increased activation after stop signal presentation. These findings from single-neuron recordings imply that the inhibition of motor commands occurs in the neural region that is also the source of activity for the response, in accordance with human studies suggesting that responses are countermanded through intra-cortical inhibitory circuits.
Groups of neurons in the frontal cortex (e.g., supplementary eye fields and anterior cingulate cortex) displayed increased activation in response to non-canceled stop trials or increased activation after reinforcement or the omission of reinforcement (Ito et al., 2003; Schall et al., 2002; Stuphorn & Schall, 2002). Intracortical microstimulation of these cells produced fewer non-canceled saccades in stop trials and increased saccade latency in the stop task while decreasing saccade latency in sessions with only go trials (Stuphorn & Schall, 2006). Thus, these areas are implicated in reward or error monitoring and seem to be critical in executive control processes.

Taken together, these data show that the countermanding paradigm is an important tool in the study of behavioural inhibition with important implications for gaining a greater understanding of inhibitory deficits underlying the symptomology of specific neurological disorders. More recently, rodent countermanding paradigms have been developed to extend knowledge of behavioural inhibition in the control of whole body movements.

1.6 Rodent Models

Rats are ideal animal models for comprehensive invasive neural investigation, such as lesioning, electrical stimulation, pharmaceutical manipulation, cell recording and gene or protein expression mapping. Moreover, there exists several animal models of neuropsychiatric disorders involving impairment of executive function that could be amenable to rigorous testing in the countermanding paradigm (Adriani & Laviola, 2004; Powell & Miyakawa, 2006). Thus, numerous researchers have recently developed stop tasks to study behavioural inhibition.

One of the first stop tasks for rats was designed by Feola and colleagues (2000) (Figure 3). In this version of the task, a trial began with centre light illumination, requiring the subject to hold its snout in a centre snout poke hole below the illuminated light. Centre light offset acted as
the go signal indicating that the subject had to make a snout poke in the right water dispenser for liquid reward. The amount of time required to break a photo-beam in this water dispenser estimated go trial response time. A tone was presented as the change signal after go signal onset in 25% of trials, indicating that the subject had to inhibit the go response and make a snout poke in the left water dispenser for liquid reward. The amount of time required to break a photo-beam in the left water dispenser estimated the change response time. Stop signal delay increased if a correct inhibition was made and decreased if an incorrect non-canceled response was made to estimate the average stop signal delay, where subjects made approximately 50% non-canceled responses. Stop response time was estimated by subtracting the average stop signal delay from the mean of the go response time distribution. Go trial response time and stop response time were estimated to be 450 ms and 125 ms respectively in this task.

Figure 3: Rodent Change Task. Rats are required to start a trial by making a snout poke into the snout poke hole. On most trials a snout poke into the right water dispenser results in liquid reward, however on a small proportion of trials an auditory change signal instructs rats to inhibit the right water dispenser snout poke and change to a left water dispenser snout poke for reward (Feola et al., 2000).

When rats were divided into fast and slow stoppers based on the length of their baseline stop response times after a median split analysis, amphetamine administration dose-dependently decreased go trial response time for all rats, but only produced a dose-dependent decrease in stop
signal response time in the slow stoppers, perhaps modeling the improved inhibitory performance shown by ADHD patients treated with methylphenidate. Alternatively, alcohol administration increased stop response time, and increase go trial response time at higher doses. These findings model amphetamine and alcohol effects in a human task of behavioural inhibition (de Wit et al., 2000). However, this task is a change task and previous research has shown that a third independent GO variable must be included to account for the change response, complicating the subsequent analysis (Camalier et al., 2007).

Eagle and Robbins (2003a) developed their own version of the stop signal task for rats (Figure 4). In their paradigm, trials were initiated with a nose poke to a central food well, which resulted in left lever presentation. Subjects were required to press the left lever to trigger right lever presentation. A right lever press resulted in food reward. The amount of time between left and right lever presses was recorded as the go trial response time, and was limited to encourage fast responding. On 20% of trials a tone (i.e., the stop signal) was presented at varying delays after left lever press, signifying that the right lever press response should be inhibited for food reward. Go trial response time in this version of the task was estimated to be approximately 800 ms, while stop signal response time was estimated at 280 ms.
Figure 4: Eagle and Robbins Stop Task. Rats are instructed to make a nose poke into a centre food well to start a trial. Left lever presentation signals the rat to make a left lever press for right lever presentation, which is only available to press for a limited amount of time. A right lever press results in food reward. On 20% of trials a tone is presented instructing the rat to cancel the right lever press to obtain food reward (Eagle & Robbins, 2003a).

This paradigm has been employed in various studies. Eagle and Robbins (2003a) reported that excitotoxic lesions of the medial striatum increased the number of go trial omissions, the proportion of non-canceled responses on stop trials and stop signal response time in the task. They further noted that low dose amphetamine administration (0.3 mg) attenuated this impairment, while a higher dose of amphetamine (1.0 mg) exacerbated the deficit. On the other hand, excitotoxic lesions of the medial prefrontal cortex or nucleus accumbens core did not produce significant deficits in task performance (Eagle & Robbins, 2003b). With a similar version of the task, Van den Bergh and associates (2006) reported that measures of stop task performance were not correlated with locomotor activity, delay aversion (i.e., choosing a small immediately available reward versus a large reward available after a delay) or sexual behaviour in untreated rats as measures of impulsive behaviour, indicating that the neural processes involved in impulsive behaviours are not related to inhibitory processes in the stop task in normally functioning rats.
Eagle and colleagues (2007) further showed a decrease in stop signal response time in rats with slow baseline stop signal response times after median split analysis with administration of modafinil, a potential therapeutic pharmacological agent for ADHD. Alternatively methylphenidate treatment decreased go trial response time in all rats, but had a modulatory effect on stop signal response times by decreasing them in slow baseline subjects and increasing them in fast baseline subjects. Administration of cis-flupenthixol, a dopamine D1/D2 receptor antagonist, increased go trial response time at higher doses but had no effect on stop signal response time. These results provide support for the hypothesis that dopamine may mediate responding while noradrenaline may be primarily associated with response inhibition (Aron et al., 2007b). Further support comes from the finding that administration of atomoxetine, a noradrenaline-specific reuptake inhibitor, decreased stop signal response time in all rats, specifically those with slow baseline stop signal response times, without significantly affecting go trial response times (Robinson et al., 2008).

Further work with this paradigm revealed that excitotoxic lesions of the orbitofrontal cortex in rats, but not the infralimbic cortex slowed stop signal response time directly (Eagle et al., 2008). On the other hand, lesions of the subthalamic nucleus did not affect stop signal response time, but decreased go trial response time and increased the probability of making a non-canceled response on stop trials as an indirect inhibitory deficit in the task. This experiment illustrates the power of the stop task because the race model allows the dissection of different inhibitory control components, whereas other behavioural inhibition paradigms do not. Effects on different processes involved in the stop task help pinpoint underlying substrates.

Although the Eagle paradigm does well in examining inhibitory processes, it does not have a clearly defined go signal, making go process activation difficult to analyze. Moreover, the go task does not contain a voluntary decision between alternative choices and always instructs the
same response, which may lead to automated responding through extensive training. Finally, 
behaviours performed in this version of the task are not analogous to behaviours performed in the 
primate version of the task where there is a random required response between left or right target 
stimuli, making it difficult to clearly translate results between species.

A third version of the stop-signal paradigm for rats was designed by Pattij and colleagues 
(2007). Their apparatus contained 3 nose poke holes with a stimulus light in each hole (Figure 5). 
At the beginning of a trial the light above the middle nose poke hole was illuminated as the start 
estimulus. A nose poke into the middle hole illuminated the stimulus light in the left or right nose 
poke hole (the go signal), and required the subject to make a nose poke into the hole with the 
illuminated light to receive a food reward. Photo-beam breaks in the nose poke holes were used to 
calculate go trial response time. An auditory tone (the stop signal) was presented on 25% of trials 
indicating that the subject had to refrain from making a nose poke to receive food reward. Mean 
go trial response time was estimated at 365 ms, while stop signal response time was 230 ms. 
Administration of the cannabinoid CB1 receptor antagonist SR141716A 30 min before stop task 
testing increased mean go response time. Administration of the CB1 receptor agonist 
WIN55,212-2 30 min before testing increased the number of errors on stop trials; however this 
was related to a slowing of mean go trial response time. This study concluded that behavioural 
inhibition was not directly under the control of cannabinoid CB1 receptors.
Figure 5: Pattij Stop Task. After subjects made a nose poke in the middle nose poke hole, a light was illuminated in either the left or right nose poke holes instructing subject to make a nose poke into that hole for food reward. On stop trials a short auditory tone instructed subjects to inhibit nose pokes, which were detected by photo-beam breaks. (Approximated from Pattij et al., 2007).

1.7 Hypothesis

It is important to develop a countermanding paradigm for rats that is highly comparable to other animal models (i.e., non-human primates) so that results can be translated between species and ultimately to humans. Moreover, current rodent stop tasks estimate stop signal response time based on the assumptions of the race model, yet no study has explicitly confirmed that the race model is valid in rats and that predictions made by the race model of countermanding performance are respected for rat behaviour in the task. The goal of the present study was to confirm various testable predications about stop task performance made by the race model in a rodent stop task that closely resembles the primate countermanding task, thus validating the model for countermanding whole body movements in rodents. First, it is
hypothesized that response time on non-canceled stop trials will be shorter than go trial response time, because non-canceled response times that escape inhibition consist of responses in a limited proportion of the response distribution. Second, it is hypothesized that non-canceled response time should lengthen with increasing stop signal delay because longer stop signal delays incorporate more of the response distribution. Third, it is hypothesized that the model will be able to predict non-canceled response times at specific stop signal delays, which should approximately be a time in the response distribution just preceding the end of the stop process.
Chapter 2
Methods

2.1 Subjects

Ten male albino Wistar rats and 2 male Long Evans rats bred by Charles River Laboratories (St. Constant, Quebec) were housed in pairs in clear plastic cages (45.0 x 25.0 x 20.0 cm high) with bedded floors (Beta Chip; Northeastern Products Corp., Warrensberg, NY). They resided in an environmentally controlled colony room with a reversed 12-h light-dark cycle, where dark began at 0700 h. Rats were given free access to water, with food (LabDiet 5001, PMI Nutrition Intl, Brentwood, MO) freely available or restricted (see procedure). As rats grew with time it became necessary to transfer pairs into larger clear plastic cages (50.0 x 40.0 x 20.0 cm high). Rats were maintained according to the guidelines of the Canadian Council on Animal Care and the Animals for Research Act. All methods were approved by the Queen’s University Committee on Animal Care.

2.2 Apparatus

Behavioural data were collected with 4 identical Skinner boxes (30.5 x 24.1 x 21.0 cm high) with a clear polycarbonate door, rear wall and roof (ENV-008, Med Associated Inc., St. Albans, VT). The floor consisted of 0.5 cm diameter parallel stainless-steel rods that were spread 1.0 cm apart. On each side wall, 4 aluminum posts separated the wall into 3 panels. On one wall, the far panel contained a 2.8-watt incandescent light bulb, 1.0 cm from the roof and 5.0 cm above a tone generator. The tone generator emitted a single tone with a frequency that differed in each box, ranging from 2400 to 3400 Hz at an intensity of 75 dB. On the same wall, the middle panel contained a food pellet receptacle (5.1 x 5.1 cm) that was 3.0 cm above the grid floor. Dustless
precision food pellets (45 mg) from Bio-serv (Frenchtown, NJ; product number: F0021) were dispensed into the food pellet receptacle from a pedestal mounted pellet dispenser located outside of the chamber. Sucrose pellets were chosen as the reward stimulus because rats are highly motivated to obtain these pellets. On the opposite wall of all 4 chambers, each of the three panels consisted of a 2.5 cm diameter, 2.8-watt stimulus light that was 4.5 cm below the roof and 5.0 cm above a retractable response lever (4.8 x 1.7 x 1.3 cm thick). Each test chamber was isolated in a sound-attenuating, light-resistant case with a fan to cool the chambers and drown out external noise. Programming and data analysis was controlled by MED-PC® IV software (Med Associated Inc.). Data were controlled and recorded by a computer located in the same room as the test chambers. These data were then collected and analyzed on computers located in a different room. Data was collected at a sampling rate of 1 ms.

2.3 Procedure

For the first week, rats were housed in pairs and had food (LabDiet 5001 Rodent Diet) and water available ad libitum, allowing them to gain weight and habituate to the colony room. From the 3rd day in the colony room until the 7th, rats were handled in pairs for approximately 5 min. Beginning on the 7th day, food access was restricted to 1 h of free-feeding per day. After approximately 3 d of food restriction, rats had been reduced to a target weight of 85% of their free-feeding weight. On this day each cage was given approximately 20 sucrose pellets, in order to habituate to the food reward.

On the next day, rats were trained to lever press for food reward in operant chambers by using a response shaping technique. In the operant chamber, the centre light was illuminated and the centre lever was made available, while the left and right levers were retracted. The house light was illuminated during all sessions except during timeout periods (see below). Sucrose pellets
were dispensed whenever progress was made by the rat toward making a lever press until the rat successfully learned to press the extended lever to dispense food pellets. During lever press training, pellets were available on a fixed-ratio 1 schedule (i.e., a pellet was dispensed each time the rat pressed the lever). A rat was considered trained (1-3 sessions) when it pressed the lever at least 30 times during a 30-min fixed-ratio 1 schedule session.

The next sessions consisted of light discrimination training. In each chamber the left and right levers were made available, while the centre lever was retracted. During a trial, the light was illuminated above the left or right lever at random. If the rat pressed the lever directly underneath the illuminated light, a food reward was presented, the light was shut off and a 5-s intertrial interval passed before the next trial began. If the rat pressed the lever that was not underneath the illuminated light, no reward was given and all lights, including the house light, were shut off for a 10-s timeout period. A 5-s intertrial interval followed the timeout period before the next trial began. Finally, if a rat did not make a lever press response on a trial before a 60-s time limit, a 10-s timeout period was given, followed by a 5-s intertrial interval before the next trial began. A rat was considered trained (3-6 sessions) when it pressed the lever directly below the illuminated light on ≥85% of the last 100 trials in a 60-min session.

After light discrimination acquisition, rats were given go trial training (4-7 sessions). For go trial training sessions, all three levers were made available for the duration of the 60-min session. Before a trial began only the centre light was illuminated. If the rat did not make a response within a 60-s time limit, or if the rat pressed the left or right levers, a 10-s timeout period was presented followed by a 5-s intertrial interval before the centre light was illuminated again. If the rat pressed the centre lever to begin a trial, the centre light shut off and either the left or right stimulus light was randomly illuminated immediately (acting as the go signal) signifying that the lever below the illuminated stimulus was the target lever. If the rat pressed the lever
underneath the illuminated stimulus, a reward was presented, the stimulus light shut off and a 5-s intertrial interval was given before the centre light was illuminated again. The amount of time from go signal onset until target lever press response was recorded as the go response time. Alternatively, if after pressing the centre lever the rat pressed one of the two levers not underneath the illuminated light, or a 60-s time limit passed, a 10-s timeout period was presented followed by a 5-s intertrial interval before the centre light was illuminated again. Once a rat made ≥ 85% correct lever press responses in the last 100 trials of a 60-min session, the time limit for pressing the target lever was decreased in the next session to encourage fast responding. This was done by shortening the time limit so that the target lever was only active for an amount of time that was slightly longer than the majority of the distribution of response times from the previous session. This shortening of the time limit continued until rats made ≥ 85% correct lever press responses in the last 100 trials of a session with a time limit between 1.0 and 1.5 s.

Once go trial responding had been acquired, rats were given a 30-min tone habituation session. All levers were retracted and only the house light was presented during this session. A short acoustic burst (1 s) was presented on a variable-interval 30-s schedule. Immediately after auditory stimulus presentation a sucrose pellet was delivered to the rat to associate the tone with reward in the absence of lever pressing behaviour.

The day after tone habituation, rats were given stop trial training (4-7 sessions). All three levers were made available for the duration of the 60-min sessions. Before a trial began the centre light was illuminated. If the rat pressed the left or right levers, or a 60-s time limit passed, the rat was given a 10-s timeout period followed by a 5-s intertrial interval before the centre light was illuminated again. If the rat pressed the centre lever to begin a trial, the light was randomly illuminated immediately above the left or right lever; however a 1-s auditory stimulus was presented concurrently (acting as the stop signal). If lever press responding was withheld for the
entire 1-s time limit, a sucrose pellet was presented and a 5-s intertrial interval preceded the illumination of the centre light. Alternatively, if the rat made a lever press during the trial, a 10-s timeout period was given followed by a 5-s intertrial interval before the centre light was illuminated again. As soon as a rat made ≥ 85% correct response inhibitions in the last 100 trials of a 60-min session, the time limit was increased in the next session until rats could withhold responses with a time limit of 2-s for ≥ 85% of the last 100 trials of a session.

The next day rats were given a 30-min go trial session using the time limit previously established in go trial training. This session was immediately followed by a 30-min stop trial session using a 2-s time limit. Go and stop trial sessions were alternated each day for 3-6 days until rats achieved ≥ 85% responding in both sessions on the same day. Once performance on the two individual tasks was correct ≥ 85% of trials, the two trial types were combined in a 60 min session the next day.

Stop task training consisted of 75% go trials and 25 % stop trials presented randomly throughout the session. The target lever was active for the time limit that was previously established in go trial training. The time limit varied for each rat between 1 and 1.5 s during stop task training until an appropriate time limit was found that incorporated most of the go response time distribution. After a number of stop task training sessions (4-7 sessions), rats made ≥ 85% correct responses in go and stop trials combined and were ready to be tested in the stop task.

Prior to each stop task session, rats were given a 10-trial go session with a time limit of 1.5-s followed by a 10-trial stop session with the same time limit. After completing these warm-up trials rats were ready to be tested in a 60-min session. The stop task was similar to stop task training in that it consisted of 75% go trials and 25% stop trials presented randomly (Figure 6).
On all trials, the light above the centre lever was illuminated indicating that the rat must press the centre lever to begin the trial. If the rat did not press the centre lever first, a 10-s timeout period was given followed by a 5-s intertrial interval before the centre light was illuminated again. Immediately after a centre lever press, the target light (acting as the go signal) was randomly illuminated above either the left or right lever. The target lever was only active for the time limit that was previously established in stop task training for each rat (1.1-1.3 s). In go trials, the rat was required to push the correct lever before the end of the time limit to be rewarded. This was followed by a 5-s intertrial interval before the centre light was illuminated again. If the target lever press was not made before the end of the time limit, a 10-s timeout period was given followed by a 5-s intertrial interval before the centre light was illuminated again. In stop trials, a

**Figure 6: Rodent Stop Task.** Before all trials the centre light is illuminated and a centre lever press begins the trial. On all trials a light is immediately illuminated randomly above the left or right lever (i.e., the go stimulus). On go trials (75%), pressing the lever directly underneath the illuminated light results in reward and the amount of time between centre and target lever presses represents the response time. On stop trials (25%) an auditory tone (i.e., the stop stimulus) is presented at varying delays from go stimulus onset (stop signal delay) and canceling the lever press results in reward, whereas a non-canceled lever press results in a timeout period.
centre lever press resulted in go signal presentation; however an acoustic burst (acting as the stop signal) was presented for the length of the time limit plus an additional 300 ms and instructed the rat to inhibit a lever press during this time period to be rewarded. This was followed by a 5-s intertrial interval before the centre light was illuminated again. All stop task sessions began with an initial stop signal delay of 100 ms. Using a staircase procedure for stop signal delay with a step of 100 ms, correctly inhibiting a lever press on a stop trial resulted in a 100 ms increase in stop signal delay on the next stop trial. Alternatively, a non-cancelled lever press after stop signal onset on stop trials resulted in a 10-s timeout period and a 5-s intertrial interval. According to the staircase procedure, a non-cancelled response on a stop trial resulted in a 100-ms decrease in stop signal delay on the next stop trial. The implementation of the staircase procedure for stop signal delay was important because it allowed the development of complete inhibition functions and the estimation of the stop signal delay where the probability of making a non-cancelled response was 0.5. Finally, if a lever press on a stop trial occurred before stop signal presentation, the trial was recorded as a non-cancelled response; however the rat was given a sucrose pellet and a 5-s intertrial interval (i.e., it appeared to be a go trial to the rat) before the centre light was illuminated again. In these instances the stop signal delay was decreased by 100 ms.

2.4 Data Analysis

For analysis of performance in the stop task the number of stop trials in a session needed to be relatively high to increase the number of stop trials at each stop signal delay. Therefore, sessions were only included in the analysis if they contained over 200 total trials (this would include more than 50 stop trials). Variability of inhibitory performance within a session also needed to be accounted for. Thus, if the stop signal delay increased by 3 steps or more in a row during stop trials later in the 1 h session and did not return back to the mean stop signal delay for
that session as an indication of a change in motivational behaviour, all trials after the increase in stop signal delay were excluded from analysis.

Each subject was tested over a number of sessions (11-33 sessions). For each rat, all response time distributions on go trials from a session were compared to each other using independent Kolmogorov-Smirnov tests (KS-Test). The sessions with the most non-significantly different comparisons that were also not significantly different from each other were pooled into one data set to analyze stop task performance for each subject. Only 5 sessions were included because this was the maximum number of response time distributions that were found to be not significantly different for one of the subjects. The number of non-cancelled responses made at different stop signal delays was compared to the total number of stop trials of that delay to calculate the proportion of non-cancelled responses at each stop signal delay. These proportions of non-cancelled responses were used to develop inhibition functions for each subject. Independent Chi-square tests were conducted on probability contingencies to determine if the proportion of non-cancelled responses differed at longer stop signal delays.

The integration method was used to estimate stop signal response time, which cannot directly be measured (Logan & Cowan, 1984). To approximate the stop signal delay at which the probability of making a non-cancelled response was 0.5, the staircase procedure was used (Levitt, 1971; Figure 7A). With the staircase procedure a grouping of stop signal delays that increase or decrease consecutively as a result of task performance is defined as a run. The average of the peaks and valleys of each run and midpoint of every second run were estimated and averaged to determine this stop signal delay. Assuming stop signal response time is a constant, the integration method estimates the time when the stop process ends - given the stop signal delay where the probability of making a non-cancelled response is 0.5 - by integrating the distribution of go trial response times until the integral equals the response time at which the probability of making a
non-canceled response is 0.5. The stop signal response time then equals this time (i.e., the instant when the stop process end) minus the stop signal delay where the probability of making a non-canceled response is 0.5 (i.e., the instant when the stop process is initiated) (Figure 7B).

Rats did not make a response on a small proportion of go trials in the stop task. The possibility therefore exists that a correctly inhibited stop trial was in fact a failed go response. To account for omission errors, the inhibition probability data were corrected using a procedure modified from Tannock et al. (1995):

\[ Y = \frac{X-O}{N-O}, \]

where \( Y \) is the corrected proportion of non-canceled stop trials at a specific stop signal delay, \( X \) is the observed number of non-canceled stop trials at a particular stop signal delay, \( O \) is the correction for the number of omission errors calculated as the number of no responses on go trials divided by the total number of go trials and \( N \) is the total number of stop trials at a particular stop signal delay.

The race model makes testable predictions of stop task performance. Data for non-cancelled response times at varying stop signal delays was combined from all subjects to increase the number of comparisons to gain greater statistical power. Paired samples t-tests were conducted to confirm these predictions of task performance. Data from a stop signal delay were excluded from analysis if there were less than 10 trials. All analysis was conducted using an alpha of 0.05.
Figure 7: Estimation of stop signal response time. A) During the stop task a correctly inhibited stop trial increased stop signal delay by 100 ms and a non-canceled stop trial decreased stop signal delay by 100 ms (staircase procedure). A series of steps in one direction was defined as a run. One method to estimate the stop signal delay where the probability of making a non-canceled response is 0.5 is to find the average stop signal delay of the peaks and valleys of all the runs. A second method is to find the midpoint stop signal delay of every second run. These two methods were averaged for each subject. Data from an individual subject is displayed above. B) The integration method estimates the time at which the stop process finished by integrating the frequency distribution of go trial response times until the integral is equal to the proportion of non-canceled trials at the stop signal delay where the probability of making a non-canceled response is 0.5. The time from stop signal presentation to this finishing time represents the stop signal response time.
Chapter 3

Results

A total of 12 subjects were tested. Two male Long Evans rats were acquired for a pilot study after testing in a different set of experiments (See Appendix A). Two Wistar rats were excluded from the analysis because their behavior did not comply with the prerequisites of the race model (i.e., they did not perform the task according to instructions). This left 8 subjects for subsequent analysis.

3.1 Response Time Distribution Comparisons

Response Time distributions of 5 sessions for each subject were assessed to ensure the data sets were similar and could be combined (Figure 8). Sessions did not have significantly different response time distributions for each individual subject (Table 1). Thus, data from each session was pooled into a single data set for each subject. Mean go trial response times (± standard error) were 609 ± 222 (subject 1), 519 ± 207 ms (subject 2), 522 ± 142 ms (subject 3), 602 ± 212 ms (subject 4), 599 ± 195 ms (subject 5), 625 ± 185 (subject 6), 580 ± 216 (subject 7) and 505 ± 138 (subject 8). The overall mean go trial response time was 570 ± 17 ms (Figure 9). Subjects did not always make a correct response on go trials. The proportion of omission errors on go trials was 0.14, 0.10, 0.07, 0.08, 0.08, 0.08, 0.16, and 0.06 for subjects 1 to 8 respectively.

3.2 Inhibition Functions

The proportion of non-canceled responses on stop trials at different stop signal delays was calculated for each subject to create inhibition functions. The probability of making a non-canceled response increased significantly as stop signal delay increased for all 8 subjects (Figure 10). This finding was supported by the results of independent chi-square tests for subject 1, $X^2(6) = 65.0, p < 0.01$; Subject 2, $X^2(6) = 59.8, p < 0.01$; Subject 3, $X^2(6) = 72.1, p < 0.01$; Subject 4, $X$
\[ \chi^2(7) = 74.8, \; p < 0.01; \text{Subject 5, } \chi^2(6) = 46.2, \; p < 0.01; \text{Subject 6, } \chi^2(7) = 53.9, \; p < 0.01; \text{Subject 7, } \chi^2(7) = 43.3, \; p < 0.01; \text{and Subject 8, } \chi^2(6) = 77.1, \; p < 0.01. \] Subjects made a greater proportion of non-canceled responses on stop trials as the stop signal delay increased.

Figure 8: Cumulative Response Time Distributions. Cumulative proportion of response times on go trials for the 5 sessions that were pooled together for further analysis for subjects 1 to 8. Sessions for each subject were combined as none of the reaction time distributions were found to differ significantly.
### Table 1

**KS-Test comparisons of response time distributions of sessions for each subject**

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Note. None of the KS-test comparisons were statistically significant, all p values are greater than 0.05.

3.3 Stop Signal Response Time

The staircase procedure for stop trials was analyzed to estimate the stop signal delay at which the probability of making a non-canceled response was 0.5 for each subject. The peaks and valleys average of stop signal delay ranged from 315 ms to 448 ms while the midpoint of each second run average of stop signal delay ranged from 316 ms to 448 ms. The average of these two estimations approximated the stop signal delay at which the probability of making a non-canceled response was 0.5 for each subject (Table 2). The go response time distribution was then integrated until the proportion of response times was equal to 0.5. The corresponding response
time at which this proportion was reached ranged from 475 to 609 ms across subjects. Stop signal response time was calculated by taking the response time at which the probability of making a non-canceled response was 0.5 for each subject, and subtracting the stop signal delay where the probability of making a non-canceled response was 0.5. The mean stop signal response time (± standard error) was 157 ± 8 ms (Figure 9).

**Figure 9:** *Mean Go response times and stop signal response times.* Mean go response times (± SEM) for all 8 subjects (gray). The dashed line represents the overall mean (570 ± 17 ms). Stop signal response times for all 8 subjects (black). The dashed line represents the overall mean (157 ± 8 ms).
Figure 10: Inhibition Functions. The probability of making a non-canceled response (± 95% confidence interval) increased as the time between go signal presentation and stop signal onset (SSD) lengthened for all 8 subjects. The red dashed line represents the point at which the probability of making a non-canceled response was 0.5. The black dashed line represents the staircase procedure estimation of the stop signal delay at which the probability of making a non-canceled response was 0.5.
Table 2

Data required to estimate stop signal response time with the integration method

<table>
<thead>
<tr>
<th>Subject</th>
<th>Peaks &amp; Valleys</th>
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<th>RT\textsubscript{p[non-canceled = 0.5]}</th>
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<td>316 ms</td>
<td>475 ms</td>
<td>159 ms</td>
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</table>

Note. The Peaks & Valleys and 2\textsuperscript{nd} Run analysis represent stop signal delays derived from the staircase procedure where the probability of making a non-canceled response is 0.5. SSD\textsubscript{p[non-canceled = 0.5]} is the average of these two analyses. RT\textsubscript{p[non-canceled = 0.5]} represents the point from the go response time distribution in which the integrated proportion of response times is 0.5 (i.e., the median response time). Stop signal response time (SSRT) equals RT\textsubscript{p[non-canceled = 0.5]} - SSD\textsubscript{p[non-canceled = 0.5]}.

3.4 Race Model Predictions

The race model makes 3 main predictions of countermanding task performance. The model predicts that mean non-canceled response times should be faster than mean go trial response times (Figure 11A). To determine whether this prediction was respected, mean go trial
response times were compared with mean stop trial non-canceled response times for all 8 subjects. Mean non-canceled response times were faster than mean go trial response times as displayed in Figure 12A & B. This finding was supported by a paired samples t-test, \( t(7) = 11.87, p < 0.01 \).

![Race Model Predictions of Stop Task Performance](image)

**Figure 11: Race Model Predictions of Stop Task Performance.** A) Mean non-canceled response time should be shorter than mean go response time because only fast go response times escape inhibition. B) Mean non-canceled response time should increase as the stop signal delay increases because more of the distribution of go response times escape inhibition. C) If stop signal response time is constant mean non-canceled response time at a specific stop signal delay is predictable.

The race model predicts that mean non-canceled response times in stop trials with shorter stop signal delays should be faster than mean non-canceled response times in stop trials with longer stop signal delays (Figure 11B). To determine whether this prediction was respected, mean non-canceled response times in trials with a particular stop signal delay were compared to mean non-canceled response times in trials when the stop signal delay was 100 ms shorter for all 8 subjects. Mean non-canceled response times were significantly longer in trials with the longer stop signal delay as displayed in Figure 12C. This was supported by the results of a one-sample t-test on difference scores (mean non-canceled response time at SSD\(_n\) – mean non-canceled response time at SSD\(_{n-1}\)\), \( t(27) = 2.96, M = 21.80 \text{ ms}, p < 0.01 \).
**Figure 12: Race Model Predictions of Countermanding Task Performance.** A) Race model predictions for one individual subject (Subject 1) showing mean observed non-canceled response times increased as stop signal delay increased, were generally predictable and were shorter than mean go response time (dashed line). B) Mean non-canceled response time in stop trials at short stop signal delays (200-400 ms) and long stop signal delays (500-700 ms) was shorter than mean go trial response time for all 8 subjects, in support of the first prediction. C) Mean non-canceled response times at a particular stop signal delay (SSDn) tended to be longer than mean non-canceled response times at a 100 ms shorter stop signal delay (SSD_{n-1}), in support of the second prediction. D) Observed mean non-canceled response times at a specific stop signal delay were longer than the predicted mean non-canceled response time at that stop signal delay. While this was the case in trials with short stop signal delays (200-400 ms), there was no significant difference in observed and predicted mean non-canceled response times in trials with longer stop signal delays (500-700 ms).
Mean non-canceled response times at different stop signal delays should be predictable by the race model (Figure 11C). To examine whether the race model was able to predict mean non-canceled response times on stop trials at particular stop signal delays, the mean predicted non-canceled response time was compared to the observed mean non-canceled response time at each stop signal delay for all 8 subjects. Observed mean non-canceled response times for a stop signal delay were generally longer than the predicted as displayed in Figure 12D. This was supported by the results of a one-sample t-test on difference scores (observed mean non-canceled response time at SSD\textsubscript{n} – predicted mean non-canceled response time at SSD\textsubscript{n}), \( t(35) = 2.80, M = 15.23 \text{ ms}, p > 0.01 \). To further analyze the difference in observed and predicted mean non-canceled response time at different stop signal delays, the difference scores were separated into two groups, scores at short stop signal delays (200-400 ms) and scores at long stop signal delays (500-700 ms). At short stop signal delays, observed mean non-canceled response times were longer than predicted, as supported by the results of a one-sample t-test on difference scores, \( t(17) = 3.92, M = 26.64 \text{ ms}, p < 0.01 \). However, at long stop signal delays, observed mean non-canceled response times were not significantly different from mean predicted non-canceled response times, as supported by the results of a one-sample t-test on difference scores, \( t(17) = 0.49, M = 3.83, p = 0.63 \).
Chapter 4
Discussion

In the present study, rats acquired the ability to inhibit a previously planned behaviour in response to an auditory stop signal in a rodent countermanding paradigm adapted from the countermanding task used in humans. As the delay between go signal onset and stop signal onset increased on stop trials in the task, rats made more non-canceled responses. As predicted by the race model, non-canceled response time was faster than go trial response time for all subjects. Overall, non-canceled response time in stop trials lengthened as stop signal delay increased. Although non-canceled response time predicted by the race model was faster than observed non-canceled response times at short stop signal delays, the race model accurately predicted non-canceled response time at long stop signal delays. These findings supported the hypothesis that the race model accounts for performance of rats in the countermanding task.

As the duration of time lengthened on a stop trial before the stop signal was presented, it became more difficult for rats to inhibit the response. This is supported by inhibition functions plotting the effect of delay, showing that rats made none to a small proportion of errors on stop trials with short stop signal delays and a large proportion of errors on stop trials with long stop signal delays until they made all errors at the longest delays. Similar inhibition functions are produced in human and non-human primate countermanding tasks (Band et al., 2003; Hanes & Carpenter, 1999; Hanes & Schall, 1995; Logan & Cowan, 1984). This shape of inhibition function reflects the outcome of the race and is a prerequisite for a race model account of countermanding performance (Logan, 1994). Thus, these results of the present study validate the countermanding task in rats as analogous to human and non-human primate countermanding paradigms.
Race model predictions of countermanding performance have previously been confirmed in human experiments. For example, various studies have found that the race model was able to predict response latencies at different stop signal delays in trials where the participant failed to inhibit the response (Asrress & Carpenter, 2001; Hanes & Carpenter, 1999; Logan & Cowan, 1984). Colonius and colleagues (2001) also noted that saccade latencies were faster in non-canceled stop trials in comparison to go trials and that saccadic latencies increased as stop signal delay increased for two out of three subjects. Mean non-canceled response time was not faster than mean go response time in the third subject, primarily due to a small number of prolonged non-canceled responses at the shortest stop signal delays. Akerfelt and colleagues (2006) reported that behaviour from one participant fit race model assumptions better than two others. Again, the major violation of the race model witnessed in these two subjects was prolonged non-canceled response times at the shortest stop signal delay. This effect was also noted by Hanes and Carpenter (1999). Thus, the finding that mean non-canceled response time in stop trials with short stop signal delays is longer than predicted has been shown in previous literature. Boucher and colleagues (2007a) have suggested that these longer non-canceled response times on trials with short stop signal delays are simply instances where the subject makes a successful inhibition, but then produces the response anyway; therefore, these outliers are outside of the race model framework. However, these trials may contain pertinent information about behavioural inhibition which is of interest, particularly in rats where this effect may be enhanced.

It is possible that differences in brain anatomy and function between primates and rats may account for the finding that predicted response times are particularly shorter than observed non-canceled response times at short stop signal delays in rats. Human imaging studies have implicated the right inferior frontal cortex as a critical region involved in countermanding a response (Aron et al., 2007a; Aron et al., 2003b). It seems likely that a stop command could be
sent from the frontal cortex to the basal ganglia through the subthalamic nucleus (Aron & Poldrack, 2006). Furthermore, recent tractography analysis has connected the inferior frontal cortex and subthalamic nucleus with the presupplementary motor area, which is thought to play a role in conflict detection and error (Aron et al., 2007a; Stuphorn & Schall, 2006). This fits with the current model of voluntary movement control which focuses on three pathways. In the direct pathway, the striatum receives excitatory cortical inputs and sends inhibitory projections to the internal segment of the globus pallidus and substantia nigra pars reticulata to produce disinhibition on thalamic neurons that, in turn, excite thalamo-cortical motor targets. In the indirect pathway the striatum sends excitatory projections to the external segment of the globus pallidus which inactivates the subthalamic nucleus, releasing the internal segment of the globus pallidus to increase inhibition onto the thalamus. In the hyperdirect pathway, signals from the inferior frontal cortex project directly to the subthalamic nucleus which sends excitatory projections to the internal segment of the globus pallidus and inhibits thalamic activity (Nambu et al., 2002). Thus, the hyperdirect pathway serves as a likely candidate for exerting executive control of motor responses. Eagle and colleagues (Eagle et al., 2008) have recently shown that lesioning of the subthalamic nucleus impairs countermanding task performance in rats by increasing the number of non-canceled responses, but does not affect stop signal response time as human imaging studies would predict. This discrepancy warrants further investigation regarding the role of the subthalamic nucleus in response inhibition. Eagle and colleagues noted impaired stop signal response time with lesions to the orbitofrontal cortex; however there is currently no evidence that the orbitofrontal cortex in rats is homologous to the inferior frontal cortex. It has been suggested that the medial prefrontal cortex in rats is homologous to the primate lateral prefrontal cortex (Birrell & Brown, 2000). Yet, medial prefrontal cortical lesions in rats had no effect on response inhibition (Eagle & Robbins, 2003b). It may be the case that the stop process...
has evolved more independence from the go processes in primates in comparison to rats, due to a more extensive cortical network. Lengthening of non-canceled response times at short stop signal delays in rats may be due to a greater amount of interaction between the go and stop processes or weakened inhibitory control. This difficulty in controlling for longer response times in the present task warrants further investigation. Clearly more studies need to be conducted to find similarities or differences between brain regions responsible for countermanding responses in humans and rats.

Two subjects in the present study were excluded from the analysis because they did not perform the task according to instructions. It is possible that these rats were not trained extensively enough to acquire the behaviour. Yet, all other subjects were able to learn the behaviour required of the countermanding task under the same amount of training. Although baseline stop signal response times in the present study were generally comparable (i.e., 6 of 8 subjects had estimates between 145 ms and 161 ms), a small degree of variability existed in two subjects. Moreover, the possibility exists that subjects that did not perform the task according to instructions simply had inhibitory control deficits. Numerous stop task experiments with rats have reported differences in baseline behavioural performance, namely that some rats had fast stop signal reaction times (i.e., fast responders), while others had slow stop signal reaction times (i.e., slow responders) (Eagle et al., 2007; Feola et al., 2000; Robinson et al., 2008). One possibility to account for these differences may be that different levels of performance monitoring are employed by individual rats. Emeric and colleagues (2007) showed that performance history influenced current countermanding task trial performance in humans and monkeys, noting that response time was faster after consecutive go trials and slower after an inhibited, but not a non-canceled stop trial. Other stop task experiments have found similar differences in response latencies depending on previous trial history (Kornylo et al., 2003). Preliminary analysis on the
effect of previous trial type and performance in the present study has not shown substantial
differences in group averaged response times; however a great deal of individual variation in
performance existed based on trial history. Thus further investigation will be necessary to identify
whether performance monitoring accounts for any of the observed individual variance in rat
countermanding task performance as an indicator of executive function and the level of voluntary
control in rats.

Several differences exist between the countermanding task employed in the present study
and other rodent stop tasks. While estimates of go and stop response time in the present task were
similar to the estimates obtained in the study by Feola and colleagues (2000), the Feola
experiment employed a change task as opposed to a stop task. A formal model has not been
developed and rigorously tested to fully account for change task performance, which likely
involves a dissociation of more processes. In Eagle and Robbins (2003b) version of the stop task,
go responding did not require a voluntary decision between alternatives and may have become
automated with extensive training, limiting the study of voluntary control of behaviour. It is
possible that longer go trial response times in the task were produced by the need for a more
extensive body movement to make the lever press response on the opposite side of the wall, or in
waiting for the response lever to become available. Longer stop signal response times in the Eagle
task may result from estimations using partial rather than full inhibition functions, which are a
prerequisite for the model. Moreover, an overestimation of response times in the Eagle task may
lead to overestimates in stop signal response time, as stop signal response time calculations are
based on go response time distributions. In a newer version of the task, Pattij and colleagues
(2007) used nose pokes as opposed to lever presses as the primary response. It is possible that go
response times in this version of the task were faster due to a simpler motor response. Stop signal
response time estimation may have been longer due to the use of short stop signal delays and not
examining full inhibition functions. In addition, longer stop signal response times may have been due to the use of the stop signal duration being only 50 ms long. Previous experiments have shown that short stop signal durations result in a reduction in stop accuracy and longer estimates of stop signal response time (Eagle et al., 2007, November). One possible way to reconcile these stop tasks with the countermanding paradigm of the present experiment is to attempt to advance previous studies with the task employed in the present experiment, perhaps by infusing dopamine or norepinephrine agonists or antagonists centrally into brain regions implicated in go responding or response inhibition immediately before testing.

A limitation of the present study is that sessions were not conducted with go-alone trials. Previous research has shown that mean go trial response time is faster in sessions with only go trials when compared to mean go trial response time in stop task sessions (e.g., Akerfelt et al., 2006; Ozyurt et al., 2003). It would be of interest to examine whether the introduction of stop trials into the response task has a substantial effect on rat response times, as potential evidence for increased interaction of go and stop processes in the rat brain during behavioural inhibition. Furthermore, there is no distinction in this task between reaction time (e.g., the time required to release the centre lever) and movement time (e.g., the amount of time required to move to and press the target lever). These two time estimates are not separated in any version of the rodent stop task to date, and future experiments will need to evaluate and compare these two estimates within the overall response time. A further limitation of the present study stems from the use of the staircase procedure to determine stop signal delay. By nature, employing the staircase procedure results in many stop trials around stop signal delays where subjects make correct inhibitions as well as incorrect non-canceled responses but only few trials at very short or very long stop signal delays (Levitt, 1971). Thus more information needs to be obtained in future studies about behaviour at short and long stop signal delays to obtain complete inhibition.
functions. A further issue with this task is that it requires a great deal of training and performance can be variable from session to session. It will be important in future studies to keep a strict schedule to limit confounding influences on countermanding task performance.

The present research has shown that rats are able to countermand a lever press in this task, but it is uncertain whether this form of stopping reflects inhibition of a small subset of muscles involved in lever pressing, or if the entire motor system receives inhibitory signals when the stop process is activated. Findings from transcranial stimulation experiments have suggested a global motor inhibition signal noting that no-go stimuli produce suppression of motor evoked potentials in both agonist, antagonist and contralateral homologous muscles at rest (Hoshiyama et al., 1997; Leocani et al., 2000). However, Coxon and colleagues (2006) have recently shown inhibition within the specific cortical area that shows limb movement-related activity. Future experiments of behavioural inhibition in rats will need to analyze performance with video or motion detectors to record behaviour and determine precisely how motor control is modified by stop signal presentation.

Voluntary control is important in normal day to day functioning. Deficits in executive function are thought to underlie various neurological disorders. For example, studies have shown that the prefrontal cortex regulates subcortical dopamine and that dysfunction in striatal dopamine is strongly related to the degree of dorsolateral prefrontal cortex neuronal deficit (Bertolino et al., 1999). Thus, cortical areas implicated in executive control have critical downstream impact on subcortical brain function and regulation. Impaired response inhibition has been observed in patients with ADHD, Parkinson’s disease and schizophrenia in the countermanding paradigm (Badcock et al., 2002; Gauggel et al., 2004; Oosterlaan et al., 1998; Tannock et al., 1989). Rodent models of psychiatric disease have been developed allowing the investigation of underlying symptoms of various disorders (Powell & Miyakawa, 2006). Future studies should
employ animal models of psychiatric disorder in the countermanding task to better study causes and treatments for inhibitory deficits.

Further studies have looked into impulsivity as a lack of inhibitory control. For example, Colzato and colleagues (2007) examined response inhibition in a key pressing task and reported that stop signal response time was significantly longer for recreational cocaine users when compared to control participants. Cocaine dependence has been shown to be correlated with impaired executive control and cognitive function (Hester & Garavan, 2004). In rats, cocaine administration did not affect cognitive impulsivity in delayed reinforcement paradigms, but did increase behavioural disinhibition in a go/no-go task, as defined by increased responding on no-go intervals (Olmstead, 2006). Moreover, stop task performance was not correlated with various behavioural measures of impulsivity in the rat (Van den Bergh et al., 2006). Future work should be conducted in an attempt to clarify the effect of acute and chronic cocaine abuse in inhibitory processing and impulsivity with the rodent stop task.

Developing a rodent model of the countermanding task that mimics the human paradigm is useful for understanding the neurological systems and neurotransmitters involved in behavioural inhibition. The task in the present study was adapted from the paradigm developed for humans and non-human primates so that findings can be easily translated between species. The same processes that mediate inhibition in the countermanding task likely control other aspects of voluntary behaviour that are dysregulated in some psychiatric illness. Furthermore, understanding similarities and differences of these behavioural processes will likely assist in advancing the study of voluntary control in the future to a number of related cognitive areas including thoughts, memories and emotions (Aron et al., 2007b).
4.1 Conclusion

In the present experiment, rats were able to inhibit a motor response as instructed by a stop signal, in a novel rodent version of the countermanding task that closely modeled human countermanding tasks. Predictions of task performance made by the race model developed for human and non-human primate countermanding tasks were generally confirmed. These findings validate the race model in rodent stop tasks. Future research should attempt to identify why some variability exists in performance of the task with individual subjects, explore brain mechanisms involved in behavioural inhibition in the rat and examine inhibitory deficits in animal models of neurological disease and impulsivity.
Appendix A

Long Evans performance in the countermanding task

A pilot study was conducted on two Long Evans Rats prior to the present experiment. Data from 5 individual sessions are presented below. Stop signal delays with less than 5 trials in the session were excluded from analysis. The subjects generally made more non-canceled responses on stop trials as stop signal delay increased (Figure A.1).

Estimates of stop signal response time varied for the two subjects between 100 and 250 ms for individual sessions (Figure A.2).

As displayed for one of the subjects in a single 1-h session, predictions of the race model were generally respected (Figure A.3).
Fig. A.3

Rat B

![Graph showing reaction time vs. SSD (milliseconds)](image)

- Go RT
- Non-cancelled RT
- Predicted
References


