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Cortical-subcortical interactions in cognitive control,
associative learning and motor control

By

Peter Andrew Butcher

A dissertation submitted in partial satisfaction of the

Requirements for the degree of

Doctor of Philosophy

in

Psychology

in the

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of the

University of California, Berkeley

Committee in charge:

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Professor Jonathan Wallis
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Fall 2014

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Abstract

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No task is accomplished by the use of only a single brain region. Areas of the brain communicate with each other in a flexible manner that allows for complex processing to occur. Over the last decade and a half functional brain imaging has proven to be an immensely popular technique in cognitive neuroscience. While this has led to enormous progress in understanding brain function, it has also led to an increasingly phrenological view of the brain, where a single brain region is proposed to be necessary for the experience of cognitive states, or the completion of tasks. Rather, processing is accomplished by a distributed network, much of which involves connectivity between cortical and subcortical regions of the brain. The purpose of this dissertation is to explore the interaction of cortical and subcortical regions and resulting effects on performance in three task domains.

In chapter 1 a novel association learning task is used to isolate learning from positive and negative reinforcement. When a low dose (1.25 mg) of bromocriptine, a dopamine (D2) receptor agonist was administered performance was impaired in learning from positive reinforcement, while there was a boost to performance in learning from negative reinforcement. It is suggested that this pattern of results on the two feedback valences is due to a reduction in phasic dopamine release due to presynaptic drug action. The effect of drug administration was further modulated by gender where males were much more affected by the drug. Individual differences in cortical and subcortical processes were examined using genetic data for two dopamine related genetic polymorphisms. Under placebo conditions, participants with the better functioning version (Val/Val) of the polymorphism associated with cortical dopamine function, COMT Val158Met, out performed the two other allele groups (Val/Met and Met/Met). Lastly, the main effect of drug administration was best predicted by the polymorphism associated with subcortical dopamine functioning, DRD2/ANKK1-Taqla, where participants with a higher DA receptor density (A1-) had less of a drop in performance when learning from negative feedback after drug administration, than did (A1+) participants. Thus, performance in learning from positive and negative reinforcement, is at least to some extent, reliant on the interaction of cortical and subcortical dopaminergic systems.

In chapter 2 the same group of participants from experiment 1, also completed a working memory (WM) task. The WM task was completed on the same sessions as the tasks for experiment 1. Of particular interest in the WM task were three trial types: low load, where a minimal amount of information was to be held in WM; high load, where the amount of items to be remembered was increased; and filter, where some items were to be ignored. After administration of bromocriptine there was an unexpected slowing in reaction time (RT) for the easiest low load trials. Participants' RTs on the low load trials slowed to such an extent from drug administration that they were then responding more quickly to the more difficult high load and filter trials. When examining the polymorphism associated with cortical DA function, COMT Val158Met, under placebo conditions participants with the better functioning Val/Val allele, surprisingly, had superior performance when the number of items to be remembered was increased, than Val/Met or Met/Met participants. Bromocriptine administration resulted in improved load performance for participants with the lower functioning COMT allele, Met/Met. Finally, when examining the polymorphism associated with subcortical DA function, DRD2/ANKK1-Taq-IA, participants with the allele associated with lower receptor density, A1+, were the most susceptible to changes in RT from drug administration. In summary, ACC in working memory performance under increased load is best predicted by the cortical dopamine polymorphism, COMT Val158Met, while the polymorphism associated with subcortical dopamine function, DRD2/ANKK1-Taq-IA, predicts the extent to which dopamine modulation affects RT.

Lastly, in chapter 3 a visuomotor adaptation task was used to explore the role of primary motor cortex (M1) in the retention of a new sensorimotor transformation. Specifically the focus was on the role that success or error in a reaching movement plays on retention of the sensorimotor transformation in M1. On each trial participants made a reaching movement in a virtual environment while a perturbation was applied around the cursor, in this case in the form of a visuomotor rotation. To investigate M1's role in consolidation single pulses of transcranial magnetic stimulation (TMS) were delivered on specific trials. In experiment 3A feedback was only given about success in reaching for the target, not about the size of any error. Participants who received a TMS pulse on target hits failed to adjust their reach angles to compensate for the perturbation, regardless of pulse timing. Due to participants in the hit and delayed hit TMS groups not adjusting for the perturbation, the effect on consolidation was not able to be investigated. In experiment 3B participants were additionally shown the endpoint feedback of their reach. Under these conditions all participants adjusted to the perturbation, however, there were no differences in consolidation of the learning. This was true whether the TMS pulse was delivered on all trials, only on hit trials, or only on miss trials. Lastly, a direct replication was attempted by the addition of endpoint feedback during the retention test. Only a no TMS and an all trials TMS group were included. While weak evidence for impaired retention in the all trials TMS group was found, if present, the effect is much smaller than previously reported. The results in chapter 3 emphasize the importance of properly controlling for the mildly aversive nature of the TMS itself when using TMS to target specific trials. This leaves open the question of the role that success and error play in the consolidation of sensorimotor learning in M1.

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This is dissertation is in memory of my grandparents: Jacqueline Humphreys, Maurice Humphreys, Joyce Butcher and especially Thomas Butcher; who encouraged my intellectual interests from a young age.

I would not be here without all of you.

Chapter 1 Dopaminergic and genetic influences on learning from positive and negative reinforcement

1.1 Introduction

Is it better to punish your child when she misbehaves or to reward her when she behaves well? Are there differences in how people respond to reward and punishment? In a neuroscience context, a first question to ask is whether reinforcement mechanisms involve bidirectional signals. If the same mechanisms code positive and negative reinforcement signals, then punishing a child for bad behavior or rewarding her for good behavior may result in similar changes in behavior. However, if positive and negative reinforcement signals are computed by different neural systems, we might expect to observe differences in learning from the two types of feedback.

Behavioral evidence has shown that humans are more sensitive to losses than gains when they are of equal magnitude (Kahneman & Tversky, 1979). Many theories have been proposed about why this loss aversion occurs. One such set of theories states that loss avoidance stems from more attention being paid to losses than to gains (Peeters & Czapinski, 1990) and further that this effect can be modulated by varying the salience of the loss, for example making it hypothetical, and is further affected by the individual's mood (Romanus et al., 1996). Others, however, have claimed the increased attention and saliency of losses is not due to secondary emotional effects, but that losses are actually calculated relative to a reference point, with a steeper value curve in the negative direction (Tversky & Kahneman, 1991).

While this evidence that humans are more sensitive to losses than gains when they are of equal magnitude (Breiter et al., 2001; Kahneman & Tversky, 1979) may seem to pose problems for a single system account of feedback learning, however, one neural system could still encode both positive and negative reinforcement, but just be more sensitive to loss information. Thus, in this case, we would not need independent neural systems for encoding losses and gains, but a single system that has a steeper value curve for negative outcomes.

In terms of neural systems, various lines of evidence suggest that negative reinforcement may be linked to different neural systems than those associated with positive reinforcement. Neuroimaging studies have shown that the hemodynamic response in the insula is correlated with the conscious awareness of errors (Ullsperger et al., 2010) and performance improvements in learning from errors (Wrase et al., 2007). Subregions within the prefrontal cortex have also been shown to play a critical role in learning from errors, with one hypothesis focusing on the idea that lateral prefrontal cortex and anterior cingulate cortex work together to adapt behavior after an error is committed (Aston-Jones & Cohen, 2005; Gehring & Knights, 2000). While the lateral orbital frontal cortex is thought to be involved in responding to aversive outcomes

(Hosokawa et al., 2007). In studies of motor learning, there is a rich history linking the cerebellum to error-based learning (Gilbert & Thach, 1977; Horn et al., 2004; Ito, 2001; Wolpert et al., 1998).

Dopamine (DA) signaling has long been recognized as a strong neural correlate of positive reinforcement (Bayer & Glimcher, 2005; Montague, 1996; Schultz, 1998). While early work focused on the idea that DA activity was the physiological correlate of reward (Yokel & Wise, 1975), more recent work has shown that DA activity can be well-described in terms of a prediction error signal, coding the difference between the received reward and the predicted reward. An increase in DA activity, or positive prediction error, is observed when the obtained reward is larger than the predicted reward. Similarly, a decrease in DA activity, a negative prediction error is observed when the obtained reward is less than the predicted reward. Others, however, have rejected entirely the notion that DA signaling is in the form of a reward prediction, theorizing instead that DA signaling is involved in pavlovian conditioning or incentive salience (Berridge, 2007).

What is less clear is the extent to which dopamine signaling is involved in learning from negative reinforcement, is the drop in DA firing rate observed after an omitted reward coding for a lack of reward, or a negative prediction error? Answering this question has been complicated by the fact that many tasks that attempt to explore learning from positive and negative reinforcement conflate a lack of reward as an error. To continue the example from above, is withholding praise from your child the same as giving them additional chores as a punishment? Further, studies that do provide both positive and negative reinforcement give both in the same block making it difficult to discriminate which valence of feedback participants are learning from.

Michael Frank and colleagues have conducted a series of studies that favor the idea that dopamine signaling is bidirectional, coding for both reward and errors. In one such study Parkinson's Disease (PD) patients performed a discrimination learning task in which pairs of Japanese symbols were presented on each trial (Frank et al., 2004). Some symbols are associated with a higher likelihood of a positive outcome, while others a higher likelihood of a negative outcome. With practice participants learn to select the stimuli with a higher probability of reward, while avoiding stimuli with a lower probability of reward.

When PD participants performed the task after abstaining from their medication, performance was poor relative to controls in choosing the good options, but they actually outperformed controls in learning to avoid the bad options. In contrast, the reverse pattern was observed when the PD participants were tested on their normal medication, where they outperformed controls in choosing the good options, but were impaired at avoiding the bad options (Frank et al., 2004). While this study indicates that the manipulation of DA has bidirectional effects on learning from positive and negative reinforcement, it is again unclear whether participants were learning from the negative reinforcement or just learning from the lack of positive reinforcement. Did participants consider the feedback "incorrect" as an error, or a lack of reinforcement?

The role of DA in learning has also been examined in studies with normal individuals. The administration of a DA agonist, bromocriptine, was found to benefit performance in participants with low working memory capacity, while hurting performance in participants with high working memory capacity (Kimberg et al., 1997). Results such as this have led people to postulate that there is an optimal level of DA that needs to be present in the brain, and that deviation from this in either direction results in impaired performance, sometimes referred to as an inverted u-shaped curve (Vijayraghavan et al., 2007).

In addition to asking about systems-level differences in sensitivity to positive and negative reinforcement, one can also consider variability between individuals. Compellingly, it has been suggested that individual differences in DA related performance can be predicted by genes associated with DA functioning (Mattay et al., 2003). People show considerable variability in how well they learn from positive and negative reinforcement (Frank et al., 2007a), and these differences have been attributed, in part, to variation in dopamine signaling. In one study, a large cohort of participants completed a computerized reinforcement learning task in which they had to learn a set of arbitrary associations in a probabilistic manner. Variation in three dopamine related genes was predictive of individual differences in learning the task, but in distinct ways (Frank et al., 2007a). DARPP-32, associated with striatal DA function, predicted the degree to which participants learned to choose the good options and avoid the bad options. DRD2/ANKK1-TaqIa, associated with D2 receptor density, predicted the degree to which participants learned to avoid the bad choices, independent of their performance in choosing the good stimuli. COMT Val158Met, a polymorphism associated with DA levels in the Prefrontal Cortex (PFC), predicted the likelihood of a participant changing their response after receiving negative reinforcement.

At this point there has been little investigation of how genes that predict baseline DA levels affect learning from positive and negative reinforcement, and more so how this interaction is modulated by administration of dopaminergic drugs. By using a task where positive and negative reinforcement can be assessed independently a more thorough assessment can be made of the role of DA in signaling reinforcement in brain.

In the current study, we sought to develop a task in which learning from either positive or negative reinforcement could be tested in relative isolation. The behavioral paradigm used in the current study was adapted from a previous imaging study to allow for a drug manipulation (Bischoff-Grethe et al., 2009). Positive and negative reinforcement were isolated in to different learning blocks with uninformative feedback being delivered on half the trials of the current valence, and all trials of opposite valence. Thus if uninformative feedback was displayed in the negative reinforcement block the participant would not know whether they had responded correctly, or if they had responded incorrectly, but the trial was not eligible for feedback. In the original imaging study using this paradigm, bilateral nucleus accumbens, caudate nucleus, anterior insula, right cerebellar lobule VI and left putamen were more active when

informative feedback of any type was given. While no regions were found to be more selective for negative reinforcement than positive reinforcement, the insula, amygdala, putamen and supplementary motor areas were found to be more selective for positive reinforcement (Bischoff-Grethe et al., 2009).

Using this task, we examined the role of DA in learning in two ways. First, by using the administration of a DA agonist, bromocriptine, to assess the effect of DA modulation on learning from positive and negative reinforcement independently across all participants. Second we looked at how individual differences in DA genetics predicted the effect of DA modulation on learning from positive and negative reinforcement.

We focused on two dopamine-related single nucleotide polymorphisms (SNPs), DRD2/ANKK1-Taqla (RS1800497) and COMT Val158Met (RS4680). The Val158Met SNP is a polymorphism on the catechol-O-methyltransferase (COMT) gene consisting of a valine-to-methionine substitution which has been associated with DA function in the PFC. COMT is an enzyme that is present in the frontal lobe and helps to break down DA in the synaptic cleft. In the striatum DA uptake is accomplished by DA transporters (DAT), however, due to the sparse presence of DAT in the frontal lobe the removal of DA from the synaptic cleft is accomplished through a combination of COMT enzyme activity and Norepinephrine transporters (Moron et al., 2002). The Met allele (methionine substitution) of the COMT gene is associated with the less stable form of the COMT enzyme, which, as DA is not as readily cleared, results in higher DA levels in the frontal lobe. The more thermostable Val allele results in lower DA levels in the PFC, while the heterozygous Met/Val is associated with an intermediate level of DA (Egan et al., 2001). The DRD2/ANKK1-Taqla SNP is a polymorphism associated with the DRD2 gene and has been shown to be related to DA functioning in the striatum. While the DRD2/ANKK1-Taqla is on the non-coding region of DRD2 it has been found to be associated with different dopaminergic phenotypes, specifically it is associated with DA D2 receptor density, with A1 carriers having a 30-40% lower receptor density than A1-participants (Pohjalainen et al., 1998; Ritchie & Noble, 2003).

Hypothesis 1A: The results of previous studies supporting DA signaling of both reward and error outcomes have been due to DA signals for a lack of reward, rather than a pure error signal. In our task, where positive (reward) and negative (error) feedback are properly separated, DA modulation will result in an effect on learning from positive reinforcement, but not from negative reinforcement. This result would contradict predictions from the bidirectional (positive and negative) hypothesis of DA signaling for feedback.

Hypothesis 1B: An alternative is that both positive and negative reinforcement learning are affected, which would show bidirectional involvement of DA signaling in encoding the valence of reinforcement.

Hypothesis 2A: When the data is examined by looking at the two DA related SNPs, differential effects of the two genes will be found. Participants with the A1+ allele of the DRD2/ANKK1-Taqla SNP (lower striatal D2 receptor density) due to having a higher DA sensitivity will have a larger benefit to performance when learning is from positive reinforcement after bromocriptine administration than A1- participants. For the COMT Val158Met SNP participants with the Met allele (lower COMT activity), due to already high tonic DA levels at baseline, will have the largest detriment in performance in negative reinforcement learning, with the Val (higher COMT activity) allele showing the least, and the heterozygous Met/Val performance being in the middle (Table 1).

Hypothesis 2B: An alternative possibility is that frontal DA levels may be irrelevant in learning from either type of feedback in the current task. If this is the case then the Val158Met SNP allele status for a participant will not have any relation to changes in learning from positive or negative reinforcement after bromocriptine administration.

SNP	Neural Locus	Prediction	Most effected allele
COMT (RS4680)	Frontal Lobe	Neg learning	Met (Low COMT)
DRD2 (RS1800497)	Midbrain	Pos learning	A1+ (Low D2 receptors)

Table 1: Drug-gene predictions

1.2 Materials and Methods

Participants

Thirty healthy participants (mean age, 25.1 ± 1.1 years, SEM; 14 females) were recruited from an existing database of participants who had been screened for the COMT Val158Met (RS4680) and DRD2/ANKK1-Taqla (RS1800497) polymorphisms (Jacobs & D'Esposito, 2011). In creating the database, saliva samples were collected using the Oragene DNA Self-Collection Kit (DNA Genotek inc., Ottawa, Ontario, Canada). DNA extraction and analysis was conducted according to standard methods and genotyping was performed using PCR on the 3'-untranslated region (Creative Genomics, Port Jefferson Station, NY). The samples were obtained from informed, consenting participants under a protocol approved by the institutional review board at UC Berkeley.

Participants in the database had also been tested on the following neuropsychological battery: 1) the National Adult Reading Test (Nelson, 1982), 2) the Beck Depression Inventory (Beck et al., 1961), 3) the Montreal Cognitive Assessment test (to assess mild cognitive impairment) (Nasreddine et al., 2005), 4) the Barratt Impulsiveness Scale (Patton & Stanford, 1995) and 5) Listening Span (Salthouse & Babcock, 1991). During the first test session of the current study, additional neuropsychological data were collected for: 6) Forward and Backward Digit Span (Kaplan, 1995), 7) Forward and Backward Spatial Span (Kaplan, 1995), and 8) a repetition of the Montreal Cognitive Assessment test.

To enable the analysis of the two genes independently, the participants were selected to form four groups with balanced COMT Val¹⁵⁸Met and DRD2/ANKK1-Taqla alleles. Exclusionary criteria included any history of psychiatric or neurological disorders, an episode involving the loss of consciousness, or the use of psychotropic drugs. Based on these criterion, participants were contacted to seek their participation in the current study. The current study was also approved by the IRB at UC Berkeley and was conducted in accordance with the Declaration of Helsinki. All volunteers gave written informed consent and were paid for their participation.

Participants came in for behavioral testing on two occasions, resulting in the acquisition of 60 data sets. The behavioral and drug analyses reported below include the data from only 23 (mean age, 25.1 ± 1.2 years, SEM; 11 females) of the 30 participants. The data from seven participants were excluded because these individuals either failed to perform above chance on one of the experimental tasks (see below, $N = 4$) or showed a change in performance between the two sessions that was more than two standard deviations of the mean change ($N = 3$). The DNA analysis turned out to be incomplete for one male participant; thus, his data is limited to the drug effect. For the 22 participants included for analysis of gene effects, the breakdown of COMT Val158Met alleles was 8:6:8 (Val/Val:Val/Met:Met/Met). The breakdown of DRD2/ANKK1-Taqla alleles was 2:9:11 (A1/A1:A1/A2:A2/A2). The A1/A1 allele of DRD2/ANKK1-Taqla is extremely rare, only occurring in roughly 3% of Caucasians (Noble,

2003). Due to its low prevalence, we combined the homozygous A1/A1 group with the heterozygous A1/A2 carriers for analysis of DRD2/ANKK1-Taqla gene effects (Stelzel et al., 2009). Thus, there were 11 participants in the A1+ group (A1/A1 and A1/A2) and 11 participants in the A1- group (A2/A2). The COMT and DRD2 allele divisions were independent in our sample when the categorical data were evaluated in a Chi-squared test ($\chi^2 = 1.58$, $p = 0.45$).

In the original selection process, efforts were made to match ethnicity between genotypic groups to reduce population stratification effects (Lander & Schork, 1994). In the final group of 22, the distribution for the two groups formed by the COMT gene was: Met/Met N = 8; 6 of whom self identified as Caucasian and 2 as Latino; 3 females; Met/Val N = 6; 4 of whom self identified as Caucasian, 1 as Asian and 1 as Latino; 3 females; and Val/Val N = 8; 7 of whom self identified as Caucasian and 1 as Asian; 5 females. When the participants are grouped by DRD2, the breakdown for gender and ethnicity was: A1+ N = 11; 8 of whom self identified as Caucasian, 2 as Asian, and 1 as Latino; 5 females, A1- N = 11; 9 of whom self identified as Caucasian and 2 as Latino; 6 females. Note that the selection process was biased to recruit Caucasians given that prior work has shown that the relationship between genotype and phenotype can vary based on ethnicity (Domschke et al., 2007).

In a post-hoc analysis, the participants were split into two groups based on working memory span. This division (High vs. Low Span) was based on a median split of the total number of words remembered in a test of listening span (Salthouse & Babcock, 1991). The span data were not available for one participant; however, this participant was placed into the Low Span group based on his score on a test of backwards spatial span (Kaplan, 1995) as the auditory and backwards spatial span tests were positively correlated in our sample (N = 22, $r = 0.43$, $p = 0.05$).

Experimental Task

Participants learned, through a modified trial-and-error method, to classify 16 abstract images into two arbitrary categories. We opted to use 16 images based on pilot testing. The intent was to have the number of stimuli to be sufficiently large to tax the capacity of working memory.

A trial started with a fixation cross displayed at the center of the screen for 800 ms. The cross was then replaced by one of the images which remained visible until a response was made or a maximum duration of 3000 ms. The participant responded to the stimulus by pressing one of two buttons with either the thumb or middle finger of the right hand. Half of the images were randomly assigned to be the category associated with the “thumb” button and the other half were paired with the “middle finger” button. The stimulus-button associations were counterbalanced across participants. Participants were not told which button to press for each image and were instructed to figure out the correct button through trial and error. After the response, feedback was displayed for 1000 ms, followed by a blank screen displayed for 500 ms before preceding to the next trial. A block consisted of 16 trials, with each image appearing

once in a random order. This procedure was repeated for ten blocks, allowing the participant to see each stimulus 10 times. At the end of each block, a screen was displayed informing the participant that the block had ended. From the second block onwards this screen also displayed their percent correct in responding to images for which they had previously received informative feedback.

Within each session, the participant completed two 10-block runs, one in which learning was based on positive reinforcement and one in which learning was based on negative reinforcement. Separate sets of 16 images were used for the positive and negative runs. We employed a partial reinforcement scheme to emphasize the two types of reinforcement signals (Bischoff-Grethe et al., 2009). To this end, informative feedback was only possible on a maximum of 50% of the trials (see figure 1); on the other 50% of the trials, the feedback was limited to the message, “??Unknown??”. On trials eligible for informative feedback, the participant only received the informative feedback if the response was appropriate for the current valence condition: In the positive runs, the message “Correct” appeared if the selected response was the one paired with the image; in the negative runs, the message “Incorrect” appeared if the selected response was the one that had not been paired with the image. If the response was inappropriate for the current valence condition, the message “??Unknown??” was presented. In this manner, the “??Unknown??” message could occur because a trial was not eligible for informative feedback or because the inappropriate response had been made. As such, the participants could not infer the correct response from the non-informative feedback. Note that the feedback structure depicted in **Figure 1** shows the outcomes for a positive reinforcement run. The negative reinforcement run had the same structure, but when eligible for feedback they would only be informed when they were incorrect.

This feedback structure produces one important difference between the two tasks: As learning occurs, the rate of informative feedback differs for the positive and negative runs. At the start of training the participant has yet to learn the stimulus-button associations. As such, informative feedback will, on average, occur on 25% of the trials given that 50% of the trials are ineligible for feedback and the participant will press the inappropriate key on 50% of the informative trials. This will hold for both the positive and negative tasks. However, as the participants learn to make the correct responses, the probability of informative feedback will increase in the positive task and decrease in the negative task. In the extreme when performance is at 100% correct, informative feedback would be given on 50% of the positive trials (all of the trials eligible for

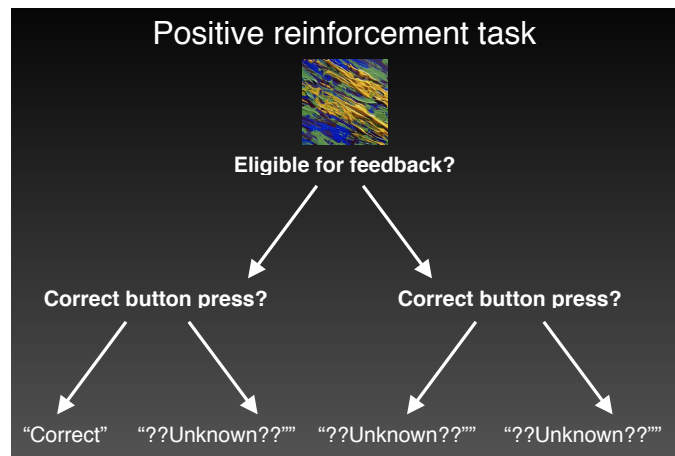


Figure 1: Positive reinforcement task structure

informative feedback) and informative feedback would never be given on the negative trials (since the participant never makes an error).

It is possible that participants would choose to select the wrong key in the negative task as a strategy to obtain informative feedback. Adopting this strategy would result in accuracy that did not represent how well the participant had actually learned the stimulus-response associations. To provide an uncontaminated assay of learning, a test phase was conducted at the end of the 10 learning blocks for each valence condition. The participant was informed that they would again see the same set of images and would now receive informative feedback on all trials (“Correct” or “Incorrect”). Note that the first block here provides a pure test of how well the participants had learned the stimulus-response associations during the learning phase as they had yet to receive completely informative feedback. The second and third blocks of the test phase also reflect additional learning that occurs from fully informative feedback.

Experimental Timeline and drug administration

Participants completed both the positive and negative tasks in each of two sessions, with the sessions separated by approximately one week. In one session they were administered a low dose (1.25 mg) of bromocriptine (Bromo) a D2 receptor agonist. In the other session, they received a lactate placebo pill (PLAC). The procedures were identical for the drug and placebo sessions and a double-blind procedure was used such that neither the participant nor experimenter knew whether the pill was the drug or placebo. The order of the two sessions and task order within each sessions were counterbalanced.

Prior studies indicate that peak blood plasma bromocriptine levels are reached in roughly 100 minutes with efficacy of the drug lasting approximately 6-8 hours after administration of bromocriptine (Price et al., 1978). In the current study, a 90 min interval separated the ingestion of the pill and the beginning of behavioral testing, and the behavioral testing was completed in approximately two hours (see below). Blood pressure was measured prior to administration of the dose, and again at the completion of the experiment to ensure there was not an unsafe drop in blood pressure due to ingestion of the drug.

At the beginning and end of each session, the participant completed two tasks: 1) The state subscale of Spielberger State-Trait Anxiety Inventory (to assess changes in anxiety over the course of the session) (Spielberger, 1983), and 2) an automated test for alertness (to assess changes in alertness over the course of the session) (Stelzel et al., 2009). The latter task has been used to assess the effect on alertness and processing speed after bromocriptine administration (Stelzel et al., 2009). For this reaction time task, there are three types of trials: 1) Simple RT following the appearance of a fixation cross; 2) Simple RT to fixation cross that is preceded by an alerting tone (150 - 750 ms prior to cross, in steps of 150 ms); 3) Choice RT in which the participant responded with the left index finger to a green cross and right index finger to a red

cross. All trial types had a variable ISI (3000 - 5000 ms, in steps of 500 ms). The task consisted of 6 blocks of trials with one trial type present per block. The simple RT task consisted of 50 trials and was completed in blocks 1 and 6, the alerting tone task was completed in blocks 2 and 5 and consisted of 50 trials, while the choice task consisted of 40 trials and was completed in blocks 3 and 4. The completion of all 6 blocks took less than 10 minutes. For the present purpose, we averaged the RTs from the three trial types as a measure of general arousal. Four participants with missing data for one or more sessions were excluded from analysis resulting in alertness data for N = 19 participants.

After completing the 10 min battery, the participant was tested on the association learning task with either positive or negative reinforcement (counterbalanced). It took approximately 25 min to complete the practice block, the 10 learning blocks, and the 3 test blocks. They then completed a 45 min test of working memory (discussed in Chapter 2). Following this, participants completed the second phase of the association learning task with the other valence task, using a new set of images. Note that the 45 min break between the positive and negative reinforcement tasks should also serve to reduce interference between the two runs of the association learning tasks.

Behavioral data analysis

Accuracy was compared across conditions, with within-subjects comparisons used for all drug effect analyses. The data were limited to trials in which the participant had previously received informative feedback for that image (known images). Note that for some images, this would mean that the data are scored for nine blocks (if informative feedback was provided on block 1); for others, the data are scored for fewer blocks (if informative feedback was not provided until a subsequent block). Accuracy data were analyzed with a repeated measures ANOVA using the general linear model framework of SPSS (IBM, 2011). The ANOVA included within-subject factors of Drug (Bromo or PLAC) and Task (Pos or Neg), while gender, session where drug was given (1st or 2nd) and, where appropriate, SNP status (COMT and DRD2) were used as between-subject factors. An additional ANCOVA analysis was ran to control for changes in arousal during drug administration using the RT data from the working memory task (median Bromo RT - median Placebo RT) as a covariate (see Chapter 2). The working memory task RT data were used rather than the RT data from the alertness task since all participants completed the working memory task in both testing sessions. Drug effects were calculated for each individual participant by subtracting their accuracy under PLAC administration from Bromo administration (Bromo ACC - PLAC ACC). In this manner a positive drug effect results from an increase in accuracy after drug administration, with the opposite being true for a negative drug effect. When necessary, sphericity was corrected with the Greenhouse-Geisser correction. All reported values are for two-tailed t-tests.

1.3 Results

Based on a debriefing questionnaire, participants were unable to determine if they were in the drug or placebo group. At the end of each session, the participant was asked to indicate if the pill taken during that session had contained an active drug or a placebo. Accuracy on this forced-choice question was 55% for the PLAC session and 37% for the Bromo session, both indistinguishable from the chance level of 50%.

Neuropsychological and test day measures

The results from the neuropsychological measures are shown in Table 2. For the most part, performance on the different measures did not differ across the genetic groups nor as a function of drug state. One exception is on the State-Trait Anxiety Inventory test, a measure of trait anxiety. For the COMT SNP, the Val/Met group showed higher trait anxiety compared to the Val/Val group (41 vs. 30, $t_{12} = 3.24$, $p = 0.01$). The association between COMT SNP allele status and anxiety is consistent with previous results indicating an increased risk for anxiety disorders in Met carriers, although the association is specific to females (Domschke et al., 2007) and is disputed by others (Wray et al., 2008).

To assess alertness, a set of three tasks was used in which participants make speeded responses in simple, choice, or cued reaction time tasks (Stelzel et al., 2009). Performance across the three was highly correlated and thus a composite score was composed by combining the RT data across the three tasks. Alertness was measured at the beginning of the testing session before the drug had taken effect and at the end of the testing session. During the PLAC session there was no change in mean RT during the testing session, with participants on average 1 ms (± 4 , SEM) slower at the end of testing session (Pre: 321 ± 8 ms; Post: 321 ± 8 ms, SEM). However, in the Bromo session, RTs were, on average, 21 ms (± 5 , SEM) slower at the end of testing session (Pre: 318 ± 8 ms; Post: 339 ± 11 ms). A repeated measures ANOVA of RT on correct trials revealed a Drug X Session interaction ($F_{1,17} = 8.86$, $p < 0.01$) where under Bromo administration RTs slowed more over the course of the experimental session than under PLAC administration.

To create High and Low working memory span groups, a median split was performed, based on listening span performance. As would be expected, the groups differed on working memory span measures. For the forward spatial span test, the High Span participants remembered 11.6 (± 0.5) items compared to the Low Span participants who remembered 10.3 items (± 0.5 , SEM). On the backwards spatial span test, the High and Low means were 10.8 (± 0.4) and 9.4 (± 0.2 , SEM), respectively. There was also a gender effect on the Backwards spatial span task, with men having a lower score than woman (9.6 vs 10.6, $t_{21} = 2.06$, $p = 0.05$). This gender effect was not observed on the other working memory span measures, and does not survive multiple comparisons correction.

Association Learning Task

Figure 2 shows the learning functions for the negative and positive reinforcement tasks when the participants were tested under placebo administration. Over all of the blocks, performance was not statistically different for the two types of feedback ($t_{22} = 0.89$, $p = 0.39$), with overall mean accuracy of 81% ($\pm 1.4\%$) and 80% ($\pm 1.6\%$, SEM) for the positive and negative tasks, respectively (**Figure 2** inset).

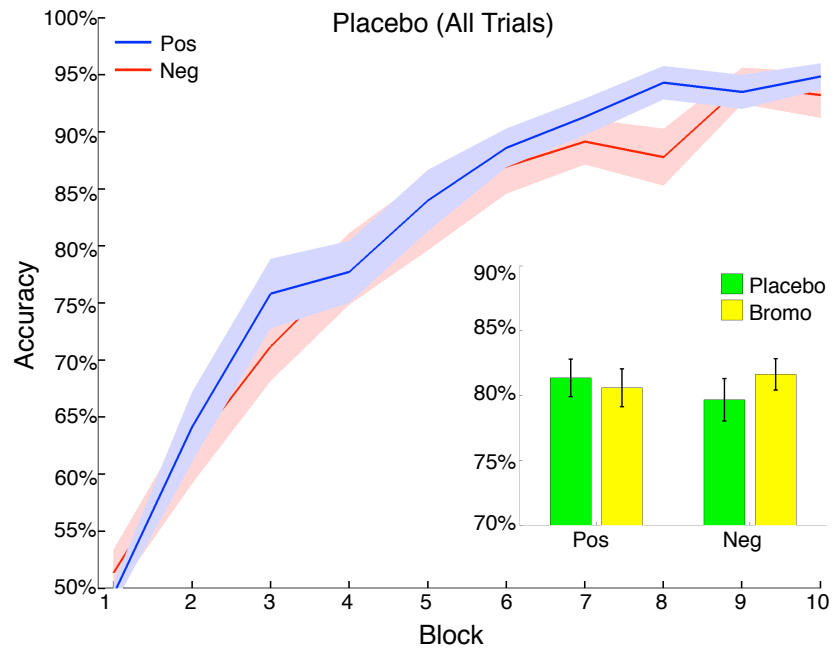


Figure 2: Placebo learning curve (Shaded area SEM)

However, when the analysis is restricted to trials involving “known” associations (items for which informative feedback had previously been provided), accuracy was higher in the positive task ($t_{22} = 2.35$, $p = 0.03$) (**Figure 3** inset). To

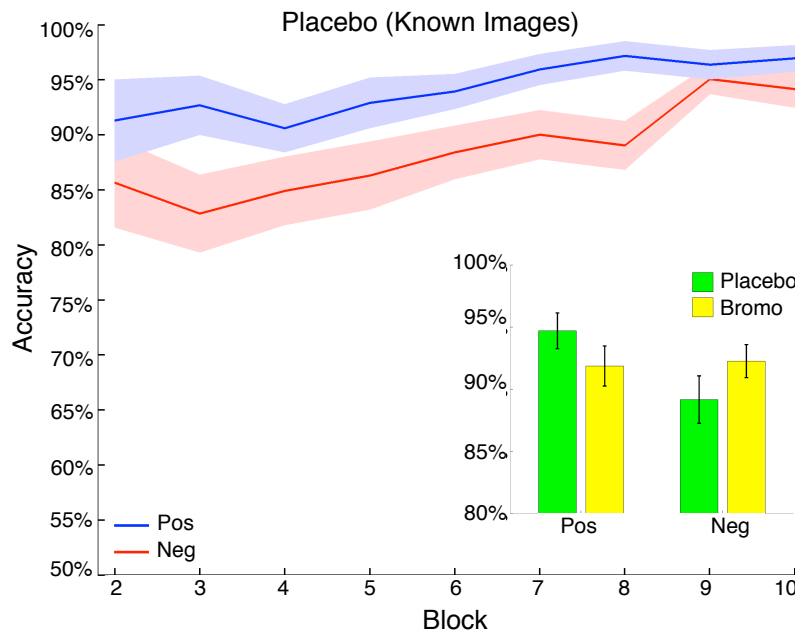


Figure 3: Placebo learning curve Known Images (Shaded area SEM)

examine whether the learning rate changed from Session 1 to Session 2, the data was averaged across the two reinforcement tasks and drug conditions for each session. The learning curves for known items are quite flat, indicating that associations were learned quite easily once informative feedback was provided. **Figure 4** depicts the learning curve averaged across both reinforcement tasks and drug conditions for the first and second sessions for all trials (top) and only

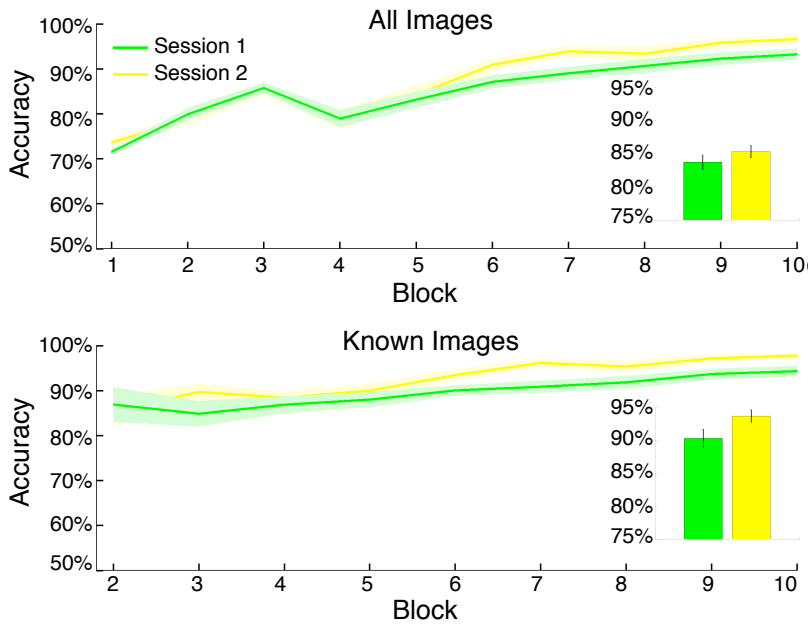


Figure 4: Session 1 and 2 learning curves (Shaded area SEM)
"Known Images" are stimuli that the participant has already received informative feedback about

"known" trials (bottom). When examining all trials, performance was not statistically different for the two sessions ($t_{22} = -1.10, p = 0.29$). When the analysis is restricted to trials involving "known" images, participants were more accurate in session 2 ($t_{22} = -2.68, p = 0.01; 94 \pm 0.9\%$ vs. $90 \pm 1.3\%$, SEM).

As mentioned previously the feedback structures of the positive and negative reinforcement tasks led to a

divergence in the rate of informative feedback for the two tasks. For the first block of trials participants received informative feedback at roughly the same rate in the positive ($23 \pm 1.5\%$) and negative reinforcement ($24\% \pm 1.8\%$, SEM) tasks (**Figure 5**). By the final block the informative feedback rate increased to 48% ($\pm 2.4\%$, SEM) for the positive reinforcement task and decreased to 2% ($\pm 1.8\%$, SEM) for the negative reinforcement task. Given that the task structure imposes this difference, the analyses described below focus on comparisons between drug state and/or gene group within a

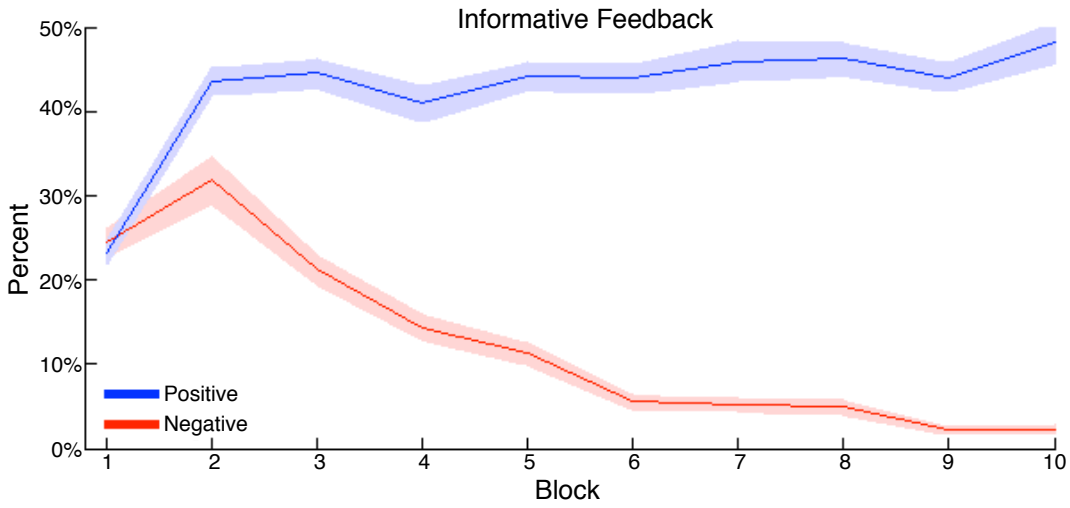


Figure 5: Rate of informative feedback (Shaded area SEM)

reinforcement task; we do not make direct comparisons between the positive and negative reinforcement tasks.

Effects of bromocriptine

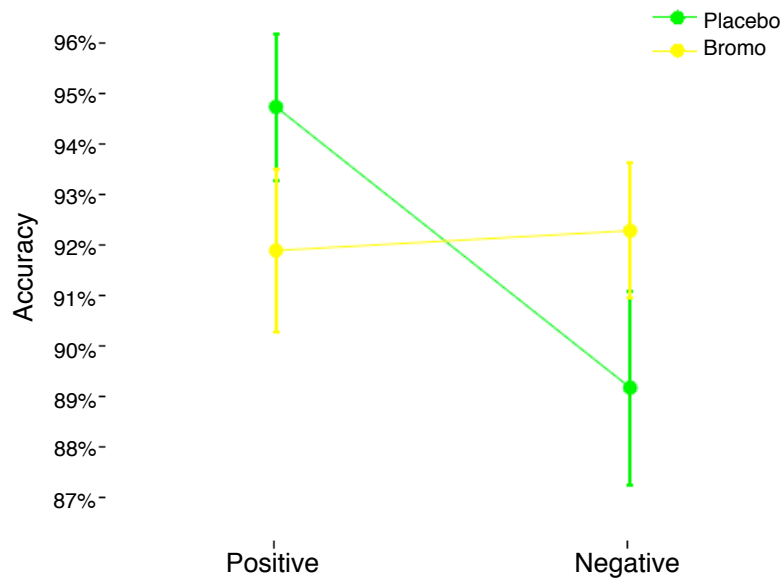


Figure 6: Feedback Type X Drug interaction (Error bars represent SEM)

We first examine if the dopamine agonist, bromocriptine, had a differential effect on learning from positive and negative reinforcement. Accuracy data, limited to trials with known stimuli were entered into an ANOVA with the factors Feedback Type (positive and negative) and Drug (BROMO and PLAC) as within-subject factors, and Gender (female or male) and Drug Session (1st or 2nd) as between-subjects factors. The Drug by Feedback

interaction was significant ($F_{1,19} = 4.46, p = 0.05$). Administration of bromocriptine was associated with improved performance in the negative reinforcement task ($3\% \pm 2.4\%$); in contrast, the drug led to a decrease in performance in the positive reinforcement task ($3\% \pm 1.7\%$) (**Figure 6**). To test whether the drug effect might be related to changes in arousal, we repeated the analysis, but used the RT difference between the two sessions (Bromo RT - PLAC) as a covariate in an ANCOVA. The interaction remains marginally significant ($F_{1,18} = 4.17, p = 0.056$).

Prior work has shown that the efficacy of DA drugs can vary with gender (Jacobs & D'Esposito, 2011). In the current study, the main effect of gender was not significant ($F_1 = 0.99, p = 0.33$). However, there was a significant Feedback Type X Drug X Gender interaction ($F_{1,19} = 6.22, p = 0.02$). As shown in **Figure 7** the Feedback x Drug effect was limited to the 12 male participants. Indeed, when tested on bromocriptine, men showed a 8% improvement in performance when given negative feedback and a 5% decrease in performance when given positive feedback. In contrast, the 11 female participants performed similarly when on bromocriptine or placebo. The 3-way interaction remains reliable ($F_{1,18} = 5.76, p = 0.03$) when the arousal covariate is included in the analysis.

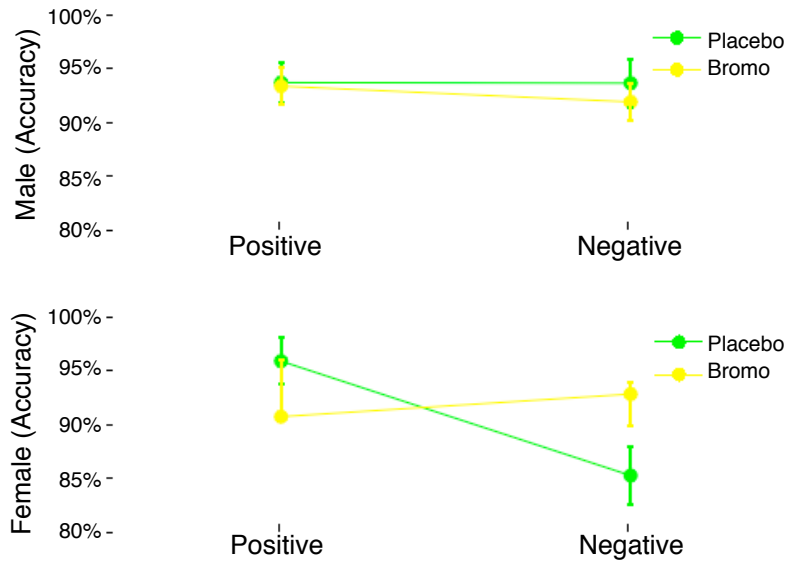


Figure 7: Gender, Feedback Type and Drug interaction (Error bars represent SEM)

Working memory span has previously been shown to interact with the administration of DA drugs (Gibbs & D'Esposito, 2005). Participants with higher working memory spans tend to show a decrease in performance after DA drug administration, while participants with relatively lower working memory spans display an increase in performance after administration of the same drug (Kimberg et

al., 1997; Mattay et al., 2000). However, there was not a significant main effect of working memory span in the current study ($F_1 = 0.69, p = 0.42$). After bromocriptine administration, both the High and Low Span groups became less accurate in the positive reinforcement task (High Span: $-4 \pm 2.5\%$; Low Span: $-1 \pm 2.4\%$) and more accurate in the negative reinforcement task (High Span: $1 \pm 2.5\%$; Low Span: $4 \pm 4.3\%$, SEM) (**Figure 8**).

Baseline gene effects

Genetic data was missing from one participant, resulting in a total of twenty two participants for the analyses of gene effects. Given the relatively small sample, we report the genetic data for COMT and DRD2 separately and do not address potential gene-gene interactions. For each gene, we first focus on performance in the placebo condition and then examine the gene x drug interactions.

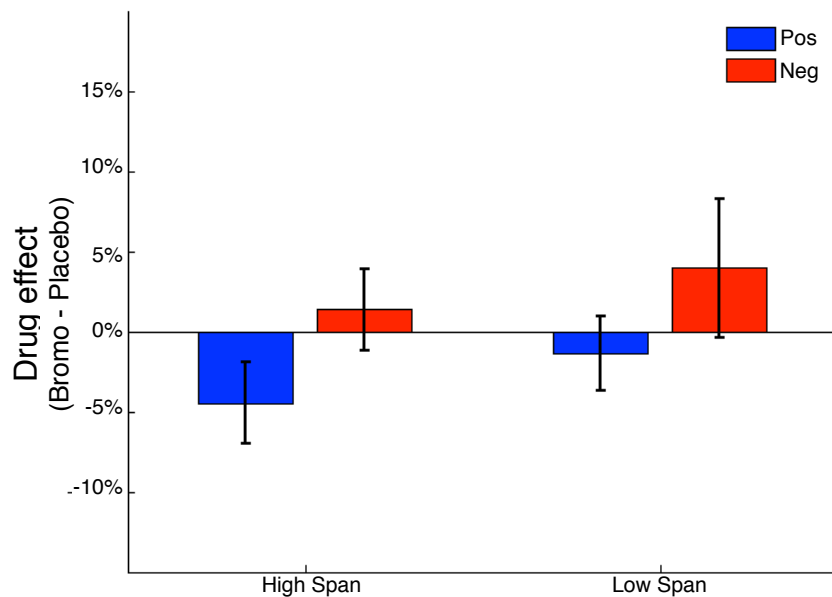


Figure 8: WM Span and Drug Effect (Error bars represent SEM)

When examining data only from PLAC sessions, the main effect of COMT was not reliable ($F_2 = 1.15$, $p = 0.39$). The intermediate (Val/Met) group showed poorer overall performance compared to the High COMT group ($p < 0.01$, $t_{12} = 3.12$) and a trend towards poorer performance compared to the Low COMT group ($p = 0.08$, $t_{12} = 1.89$).

For the DRD2 group, the participants were divided into two groups: Low D2 density (A1+, N = 11) and High D2 density (A1-, N = 11). In terms of performance under placebo, the main effect of gene was not significant ($F_1 = 0.61$, $p = 0.82$) nor did this factor interact with feedback ($F_{1,5} = 1.33$, $p = 0.30$) or gender ($F_1 = 0.78$, $p = 0.42$).

Gene-drug interactions

To simplify the analyses designed to simultaneously look at both drug and gene effects, we derived a difference score in which accuracy in the placebo session was subtracted from accuracy in the bromocriptine session. For this measure, a positive value means that accuracy increased in the drug session.

COMT-drug interactions

The Feedback Type X COMT interaction was marginally reliable ($F_{2,11} = 3.84$, $p = 0.054$). In the negative reinforcement task, the Val group performed significantly better ($95 \pm 1.6\%$, SEM; $t_{14} = 2.15$, $p = 0.05$) and Val/Met ($86 \pm 0.9\%$, SEM; $t_{12} = 4.54$, $p < 0.001$) when the data are averaged over the Bromo and PLAC sessions. In the positive reinforcement task, the three COMT groups performed similarly (**Figure 9** top). When the analysis is

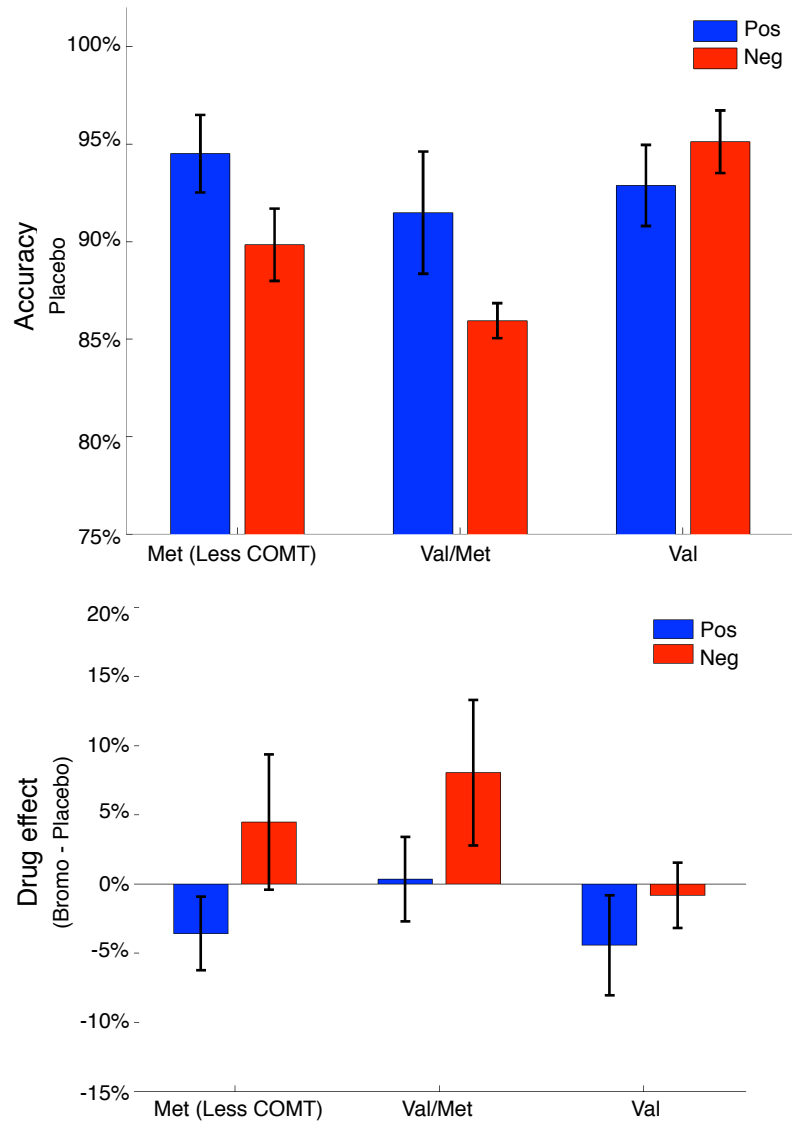


Figure 9: COMT drug effect
Placebo performance (top) and drug effect (bottom)
(Error bars represent SEM)

limited to the bromocriptine session, no differences were found for the three COMT groups in either reinforcement task (**Figure 9** bottom).

DRD2-drug interactions

There was a significant Drug x DRD2 interaction ($F_{1,14} = 19.48$, $p = 0.001$). The accuracy scores for the A1+ group were significantly lower when tested in the drug state compared to placebo ($p = 0.04$, $t_{10} = 2.31$). This reduction was largest in the positive ($-6 \pm 3.1\%$) reinforcement task, with no measurable change in the negative reinforcement task ($-1 \pm 2.4\%$, SE). In contrast, the A1- participants showed a marginal improvement in performance when tested on bromocriptine ($p = 0.07$, $t_{10} = 1.99$) (**Figure 10** top). The interaction remains significant ($F_{1,13} = 17.65$, $p = 0.001$) even when the RT data from the arousal task are used as a covariate.

When the positive and negative reinforcement tasks are included in the ANOVA, the Feedback x Drug x DRD2 interaction is not reliable ($F_{1,14} = 0.81$, $p = 0.38$). The A1+ group tended to show a larger drop in accuracy in the positive reinforcement task ($t_{20} = 1.83$, $p = 0.09$), while the A1- group tended to show a larger increase in accuracy in the negative reinforcement task ($t_{20} = 1.90$, $p = 0.07$) (**Figure 10** bottom).

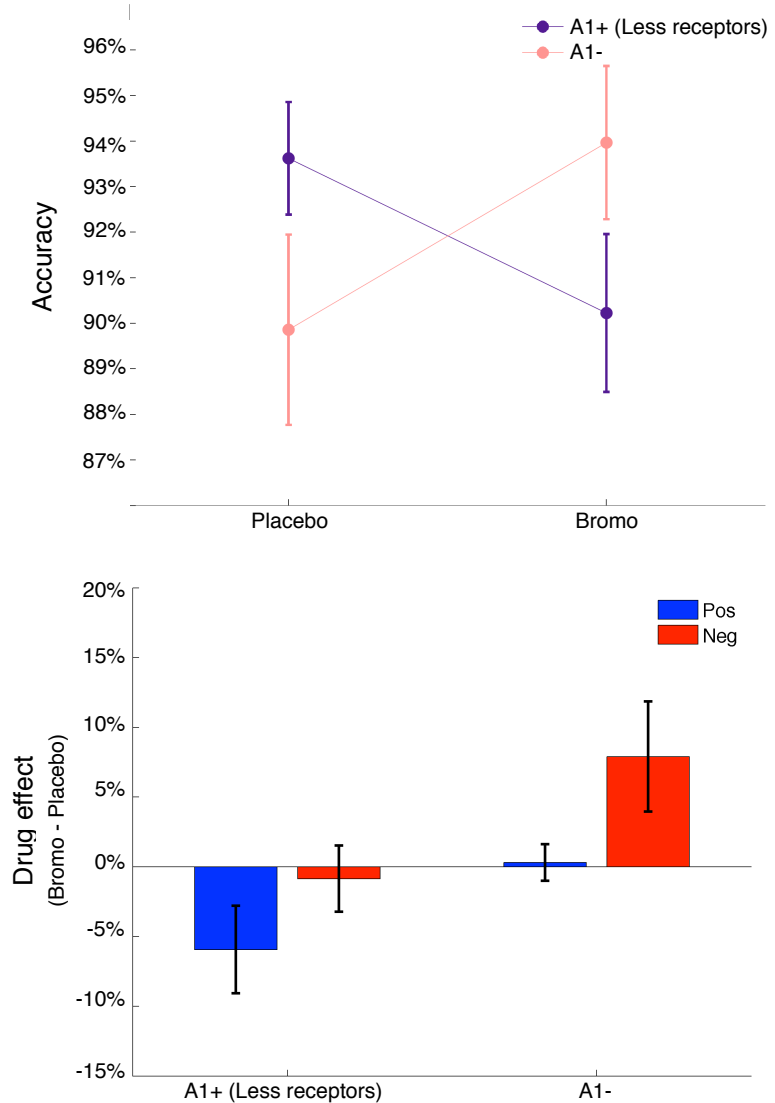


Figure 10: Drug X DRD2 interaction
Average of Pos and Neg task ACC (top) and
Drug effect (bottom)
(Error bars represent SEM)

Summary Tables mean (SEM)	All		Female		Male		High Span	Low Span	COMT Met	COMT Val/Met	COMT Val	DRD2 A1+	DRD2 A1-
	23	11	12	11	12	11							
N	23	11	12	11	12	11							
Age	25.1 ± 1.2	24.7 ± 1.0	25.5 ± 2.1	23.8 ± 1.0	26.3 ± 2.0	25.8 ± 2.6	22.8 ± 1.3	24.5 ± 1.1	23.3 ± 1.5	25.7 ± 1.5			
Weight (lbs)	159 ± 7.3	145 ± 6.3	174 ± 12.0	169 ± 14.0	150 ± 6.0	152 ± 8.8	157 ± 9.7	167 ± 18.2	152 ± 9.1	165 ± 12.0			
Height (inches)	68 ± 0.8	65 ± 0.8	71 ± 0.9	68 ± 1.6	68 ± 0.9	69 ± 1.7	67 ± 1.1	67 ± 1.3	68 ± 1.6	68 ± 0.7			
BMI	24.2 ± 1.1	24.0 ± 1.0	24.3 ± 1.9	26.0 ± 2.1	22.8 ± 0.9	22.1 ± 0.4	24.5 ± 2.0	26.6 ± 2.7	22.9 ± 0.9	25.6 ± 2.0			
MOCA	29.0 ± 0.2	29.2 ± 0.4	28.8 ± 0.3	29.5 ± 0.3	28.5 ± 0.3	28.8 ± 0.3	29.2 ± 0.5	29.4 ± 0.4	29.0 ± 0.3	29.2 ± 0.3			
Listening Span total words	54.7 ± 2.4	58.3 ± 2.7	51.2 ± 3.8	62.9 ± 1.8	46.5 ± 2.9	55.1 ± 2.3	54.5 ± 4.9	58.6 ± 3.4	53.6 ± 2.8	58.7 ± 2.7			
NAART errors	10.0 ± 0.7	10.9 ± 1.2	9.1 ± 0.8	9.7 ± 0.9	10.3 ± 1.2	11.6 ± 1.4	9.5 ± 1.5	9.1 ± 1.1	10.5 ± 1.0	9.6 ± 1.2			
BDI	1.7 ± 0.4	2.1 ± 0.6	1.4 ± 0.4	1.8 ± 0.7	1.6 ± 0.4	1.0 ± 0.4	1.8 ± 1.0	2.0 ± 0.7	1.9 ± 0.6	1.5 ± 0.5			
Forward digit span	12.6 ± 0.3	12.3 ± 0.6	12.9 ± 0.3	12.3 ± 0.6	12.9 ± 0.4	12.8 ± 0.4	12.2 ± 0.9	13.0 ± 0.6	12.9 ± 0.6	12.5 ± 0.4			
Backward digit span	9.8 ± 0.5	10.3 ± 0.6	9.3 ± 0.7	10.1 ± 0.4	9.5 ± 0.8	9.9 ± 0.5	9.7 ± 1.3	10.3 ± 0.7	10.6 ± 0.6	9.3 ± 0.6			
Forward spatial span	10.9 ± 0.4	11.3 ± 0.5	10.6 ± 0.5	11.6 ± 0.5	10.3 ± 0.5	11.1 ± 0.5	10.5 ± 1.0	11.1 ± 0.6	11.3 ± 0.5	10.6 ± 0.6			
backwards spatial span	10.1 ± 0.3	10.6 ± 0.3	9.6 ± 0.4	10.8 ± 0.4	9.4 ± 0.2	9.8 ± 0.5	10.0 ± 0.4	10.6 ± 0.5	10.3 ± 0.4	10.0 ± 0.4			
STAI trait (2 session avg)	34.5 ± 2.1	31.7 ± 2.1	37.1 ± 3.4	32.5 ± 2.0	36.1 ± 3.7	33.4 ± 4.8	41.4 ± 2.8	30.3 ± 2.1	35.2 ± 3.2	33.8 ± 3.1			
STAI state change (PLAC)	1.9 ± 0.9	0.8 ± 0.9	3.0 ± 1.5	1.8 ± 1.4	2.0 ± 1.2	3.0 ± 1.7	-0.7 ± 1.8	2.5 ± 1.2	3.4 ± 1.5	0.3 ± 0.9			
STAI state change (Bromo)	1.9 ± 1.0	1.2 ± 1.7	2.5 ± 1.2	3.2 ± 1.1	0.7 ± 1.7	-0.8 ± 2.3	2.7 ± 1.2	3.9 ± 1.4	3.2 ± 1.3	0.5 ± 1.7			
STAI state change (Br-PL)	0.2 ± 1.3	0.4 ± 2.1	0.0 ± 1.7	2.0 ± 1.3	-1.3 ± 2.1	-3.4 ± 3.1	3.3 ± 1.4	1.4 ± 1.7	0.4 ± 1.7	0.3 ± 2.2			
Barratt score	56.0 ± 1.9	56.3 ± 2.9	55.7 ± 2.5	53.7 ± 2.6	58.9 ± 2.6	56.3 ± 3.4	57.2 ± 3.6	55.3 ± 3.7	53.6 ± 2.0	58.1 ± 3.2			
RS-RT DE (ms)	-4 ± 22	11 ± 42	-18 ± 21	-28 ± 35	18.0 ± 29.2	37 ± 37	-38 ± 42	-15 ± 43	-5 ± 28	1 ± 39			

Table 2: Neuropsychological and test day measures

COMT: STAI trait (Val/Met vs Val) p = 0.01 High vs Low Span: MOCA p = 0.02,
 Listening span p < 0.01, Forward spatial span p = 0.05, Backwards spatial span p = 0.01
 Male vs Female: Height p < 0.01, Backwards spatial span p = 0.05

1.4 Discussion

In the current study a dopaminergic drug manipulation (in this case the D2 receptor agonist bromocriptine) was used to explore the relationship between dopaminergic signaling and learning from reward (positive reinforcement) and error (negative reinforcement) in an associative category learning task. By separating out the two types of feedback into two separate runs we are able to explore more directly the distinct involvement of DA signaling in learning from positive and negative reinforcement, without having to contend with participants having the ability to make inferences about the likelihood of success or failure when neutral (uninformative) feedback was given. A Feedback Type X Drug interaction was found where after Bromo administration performance improved 3% relative to PLAC in the negative reinforcement task and dropped -3% in the positive reinforcement task. These results are in opposition to predictions made from the basic model of DA function where learning from positive reinforcement would be improved with increased DA signaling. In the current experiment after administration of 1.25mg of bromocriptine participants performed more poorly in the positive reinforcement task and improved in the negative reinforcement task. Many other studies have found conflicting data where DA modulation can be both beneficial (Kimberg et al., 1997; Mattay et al., 2000; Mehta et al., 2005) or detrimental to performance (Frank & O'Reilly, 2006; Richfield et al., 1989; Schoemaker et al., 1997). This has resulted in the suggestion of an inverted U-shaped curve for DA function, where with high DA levels there is an overdose effect which actually results in a decrease in functioning of the DA system (Kimberg et al., 1997).

Why might a DA agonist hurt performance when learning is dependent on positive reinforcement? One compelling suggestion comes from looking at the difference between presynaptic and postsynaptic D2 receptors. D2 receptors have two different isoforms, the short form, S, and the long form, L. The S isoform has a higher affinity for DA and has more presynaptic expression. The L isoform has a lower affinity for DA and has more postsynaptic expression (Usiello et al., 2000). The D2 receptors on the presynaptic side are autoreceptors, which regulate the phasic release of DA, while the receptors on the postsynaptic side regulate tonic DA levels (Grace et al., 1991). At a lower dosage it is possible that a DA agonist would bind mostly to presynaptic D2 receptors (autoreceptors), which would then result in a decrease in phasic DA release, leading to a detriment in signaling positive reinforcement. At higher concentrations DA agonists would begin to have postsynaptic effects and result in improved signaling of positive reinforcement. It has been suggested that the two different isoforms of the D2 receptor and the mixture of pre- and postsynaptic effects, may result in the inverted U-shaped performance that has been reported in participants (Frank & O'Reilly, 2006).

A low dose of bromocriptine (1.25 mg) was used in the current study to preserve blinding by reducing possible side effects. One possibility is that this low dose of bromocriptine led to more presynaptic binding, resulting in decreased phasic DA release (a net antagonistic-like effect). This framework would help to explain the results, as the lower phasic DA release would result in difficulties in learning from positive reinforcement. Interestingly, although unintentionally, the current study actually provides

more direct evidence for the bidirectional coding of reinforcement by DA signaling. There was an improvement in learning from negative reinforcement after drug administration, which only bidirectional coding of reinforcement signals by DA would predict. It must be noted however that this a post-hoc interpretation, and the directionality of DAs effect on reinforcement learning was counter to initial predictions. The initial expectation was that Bromo administration would improve performance only in the positive reinforcement task. To appropriately test the hypothesis that low dose DA agonists can result in presynaptic D2 receptor binding, it would be necessary to vary the dosage level of bromocriptine. To do so, the same participant would have to be given different dosages levels of the same DA agonist, and performance measured on a feedback-learning task (such as the one used here), to see if the impaired learning from positive reinforcement at low doses is reversed at higher doses. Until good dose responses studies are completed it is going to continue to be difficult to interpret results from studies involving the administration of DA drugs, due to the many possible mechanisms of action for DA drugs.

Previous studies have found that working memory span both affects learning from positive and negative reinforcement (Frank & O'Reilly, 2006) as well as predicting DA synthesis in the striatum (Cools et al., 2008). In the current study, however, no relation was found between working memory span and either performance under PLAC conditions nor on the effect that administration of a DA agonist had on performance.

Under PLAC conditions a main effect of COMT Val158Met allele status was present. Heterozygous Val/Met (intermediate COMT activity) were less accurate overall than either of the Val/Val or Met/Met homozygous participant groups. While a Feedback Type X COMT interaction was not present, the main effect of COMT appears to arise from superior performance of Val/Val homozygous participants in the negative reinforcement task. Negative reinforcement or a negative prediction error is coded in DA signaling by a reduction in phasic DA release. Two mechanism could lead to enhanced learning from negative reinforcement in Val/Val participants. The more active form of the COMT enzyme (Val/Val participants) would result in overall lower tonic levels of DA in the PFC (Egan et al., 2001) allowing more sensitivity to negative prediction errors. This mechanism would be inline with results in PD patients where they outperform controls in learning from negative reinforcement when abstaining from their regular DA medication (Frank et al., 2004). A second possibility is that the more active form of the COMT enzyme resulted in DA being cleared more quickly from the synaptic cleft, resulting in better temporal separation of DA reinforcement signals. This mechanism would be inline with hypotheses relating DA levels in the PFC and task switching and maintenance (Cohen et al., 2002; Cools & Robbins, 2004). The effect would be isolated to negative reinforcement as in the striatum, where positive reinforcement signals are most prevalent, DA is cleared much more quickly from the synaptic cleft by DA transporters (Garris & Wightman, 1994).

Consider next the effect of drug administration on the alleles for the two DA related SNPs that were of interest. In interpreting the gene-drug results, we will frame it under the assumption that bromocriptine at the low dosage used here resulted in a

reduction of phasic DA release (net antagonistic-like effects). However, regardless of the mechanism that is accepted there were differential effects of bromocriptine for the two DA related SNPs that were studied. Suggesting that individual differences in the effects of DA drugs can be predicted using genetics.

The results from analysis of the COMT Val¹⁵⁸Met SNP provide some interesting insights related to the dual account of DA signaling of reinforcement. A trend was present for a Feedback X COMT interaction where when performance is combined across the Bromo and PLAC sessions Val/Val participants' accuracy in the negative reinforcement task was significantly higher than that of Val/Met or Met/Met participants. No difference was present between the allele groups in the positive reinforcement task. As noted in the discussion of baseline effects of COMT this suggests an advantage in having the more efficient COMT enzyme (Val/Val participants) for learning from negative reinforcement in particular. The lack of a significant drop in performance in negative reinforcement learning in Val/Val participants after Bromo administration suggests that this may be resistant to increases in DA D2 signaling. This is perhaps due to low D2 receptor availability in the PFC, and that negative reinforcement processing in the PFC is likely reliant on D1 receptor processing. This runs counter to the dichotomy of direct (D1) and indirect (D2) processing of reward and punishment respectively in the striatum. In the striatum positive reinforcement is thought to be processed in the direct pathway, where D1 receptors are the primary post-synaptic receptor and D2 receptors are only on the presynaptic neuron (autoreceptors), while negative reinforcement is signaled via the indirect pathway where D2 receptors are the primary postsynaptic receptor. One caveat, however, is that the initially poor performance of Val/Met participants in learning from negative reinforcement was ameliorated somewhat after bromocriptine administration. This resulted in performance of Val/Met participants being closer to that of the homozygous Val/Val and Met/Met participants, although this increase was not statistically significant. The result implies that the PFC may play an integral role in learning from negative reinforcement, which contradicts some of the assumptions of the bidirectional hypothesis of DA signaling. Although, as noted above there has been evidence that the VMPFC may be critical in learning from negative reinforcement (Wheeler & Fellows, 2008), but the involvement of DA signaling had not been explored.

Analysis of the DRD2/ANKK1-Taqla SNP found a Drug X DRD2 interaction where participants with lower D2 receptor (A1+) were generally impaired in their performance after Bromo administration, while those with higher D2 receptor density (A1-) had generally improved performance. This effect appears to be driven by a trend towards A1+ participants to have a drop in performance in the positive reinforcement task after Bromo administration, with little change in the negative reinforcement task. While A1- participants, on the other hand, show a trend for the opposite effect, a boost to performance in learning from negative reinforcement, and little change in learning from positive reinforcement. The pattern of results suggests that high D2 receptor density (A1- participants) may protect against a drop in performance in learning from positive reinforcement when phasic dopamine release is reduced, and a benefit to performance in learning from negative reinforcement. This would fit into the direct vs indirect pathway mechanism for processing of reinforcement in the striatum. It is

possible that in A1- participants bromocriptine may be binding to a smaller percentage of D2 autoreceptors in the direct pathway, causing a smaller drop in phasic DA release, and less impairment in positive reinforcement learning. While in the indirect pathway the higher D2 receptor density in A1- participants allows for more sensitivity to the dips in DA associated with negative reinforcement, despite the drop in phasic DA release being less than that of A1+ participants.

Gender was found to be an important mediator of the Feedback Type X Drug interaction, where in general women were less affected by drug administration. Given the long and conflicting history of the exploration of gender differences in behavior, caution must be taken in overly interpreting these results, however, it does suggest an interesting avenue for future research. One possible mechanism for these differences could be differences in estradiol levels between males and females. Endogenous fluctuations of estradiol levels in women has been shown to interact with working memory related PFC activity (Jacobs & D'Esposito, 2011). It has been suggested that estradiol levels are higher in the PFC than any other areas of the brain (Bixo et al., 1995), and as noted above, previous studies have suggested that areas of the PFC may be integral in learning from and responding to negative reinforcement (Wheeler & Fellows, 2008). As the baseline gender difference was found mainly in learning from negative reinforcement, as well as interacting with DA drug administration, this would fit into a general framework of DA and estradiol levels modulating PFC function. The increased variability that would be caused by endogenous estradiol fluctuations in female participants would help to explain why the drug effect were much smaller in females than males.

Conclusions

We have shown here that a low dose of bromocriptine may result in reduced phasic DA release, resulting in impaired performance in learning from positive reinforcement with a concomitant benefit to learning from negative reinforcement in an associate learning paradigm. This effect is modulated by gender where males had a much larger effect of drug administration. Further, under baseline conditions (PLAC), the COMT Val158Met SNP predicts performance where participants with the more active form of the COMT enzyme (Val/Val) out perform the other two allele groups (Val/Met and Met/Met). After drug administration the superior performance of those with the more active form of the COMT enzyme seems to be particularly driven by performance in learning from negative reinforcement, an effect which is further modulated by gender. Finally, the main effect of drug administration is best predicted by the DRD2/ANKK1-TaqIa SNP, where high D2 receptor density (A1-) protects against a drop in performance, and may result in improved performance in learning from negative reinforcement. In summary, a systematic relation has been shown between striatal and frontal dopaminergic signaling, where the effect of DA D2 receptor modulation is driven mainly by the striatum, while a participants' ability to respond to negative reinforcement is reliant on DA signaling in the PFC, the effects of which are all further modulated by gender.

Chapter 2 Component analysis of the effect of a dopamine agonist on working memory: Load and filtering

2.1 Introduction

Working memory (WM) is the ability to maintain task relevant information while ignoring task irrelevant information, such as remembering all the items on a shopping list while walking through a grocery store. WM memory can be broken down in to three distinct subcomponents. First the information to be held in working memory must be selected, such as which items you need at the grocery store. Second, the information must be maintained in working memory while resisting distractions. Lastly, the information can be manipulated, for example, reordering your grocery list based on where things are located in the store. While intuitively it might seem that performance on WM tasks would be best predicted by the number of items an individual is able to maintain in working memory, performance appears to be more reliant on the ability to select the relevant information to hold in working memory while ignoring irrelevant information (Vogel et al., 2005).

Early work in monkeys suggested a critical role for areas of the prefrontal cortex (PFC) in spatial working memory tasks, or what were referred to as delayed response (DR) tasks (Goldman & Rosvold, 1970). In a typical DR task the monkey is shown food being put into a small well in front of their cage, and then the monkey's vision is obscured for a short delay. At the end of the delay period, the monkey must choose the well that contains the food in order to receive the reward (the food in this case). When specific areas of the PFC were lesioned monkeys were shown to have a deficit in correctly identifying the baited well even over a short delay, with the deficit increasing with the length of the delay (Goldman et al., 1970; Goldman et al., 1971; Jacobsen & Nissen, 1937). The monkeys were, however, able to perform the task when slight alterations were made to the procedure, to reduce the working memory demands (Kubota & Niki, 1971), such as minimizing distraction (Malmo, 1942) or making the task a go no-go response (Mishkin & Pribram, 1956). In a set of key experiments reversible deficits in a DR task were shown when areas of the PFC were subjected to temporary cooling (Bauer & Fuster, 1976; Fuster & Alexander, 1970). An important advantage of this technique is that the cooling can be turned on and off, allowing for a monkey to repeatedly perform as it's own control (Fuster & Alexander, 1970). Further work has extended the deficits in WM seen in monkeys to humans with damage to the PFC (Owen et al., 1990).

Subsequent research focused on the question of how to characterize the PFC contribution in DR tasks. One hypothesis is that PFC encodes the information into short term memory, and this information must be retrieved at the time of the response. Alternatively, PFC might provide a mechanism for constantly maintaining stimulus

information over the delay period. Electrophysiological recordings in primates have suggested that the PFC is maintaining a representation of the stimulus through sustained neural firing during the delay period (Kubota & Niki, 1971; Miller et al., 1996). Neurons in the PFC respond to various other task related events, including the beginning of the delay period (Kojima & Goldman-Rakic, 1982) and the initiation of a motor response (Fuster, 1973). Activity in the PFC has been shown to predict individual differences in WM performance (Rypma & D'Esposito, 1999), however, the PFC is also clearly involved in other aspects of task performance such as the encoding of abstract rules (Wallis et al., 2001), and directing attention to task related representations (Curtis & D'Esposito, 2003). Thus, it is not totally clear if the relation between PFC activity and individual differences is due to a direct role of the PFC in performance of WM tasks, or whether it is due to individual differences in non-WM related aspects of the tasks.

The involvement of the PFC in WM tasks is, at least to some extent, reliant on DA modulation (Brozoski et al., 1979). When DA (D1) antagonists are injected locally in the PFC of monkeys performing an oculomotor DR task there is a deficit to performance, while a control task without a delayed response is unaffected (Sawaguchi & Goldman-Rakic, 1991). Further evidence for the functional necessity of DA modulation comes from the deficit in performance in the DR task increasing with both length of delay, and dosage of the DA (D1) antagonist (Sawaguchi & Goldman-Rakic, 1994).

The reliance on DA signaling in the PFC for WM appears to be D1 specific as injection of D2 antagonists and 5HT-2 (serotonin) antagonists into the same PFC sites failed to produce measurable deficits in performance (Sawaguchi & Goldman-Rakic, 1994). Moreover, working memory impairments are observed when DA levels in the PFC are elevated above normal levels, either through stress (Arnsten & Goldman-Rakic, 1998) or pharmacological manipulation (Murphy et al., 1996; Zahrt et al., 1997). This has led to the hypothesis that the relationship between PFC DA and performance reflects an inverted-U-shaped function where too much or too little DA results in impaired performance. One possible mechanism for this involvement is cyclic AMP intracellular signaling effecting tuning of DA neurons (Vijayraghavan et al., 2007). It is likely that there is not a single optimal level of DA across all tasks, but that different baseline levels of DA in the PFC may be optimal for different tasks (Cools & Robbins, 2004; Floresco, 2013). For example, performance on a task that requires frequent switching between rules or goals benefits from lower DA levels in the PFC (Roberts et al., 1994), while performance on a task that requires sustained maintenance and resistance to distraction, benefits from higher DA levels (Cools & D'Esposito, 2011; Robbins, 2005).

One possibility that follows from the idea of task-specific inverted-U-shaped functions is that between-subjects differences in performance may be constrained by differences in baseline DA levels. In one experiment exploring this idea rats performed an attention task and were median split into two groups based on performance. A DA D1 receptor agonist was then given and rats performed the attention task again. The rats with relatively inferior performance at baseline improved their accuracy after drug

administration, while there was a reduction in accuracy for the rats with relatively superior performance at baseline (Granon et al., 2000). In human participants similar contrasting effects of DA drug administration have been found. Individuals with lower working memory span (assumed to result from lower DA levels) show improved performance and neural function after administration of a DA agonist. In contrast a detriment to performance is found after drug administration in participants with relatively higher working memory spans (Gibbs & D'Esposito, 2005; Kimberg et al., 1997; Mattay et al., 2000).

The differing effects on performance after DA administration have been suggested to be mediated by differences in COMT enzyme activity in the PFC. In the frontal lobe COMT enzyme activity helps to break down DA in the synaptic cleft. Thus with higher enzyme activity less DA remains in the PFC, and with lower enzyme activity more DA remains in the PFC (Mattay et al., 2003). In some humans the COMT gene is found to have undergone a valine to methionine (Met allele) substitution resulting in a less stable form of the COMT enzyme. This less stable form of COMT (Met allele) has lower enzyme activity resulting in higher DA levels in the frontal lobe compared to the ancestral (Val allele) gene (Egan et al., 2001). These genetic differences in COMT enzymatic activity have also been extended to predict baseline differences in performance on WM tasks (Meyer-Lindenberg et al., 2006). The individual differences in working memory capacity (Cools et al., 2008) and the effect of DA drug administration (Cools et al., 2009) have been suggested to extend to the striatum.

As with the reinforcement learning experiment presented in Chapter 1, the present study was designed for a preplanned genetic comparison between the two dopamine related Single Nucleotide Polymorphisms (SNPs), DRD2/ANKK1-Taq-IA (RS1800497) and COMT Val158Met (RS4680). The TaqIA SNP has been found to be associated with D2 receptor density (Pohjalainen et al., 1998), while the COMT SNP is related to the breakdown of DA in the frontal lobe (Egan et al., 2001). A compelling possibility is that it may be the interaction between striatal and PFC DA related genes that predict differing aspects of WM performance. For example in one study it was found that performance on an array of WM tasks was not predicted by COMT Val158Met allele status alone, but it was only when DRD2/ANKK1-TaqIA (striatum) allele status was taken into account that the COMT Val158Met SNP predicted relatively superior performance in the active manipulation of information held in working memory (Stelzel et al., 2009).

Performance on working memory tasks is influenced not just by the amount of information to be maintained (load), but also the ability to ignore task irrelevant information (filter). Indeed, the latter may be the most relevant for predicting individual differences in working memory performance. Participants who perform better on WM tasks do not have an ability to hold more items in WM, but are better in filtering out task irrelevant information (Vogel et al., 2005). Computational modeling work has suggested that DA signals in the basal ganglia may be important in filtering task-relevant from task-irrelevant information, allowing only task-relevant information to enter working memory (Frank et al., 2001; O'Reilly & Frank, 2006). Under this computational framework the

direct pathway of the BG is responsible for allowing items to enter WM, while the indirect pathway inhibits the direct pathway, filtering out irrelevant items. The direct pathway primarily contains postsynaptic D1 receptors, while the indirect pathway primarily contains postsynaptic D2 receptors. This creates a dichotomy where D1 activity is primarily related to load component processes of WM, while D2 activity is mainly involved in filtering processes. These theories fit into a larger body of work that extends early theories of the role of BG in the selection and inhibition of motor commands (Albin et al., 1989; DeLong, 1990) to the control of non-motor aspects of behavior (Doya, 1999; Frank et al., 2007b; Hazy et al., 2007).

Evidence from both lesion studies (Baier et al., 2010) and neuroimaging studies (McNab & Klingberg, 2008) supports the importance of a network between the BG and PFC in working memory performance. McNab and colleagues used functional magnetic resonance imaging to examine the effects of load and filtering in a working memory task. An increased blood oxygen level dependent (BOLD) signal associated with load was found in the right posterior parietal lobule (McNab & Klingberg, 2008). On trials when the displays included information that had to be ignored (filter), an increase in BOLD response was found bilaterally in the middle frontal gyrus (MFG), however, on trials where participants managed to ignore the irrelevant information there was an increased BOLD response in the basal ganglia (BG) (McNab & Klingberg, 2008). This suggests a mechanism where the MFG is sending a signal that filtering is necessary to the BG, and subsequently the BG is acting to suppress the irrelevant information.

The goal of the current project was to use WM the task from (McNab & Klingberg, 2008) to explore the influence of load and filtering component processes on individual differences in WM performance. Specifically the focus was on how individual differences in DA related genes may contribute to individual differences in filtering and load WM subcomponents. As previously mentioned, both the MFG and BG are strongly modulated by the DA system. As COMT activity is more associated with frontal DA levels (MFG) and DRD2 is related to striatal DA levels (BG), COMT Val158Met may then be predictive of performance in WM load conditions, while DRD2/ANKK1-Taqla may be more related to filtering out irrelevant information from entering WM.

Hypothesis 1: The administration of Bromocriptine, a D2 agonist, will primarily affect WM performance when task-irrelevant information must be ignored (filter). Given that the results of the experiment in Chapter 1 are best explained by a mainly presynaptic DA effect of Bromocriptine administration, reduced phasic DA release due to presynaptic drug effects is also expected in the current study. Reduced phasic DA release would result in impaired accuracy when WM load is high, based on the assumption that the direct pathway of the basal ganglia will be affected by the pharmacological manipulation. However, a concomitant disinhibition of the indirect pathway would also be expected after Bromocriptine administration, and this should result in enhanced accuracy when task irrelevant stimuli need to be filtered from entering WM. To the extent that postsynaptic effects are found, this would result in increased tonic D2 signaling in the indirect pathway. As the direct pathway is normally inhibited by the indirect pathway, a Bromo-induced increase in D2 activity should disrupt

the indirect pathway and disinhibit the direct pathway, resulting in relatively faster RTs under high WM load. Under filter conditions, where task irrelevant stimuli need to be stopped from entering WM, the inefficient indirect pathway will also result in faster RTs, but impaired performance due to ineffective gating in to WM (Table 3).

Drug Action	Biological Effect	Load	Filter
Presynaptic	Reduced phasic DA release (both D1 and D2)	Impaired ACC (reduced direct pathway)	Enhanced ACC (improved indirect pathway)
Postsynaptic	Reduced phasic D1 release Increased tonic D2	Impaired ACC and faster RT (disinhibited direct pathway)	Impaired filter and faster RT (impaired indirect)

Table 3: Main drug effect predictions

Hypothesis 2: When the data are examined by dividing participants based on the two DA related SNPs, allele status for DRD2/ANKK1-Taqla will best predict the effect of drug administration. Under baseline conditions (PLAC) A1- participants (higher D2 receptor density) will have better performance in filtering of irrelevant items from WM when compared to A1+ participants (lower D2 receptor density). Presynaptic effects of the drug will have the largest effect on A1+ participants due to lower receptor density resulting in relatively more impairment in load component processes, and more enhancement to filtering of irrelevant stimuli when compared to A1+ participants. To the extent that postsynaptic effects of drug administration are present they would be more likely to be present in A1+ participants due to their lower D2 receptor density, and hence less presynaptic receptors for the drug to bind to. Participants with the Met allele COMT Val158Met SNP have lower COMT enzyme activity which is thought to lead to higher DA levels in the PFC. Based on this relationship Met participants will show a main effect of better performance under conditions where load component processes are important, with Val participants' performance the lowest and Val/Met with intermediate performance. Drug administration will not affect the relationship of COMT Val158Met allele status on performance under load conditions (Table 4).

SNP	Baseline	Presynaptic	Postsynaptic
<u>COMT (RS4680)</u>	(Met) better Load performance	No change	No change
<u>DRD2 (RS1800497)</u>	(A1-) better Filter performance	(A1+) drop in Load performance increased Filter performance	(A1+) drop in RT for Load and Filter

Table 4: Gene predictions

2.2 Material and methods

Participants

The same group of 23 participants from the experiment in chapter 1 were tested on the WM task. Participants completed both the associative learning task and WM task in each test session. The WM task was always completed between the versions of the associative learning tasks (positive or negative reinforcement). This provided a break and also served to reduce interference between the two associative learning tasks. Performance of the WM task began roughly two hours after initial administration of bromocriptine (Bromo) or the placebo (PLAC), depending on the session. More detailed descriptions of participant demographics, genetic analysis, test day procedures and neuropsychological measures can be found in the methods section of Chapter 1.

Experimental Task

Participants performed a spatial WM task similar to the design of (McNab & Klingberg, 2008). On each trial, participants viewed a display composed of 14 squares arranged along a virtual circle about a fixation point. Three different conditions were verbally cued at the start of each trial. In the "Low Load" condition, participants were instructed to remember the location of 4 red circles. In the "High Load" condition, the memory load was increased to six items, 4 red and 2 yellow circles. In the "Filter" condition, 4 red and 2 yellow circles were presented but participants were instructed to only remember the locations marked with red squares (**Figure 11**). The red circles were balanced across visual hemifields such that two were presented on each side. Additionally the red circles were arranged so that two of the circles, but only two, had to have only one blank square between them. When the yellow circles were present (High Load and Filter trials), one circle was presented in each hemifield. One of the two yellow circles was adjacent to a red circle, while the other was not adjacent to a red circle. The placement constraints were included to reduce the effectiveness of heuristics in memorizing the colored circle locations. For example, knowing that a red circle was presented next to a probe location, does not inform about whether a yellow circle was present at the probe location.

A trial started with the presentation of a blue fixation cross at the center of the screen for 700ms. One of two task instruction cues then appeared. The cue "ALL" signified that all the locations with colored circles should be remembered. The cue "RED ONLY" signified that only the locations with red circles needed to be remembered. Critically, the "RED ONLY" cue could indicate a Low Load or Filter trial. A white fixation cross was then presented for 700ms to ensure fixation before presentation of the memory array. The fixation cross remained on the screen through the memory stimuli and the delay period.

The memory set was presented for 200 ms, followed by a 2 s delay period where the stimulus had to be maintained. After the delay period, a probe was presented, either within one of the to-be-remembered locations, or at a non-target location (empty or

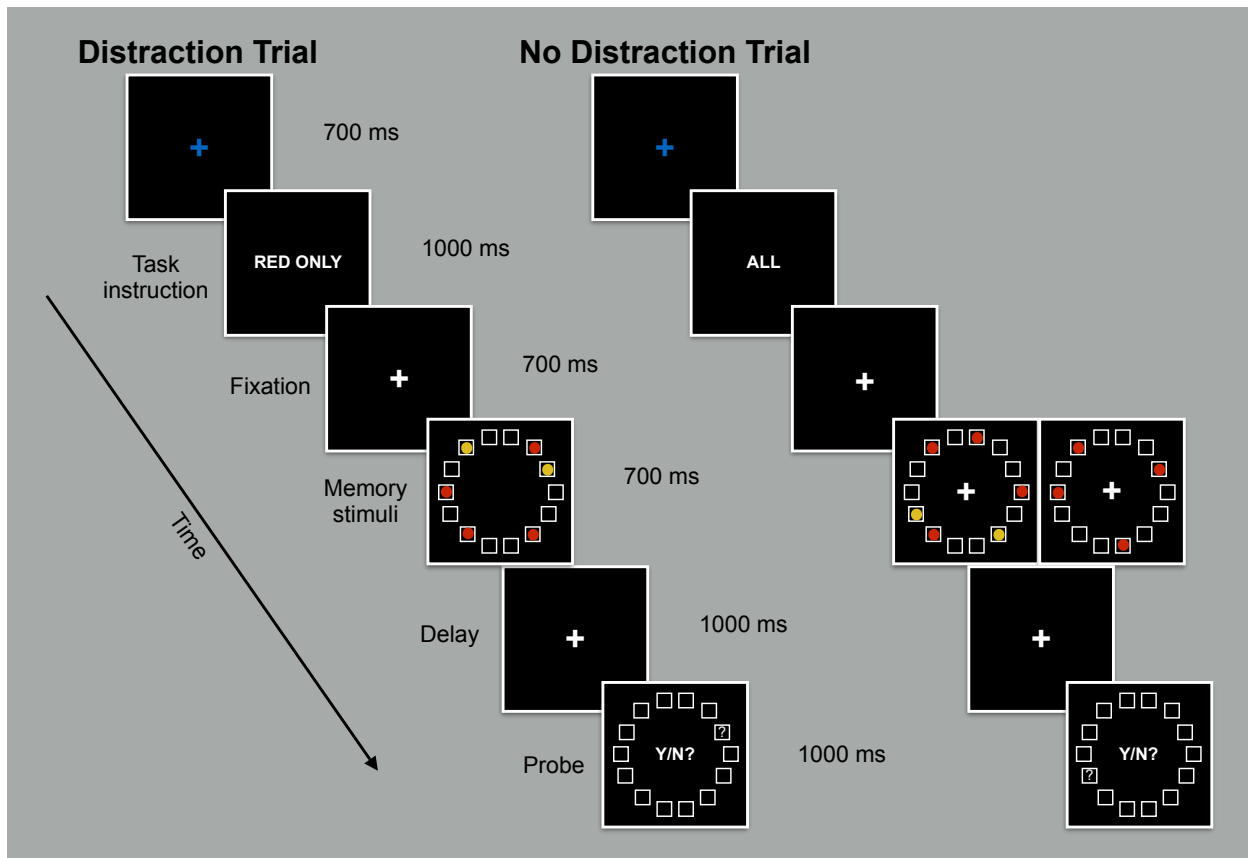


Figure 11: Working memory task design

Both trial types had the same timing structure. For the Filter trial example shown the participant would respond No as the probe appears at a location that should have been ignored. In the High Load trial (left side of the no distraction examples) the participant would respond Yes as the probe appears at a location to remember. In the Low Load trial (far right) the participant would respond No as the probe appears at an empty location. Note that the verbal instruction “Red Only” would also appear with Low Load trials so that the instruction did not cue the trial type.

ignored location). When the probe appeared in an empty location it was always an empty location that was located next to where a colored circle had been presented. This was to ensure that participants had to remember the exact spatial locations of the colored circles. Participants indicated whether the probed location was one of the target locations, pressing one of two buttons on a custom button box. Following the response, a blank screen was presented for 1000ms before proceeding to the next trial.

Within each session, the participants completed 240 trials. Half of the trials were the Filter condition and the other half were divided evenly into High and Low Load conditions. This resulted in the completion of a total of 120 trials in the Filter condition, and 60 trials each for Low Load and High Load conditions. More Filter trials were included as there was a specific interest in this subcomponent. Three breaks were provided across the 240 trials to reduce fatigue, during which participants were provided with accuracy feedback for the preceding block of 60 trials. At the completion of the experiment participants received accuracy feedback for the final 60 trials.

Participants completed the WM task on two separate sessions, one in which a lactate placebo was administered (PLAC) and a second where a low dose (1.25mg) of the DA D2 receptor agonist bromocriptine (Bromo) was administered. The order of the sessions was counterbalanced across individuals, and the drug was administered in a double blind fashion, where neither the experimenter nor the participant knew in which session they received the drug. This design led to two fully crossed factors, Drug (Bromocriptine and Placebo) and WM load (Low, High and Filter).

Behavioral data analysis

Average accuracy (ACC) and average median (within-subject) reaction time (RT) on correct trials was compared across conditions, with within-subject comparisons used for all drug effect analyses. For calculating drug effects, the accuracy under PLAC administration was subtracted from Bromo administration (Bromo ACC - PLAC ACC) for each individual participant. For RT, the drug effects were calculated the other way, subtracting the RT under Bromo administration from PLAC administration (PLAC RT - Bromo RT). This was done so that for both measures, a positive number indicates a benefit in performance after Bromo administration (and vice versa for negative numbers).

A cost measure was established for analysis of load and filtering performance. For accuracy, Load Cost was calculated by subtracting ACC on High Load trials from the ACC on Low Load trials (Low Load ACC - High Load ACC). For Filter Cost a similar calculation was made, where ACC on Filter trials was subtracted from ACC on Filter Trials (Low Load ACC - Filter ACC). For RT cost calculations were performed in the opposite direction. Thus larger Load Costs on both measures indicate performance decrements as WM load increased.

In calculating the effect of drug administration on these cost measures, the cost in the Bromo session was subtracted from the cost in the PLAC session (PLAC - Bromo). As a result for both measures, a negative number means that the cost increased following drug administration and a positive number means a reduction in the cost. In this manner, the drug effect calculations are consistent across all measures with positive numbers indicative of better performance after drug administration.

Statistical analyses were carried out with a repeated measures MANOVA with ACC and RT for the three trial types using the general linear model framework of SPSS (IBM, 2011). Within-subject factors were drug (Bromo and PLAC) and trial type (Low load, Filter and High load), while gender, drug session (1st or 2nd) and, where appropriate, SNP status (COMT and DRD2) were used as between-subject factors. A separate MANOVA was done for main drug effects, and for each SNP.

As noted in the results section of Chapter 1 a significant reduction of arousal was found due to drug administration. To examine the effects of the drug (and genes) above and beyond an overall change in arousal, we used the RT data from the associative learning task as a covariate. For this measure, we took the change in median RT due to

drug administration (PLAC RT - Bromo RT) using the data from the “known” trials, averaged across the positive and negative feedback conditions. Where appropriate, sphericity was accounted for by using the Greenhouse-Geisser correction. All reported values are for two-tailed ttests.

2.3 Results

Working Memory Filtering Task

The WM task was demanding, and both the Load and Filter manipulations proved to be effective. In the placebo (PLAC) condition participants were correct on 84% ($\pm 1.5\%$, SEM) on low load trials. Performance dropped to 67% ($\pm 1.8\%$; $t_{22} = 11.2$, $p < 0.001$) when the load was increased to six items and to 78% ($\pm 1.7\%$, SEM; $t_{22} = 4.8$, $p < 0.001$) when two irrelevant items were added to the display in the filter condition (**Figure 12**).

Additionally participants were significantly more accurate ($t_{22} = 8.2$, $p < 0.001$) on filter trials than high load trials. When the cost measures are examined the Filter Cost of 6% ($\pm 1.2\%$) was smaller than the Load Cost of 17% ($\pm 1.5\%$, SEM; $t_{22} = 8.3$, $p < 0.001$), suggesting participants were successfully able to filter out irrelevant items (**Figure 12 inset**).

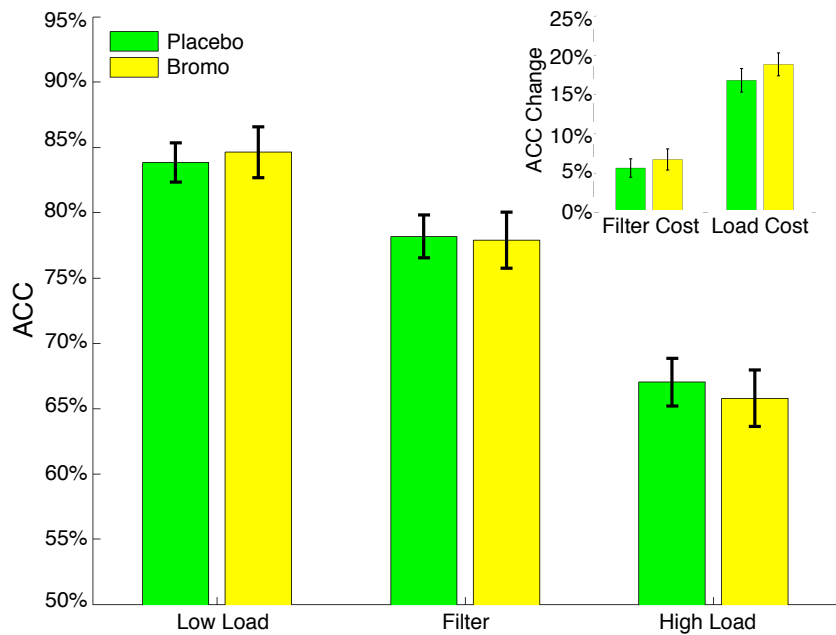


Figure 12: Average ACC Bromo and PLAC (error bars represent SEM)

When RT is considered the same general pattern of results is present although the differences between the trial types is smaller. In the placebo (PLAC) condition there was a trend towards the mean RT of 741ms (± 22 ms) on low load trials to be faster than the mean RT for high load trials of 764ms (± 21 ms, SEM; $t_{22} = 2.0$, $p = 0.054$) (**Figure 13**). Response times also slowed when the two irrelevant items were added to the display in the filter condition with the mean RT of 750ms (± 21 ms, SEM) between that of low load and high load, however, there was no statistically significant differences present. When examining the cost measures there was a marginal trend for the mean Filter Cost of 11ms (± 8 ms) to be smaller than the mean Load Cost of 26ms (± 13 ms, SEM; $t_{22} = 1.7$, $p = 0.104$) (**Figure 13 inset**).

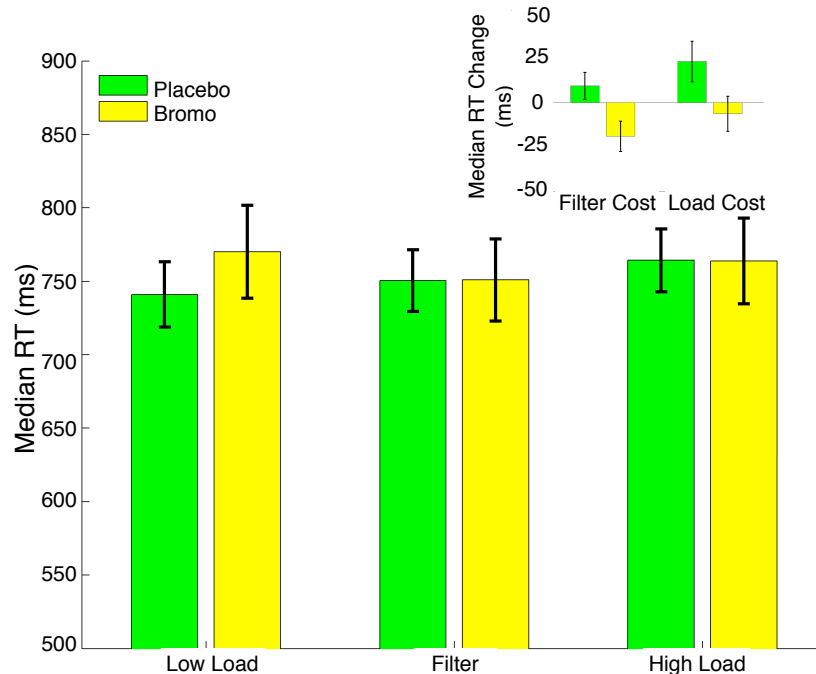


Figure 13: Median RT Bromo and PLAC (error bars represent SEM)

Effects of Bromocriptine

Next we turn to the analysis of the effect of Bromocriptine, looking at whether administration of this dopamine agonist has a differential effect on the three WM trial types. In the MANOVA multivariate analyses revealed a main effect of Trial Type ($F_{4,18} = 59.77, p < 0.001$). From examination of the univariate results the multivariate effect stems from a significant main effect

of Trial Type in ACC ($F_2 = 168.55, p < 0.001$). The basic pattern observed in the Placebo condition was also observed after administration of Bromo. Post hoc tests (Bonferroni corrected) revealed, that when averaging across Bromo and PLAC conditions, accuracy on Low Load trials ($84 \pm 1.6\%$) was significantly ($p < 0.001$) higher than that of both Filter ($78 \pm 1.8\%$) and High Load ($66 \pm 1.9\%$, SEM) trial types. Additionally, accuracy was significantly ($p < 0.001$) higher on Filter than High Load trials. The main effect of Trial Type remains reliable ($F_{4,13} = 45.45, p < 0.001$) when the overall change in RT after drug administration from the associative learning task in chapter 1 is used in an additional MANCOVA to control for changes to arousal due to drug administration.

In the MANOVA a trend ($F_{4,16} = 2.38, p = 0.090$) was present for a Trial Type X Drug interaction. The Trial Type X Drug interaction is significant ($F_{4,17} = 4.64, p = 0.01$) when a MANCOVA is performed using PosNegRTchange as a covariate. When examining the univariate results the interaction in the multivariate analysis does not arise from ACC as there was no significant effect of drug administration for Low Load, Filter, nor High Load trial types (**Figure 12**). Additionally for ACC there was no significant effect of drug administration on Load Cost nor Filter Cost.

From inspection, the interaction present in the multivariate analysis comes from a significant TrialType X Drug interaction ($F_2 = 4.45, p = 0.02$) for RT. As in the analysis without the covariate in examining the univariate analyses the interaction is present in the RT measure where the Trial Type X Drug interaction ($F_{1,543} = 5.55, p = 0.01$) and Trial Type X Drug X PosNegRTchange interaction ($F_{1,543} = 6.44, p = 0.01$) are both significant. Notably in the Bromo session participants actually responded faster on High Load and Filter trials than on Low Load trials. This was due to a slowing of RT in Low

Load trials after drug administration (PLAC RT - Bromo RT) of -29ms ($\pm 24\text{ms}$). The slowing of RT after drug administration on Low Load trials was significantly more than that on Filter trials ($0 \pm 21\text{ms}$; $t_{22} = 2.3$, $p = 0.04$) or on High Load trials ($1 \pm 23\text{ms}$, SEM; $t_{22} = 3.0$, $p = 0.001$). For RT there was a significant effect of drug administration for a reduction in both Load Cost ($t_{22} = 2.3$, $p = 0.04$) and Filter Cost ($t_{22} = 3.0$, $p = 0.001$) (**Figure 13**). Notably these changes in RT cost are present as RT slowed down for Low Load trials, without a measurable change for High Load or Filter trials. There was not a significant difference between the effect of drug administration on Load and Filter Cost.

Working memory span-drug interaction

Working memory span has been shown to predict whether a DA agonist will benefit or impair a participants performance, with participants with low working memory spans generally showing an improvement in performance. When participants are median split based on working memory span multivariate analyses show no main effect of working memory span in the current study. Additionally no Trial Type X Span Group interaction was present nor a Trial Type X Drug X Span Group interaction. When accuracy and RT measures are analyzed separately there is a significant difference in the effect of drug administration on ACC for Low

Load trials ($t_{21} = 2.63$, $p = 0.02$), where participants in the High Span group had a larger improvement in ACC after Bromo administration than did participants in the Low Span group (**Figure 14** top). When the cost measures are examined a difference was present in the effect of drug administration on Filter Cost where there is a significant detriment to

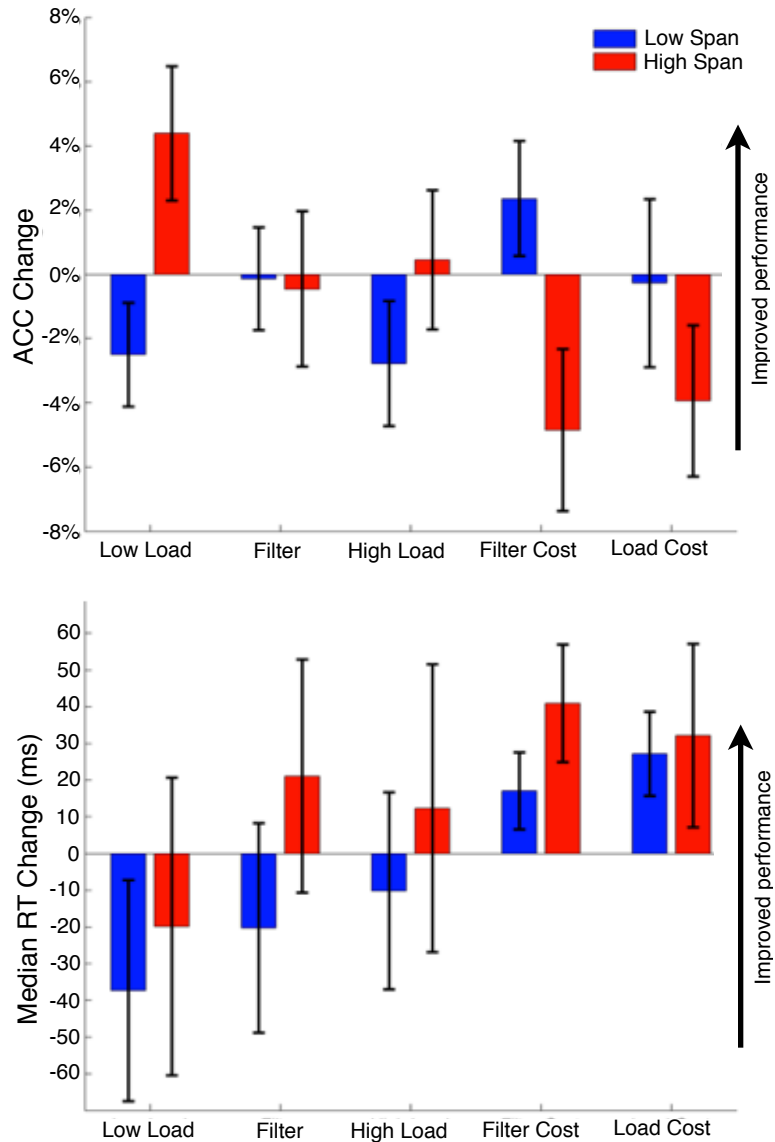


Figure 14: WM Span Drug effect ACC and RT (error bars represent SEM)

performance in the High Span group (mean, $-5 \pm 2.5\%$) compared to the Low Span group (mean, $2 \pm 1.8\%$, SEM; $t_{21} = 2.4$, $p = 0.03$).

Next we turn our focus to RT. There was no significant effect of drug administration for any of the three trial types between Low Span and High Span participants (**Figure 14** bottom). When only the PLAC session is considered there was a small effect in the Load and Filter Cost measures, with marginal trends toward smaller costs for the Low Span group. The mean Load Cost in the PLAC session for Low Span participants ($2 \pm 9\text{ms}$) was marginally significantly smaller than for High Span participants (mean, $46 \pm 20\text{ms}$, SEM; $t_{21} = 2.0$, $p = 0.055$). The mean Filter Cost for Low Span participants ($-4 \pm 10\text{ms}$) had a trend to be smaller than for High Span participants ($24 \pm 11\text{ms}$, SEM; $t_{21} = 1.9$, $p = 0.07$). Notably the small Load Cost and small negative Filter Cost values for Low Span participants results from their RTs being very similar across all three trials types, despite Low Span participants having non-significantly faster RTs overall. There was no difference between Low Span and High Span subjects in the change to Filter and Load Costs due to Bromo administration

Administration of Bromo resulted in High Span participants having improved accuracy on Low Load trials compared to Low Span participants who actually show a detriment in performance. High Span participants also showed an increased Filter Cost after drug administration, however, this was due to the improvement on Low Load trials, rather than a detriment to Filter trial performance.

Gene-drug interactions

To examine how the drug effect varied as a function of different genetic phenotypes, the analysis focused on the cost measures where positive numbers indicate that the administration of Bromo led to improved performance relative to placebo.

COMT-drug interactions

For the COMT Val158Met SNP (RS4680) no significant interactions were found for either the MANOVA nor the MANCOVA. When ACC is examined in the PLAC session, there was no significant differences in performance between the Met, Val/Met and Val participant groups in any of the three trial types. When Bromo and PLAC session are compared drug administration resulted in Met participants having a mean drop in performance ($-4 \pm 2.0\%$) compared to both Val/Met ($6 \pm 3.1\%$; $t_{12} = 2.72$, $p = 0.02$) and Val ($3 \pm 1.5\%$, SEM; $t_{14} = 2.41$, $p = 0.03$) participants.

For the cost measures in the PLAC session a trend was present for Met ($21 \pm 2.7\%$) participants to have a larger mean Load Cost than Val ($14 \pm 2.4\%$, SEM) participants (**Figure 15** top). Val/Met (mean, $15 \pm 2.3\%$, SEM) participants had a Load Cost that fell between Met and Val participants, although it was not significantly different from either group. In the PLAC session there was no significant differences in Filter

Cost between any of the Met (mean, $7 \pm 2.5\%$), Val/Met (mean, $5 \pm 2.5\%$), or Val (mean, $5 \pm 1.5\%$, SEM) participants. After Bromo administration the Val participants had a marginally significant increase in Load Cost ($-5 \pm 2.1\%$, SEM; $t_7 = 2.34$, $p = 0.052$) (**Figure 16 top**). There was no significant change in mean Load Cost due to drug administration for either Met ($3 \pm 2.8\%$) or Val/Met ($-6 \pm 4.1\%$, SEM) participants. The change in Load Cost due to Bromo administration, did however, result in a significant ($t_{14} = 2.15$, $p = 0.05$) improvement for Met participants relative to Val participants, and a trend ($t_{12} = 1.85$, $p = 0.09$) for an improvement compared to Val/Met participants (**Figure 16 top**). There was no significant change due to Bromo administration in mean Filter Cost for ACC in Met ($2 \pm 2.4\%$), Val/Met ($-5 \pm 4.5\%$) nor Val ($-3 \pm 1.9\%$, SEM) participants.

When RT data is examined there were no significant differences between the COMT allele groups for any of the trial types, nor were there any changes due to Bromo

administration. For the RT Cost measures in the PLAC session Met participants (mean, $-12 \pm 12\text{ms}$, SEM) had a significantly smaller Filter Cost than Val participants (mean, $29 \pm 14\text{ms}$, SEM; $t_{14} = 2.23$, $p = 0.04$) (**Figure 15 bottom**), with Met participants responding more quickly to Filter trials than Low Load trials. Val/Met (mean, $8 \pm 10\text{ms}$, SEM) participants had a Filter Cost that fell between that of Met and Val participants, but was not significantly different from either group. In the PLAC session there was no significant difference in Load Cost between Met, Val/Met and Val participants.

Administration of Bromo resulted in a significantly reduced mean Filter Cost for Val/Met ($18 \pm 11\text{ms}$, SEM; $t_5 = 2.92$, $p = 0.04$) participants (**Figure 16 bottom**). There was not significant change in Filter Cost for either Met (mean, $21 \pm 15\text{ms}$) nor Val ($45 \pm$

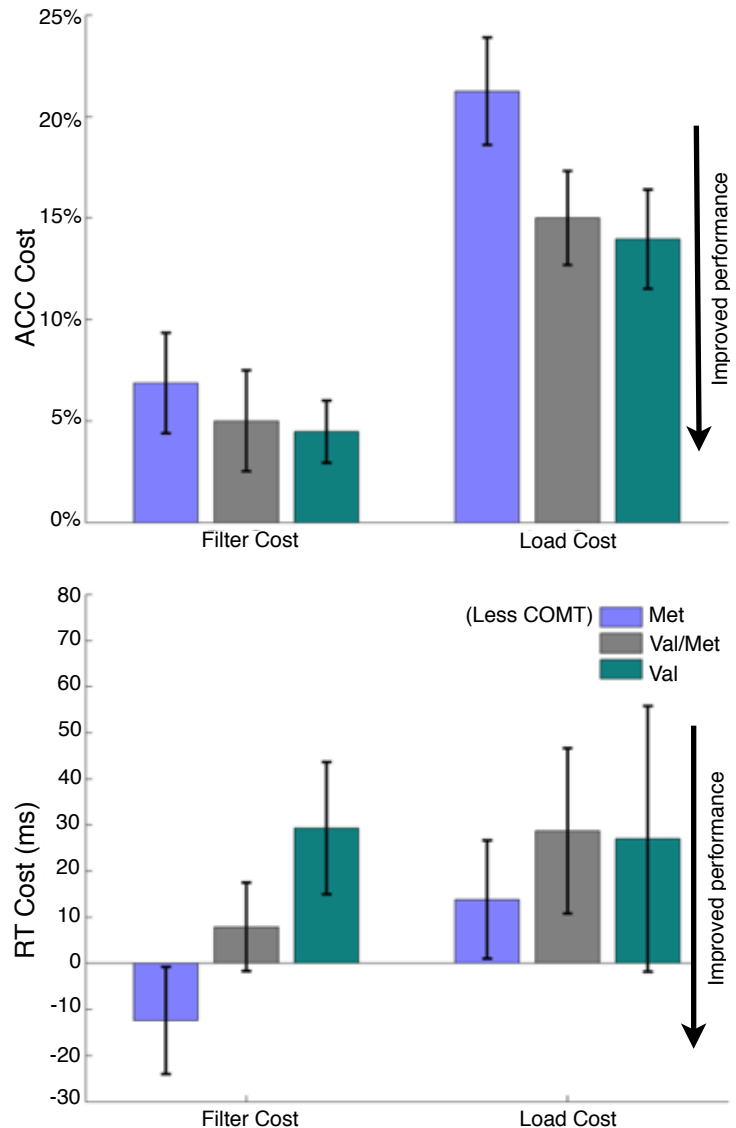


Figure 15: COMT placebo ACC (top) and RT (bottom) costs (error bars represent SEM)

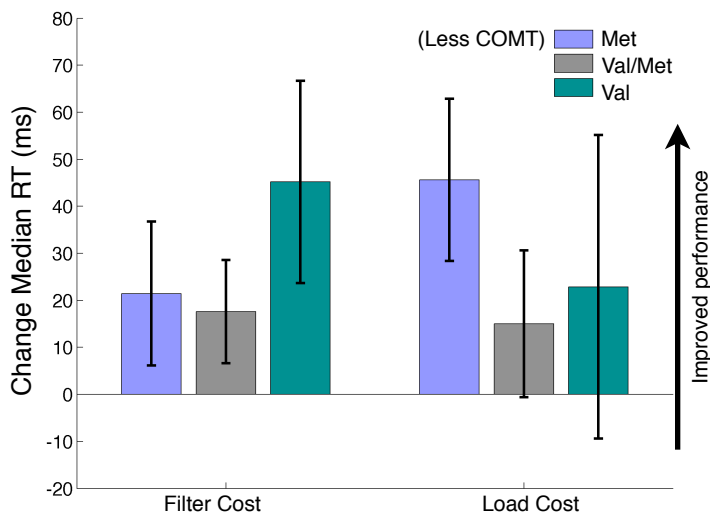
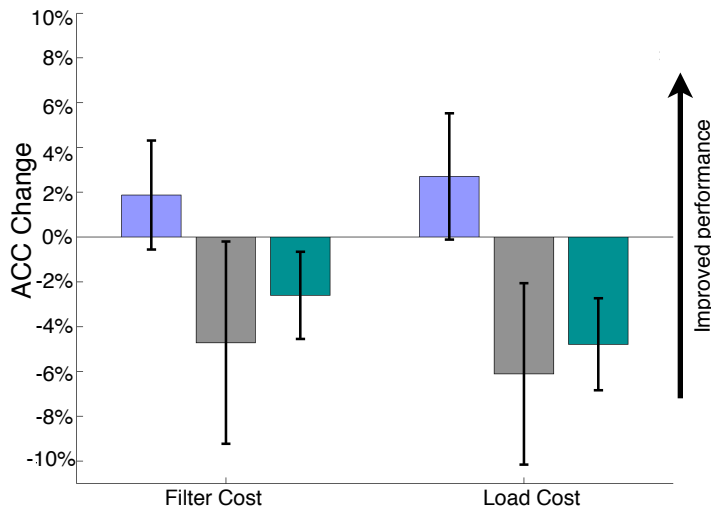


Figure 16: COMT Drug effect (error bars represent SEM)

22ms ,SEM) participants. For Load Cost, administration of Bromo resulted in a trend for a reduction in the Cost to RT of remembering more items in Met participants(mean, 46 ± 17 ms, SEM; $t_7 = 1.97$, $p = 0.09$). No significant change in mean Load Cost existed for Val/Met (15 ± 16 ms) nor Val (23 ± 32 ms, SEM) participants.

Thus, analysis of the data based on the COMT allele status revealed that after drug administration participants with the Met allele had an overall drop in accuracy compared to Val/Met and Val participants. Val participants had a relatively larger accuracy Load Cost under placebo conditions, which became larger after drug administration. When looking at reaction time, under placebo conditions Met participants had a smaller RT Filter Cost than Val participants, with Val/Met participants falling in between.

Drug administration reduced the RT Filter Cost for Val/Met participants while the costs remained unchanged for the other two allele groups.

DRD2-drug interactions

No significant interactions are found when participants are divided by the DRD2/ANKK1-Taq-IA (RS1800497) SNP in a MANOVA, however, a significant ($F_{4,6} = 7.09$, $p = 0.02$) Trial Type X Drug X Gender X DRD2 interaction is present in the multivariate analysis in a MANCOVA using PosNegRTchange as a covariate. When the univariate tests are examined the interaction is not significant for either ACC ($F_2 = 1.32$, $p = 0.29$) nor RT ($F_2 = 0.39$, $p = 0.69$), suggesting that the multivariate effects result from the combined effect of the two dependent variables.

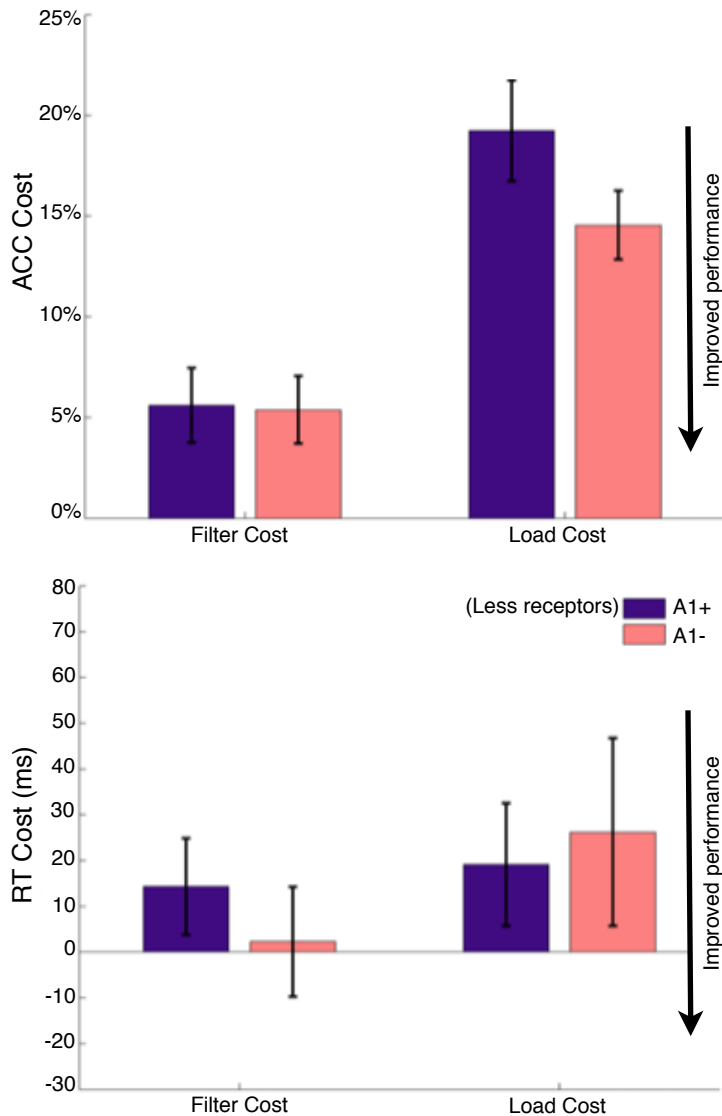


Figure 17: DRD2 placebo ACC (top) and RT (bottom) costs (error bars represent SEM)

In the PLAC session accuracy was not significantly different between the two allele groups on any of the Low Load (A1+: mean, 86 ± 2.0%; A1-: mean, 82 ± 2.4%, SEM), Filter (A1+: mean, 80 ± 2.3%; A1-: mean 77 ± 2.4%, SEM), nor High Load (A1+: mean, 67 ± 2.9%; A1-: mean, 68 ± 2.6%, SEM) trials types. There were additionally no differences between the allele groups for the accuracy cost measures (Figure 17 top). Administration of Bromo did not result in a significant change in accuracy between the two allele groups for any of the three trial types, or on the cost measures (Figure 18 top).

When RT in the PLAC session is examined the two DRD2 allele groups had similar response times for Low Load (A1+: mean, 710 ± 28ms; A1-: mean, 775 ± 36ms), Filter (A1+: mean, 724 ± 32ms; A1-: mean, 777 ± 30ms) and High Load (A1+: mean 729 ± 31ms; A1-: mean, 801 ± 30ms, SEM) trial types. Under PLAC conditions there were no

differences between the two allele groups for the RT cost measures (Figure 17 bottom). Administration of Bromo did, however, result in a significant decrease in Filter Cost ($t_{10} = 3.96$, $p = 0.003$) for A1+ leading to a larger decrease in Filter Cost compared to A1- participants ($t_{14} = 2.16$, $p = 0.04$) (Figure 18 bottom). Drug administration did not differently effect Load Cost for the two allele groups.

In the MANCOVA a significant multivariate Trial Type X Drug X Gender X DRD2 interaction was present, without a univariate effect for either RT or ACC. This interaction appears to be best represented by a change in the cost measures between genders in the two DRD2 allele groups, most notably in the RT costs. When the effect of Bromo administration on ACC is examined for A1+ participants no gender differences were present between the two allele groups (Figure 19).

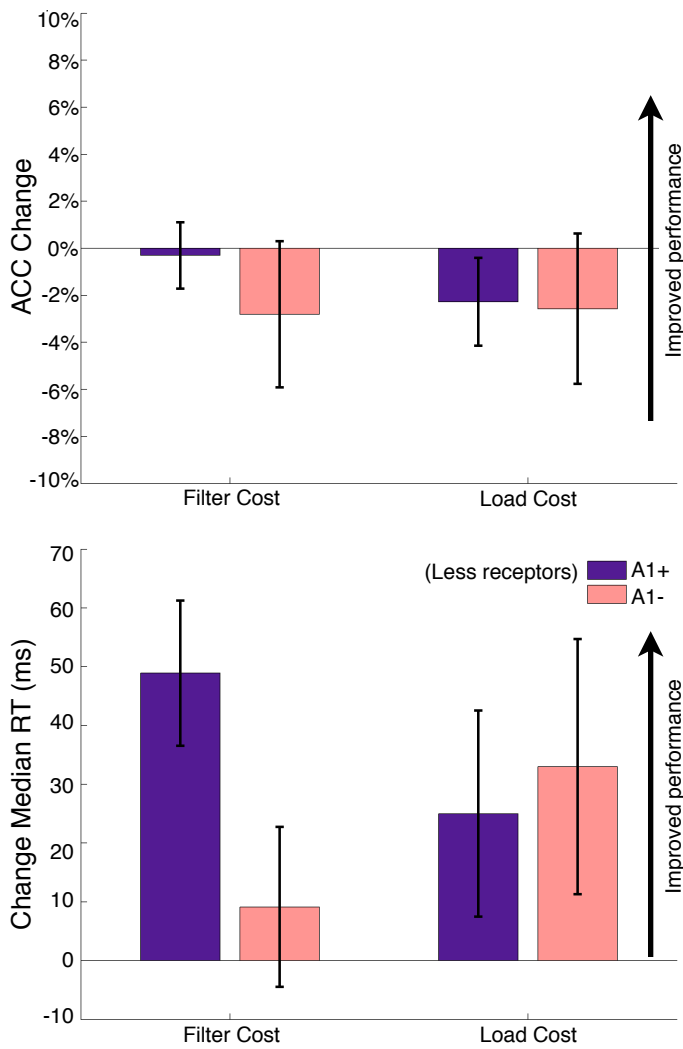


Figure 18: DRD2 Drug effect (error bars represent SEM)

When RT is examined, significant differences are present. After Bromo administration male A1+ participants had a significantly reduced Load Cost ($t_5 = 3.0$, $p = 0.03$) and Filter Cost ($t_5 = 4.3$, $p < 0.01$) compared with the PLAC session (**Figure 20**). The reduction in RT Load Cost for Male A1+ participants was also significantly larger than Female A1+ participants ($t_9 = 2.4$, $p = 0.04$) and Male A1- participants ($t_9 = 2.3$, $p = 0.04$). Importantly though, the drop in Load and Filter Cost for the Male A1+ participants is due largely to an increased reaction time on Low Load trials. No further differences were found between genders in the two DRD2 allele groups.

In summary, in a MANOVA there was a trend for a Trial Type X Drug interaction, which reached significant in a MANCOVA where the a covariate to account for changes in arousal was used.

Examining the effect of drug administration across all participants suggests a mixture of pre- and postsynaptic effects. While there was no measurable change in ACC due to drug administration, some of the RT effects resulting from postsynaptic binding were found. After drug administration there was a significant reduction in Load Cost and Filter Cost for RT, which was a predicted consequence of postsynaptic binding, however, the reduction in costs was actually due to a slowing in responding to Low Load trials, rather than a faster RT for High Load and Filter trials. When participants were median split based on WM span, High Span participants showed a larger improvement on Low Load trials than did Low Span participants. Dividing participants by the COMT Val158Met SNP (RS4680) found that initially superior ACC Load Costs in Val participants, were reduced after drug administration. Met participants, had relative improvement in Load Cost after drug administration compared to Val/Met and Val participants. Drug administration also resulted in a reduced RT Filter Cost for Val/Met participants. Lastly,

when the DRD2/ANKK1-Taq-IA (RS1800497) SNP a Trial Type X Drug X Gender X DRD2 interaction for the MANCOVA. The interaction appears to stem from changes in the RT cost measures. Male A1+ participants had a significant reduction in both Load and Filter RT Cost. This was a larger drop in RT cost than was seen for either Female A1+ participants, or Male A1- participants.

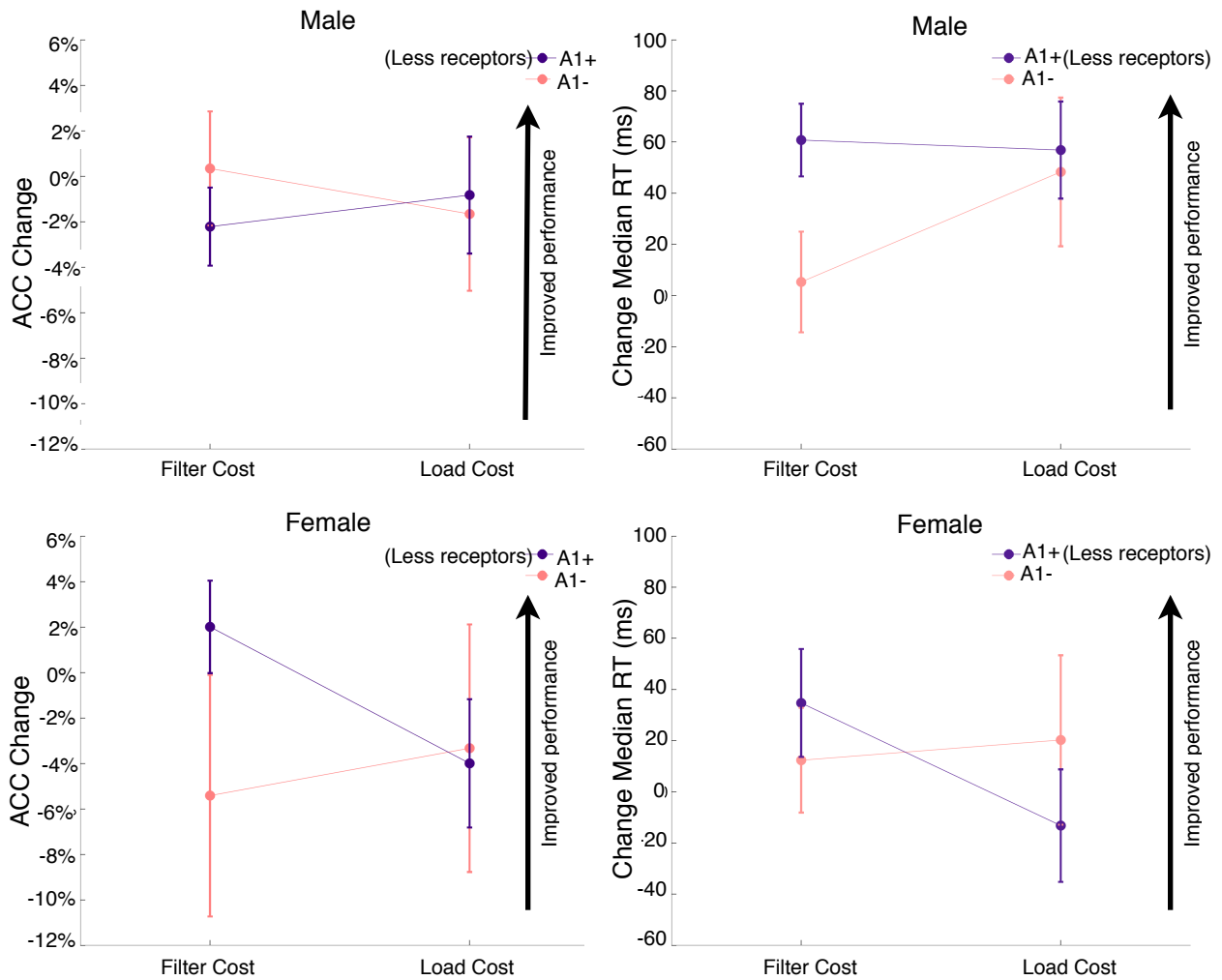


Figure 19: DRD2 and Gender ACC Drug effect
Bromo Cost - PLAC Cost
A1+ (5 females, 6 males), A1- (6 females, 5 males)
(error bars represent SEM)

Figure 20: DRD2 and Gender RT Drug effect
PLAC Cost - Bromo Cost
A1+ (5 females, 6 males), A1- (6 females, 5 males)
(error bars represent SEM)

2.5 Discussion

A dopaminergic drug manipulation (the D2 receptor agonist Bromocriptine) was used to investigate the involvement of dopamine in load and filter component processes of working memory, along with the role of DA genetics through two DA related SNPs: COMT Val158Met SNP (RS4680) and DRD2/ANKK1-Taq-IA (RS1800497). We focused on two WM component processes by calculating two cost measures, a Load Cost — where the effect of increased WM load was calculated by comparing performance on Low Load trials to High Load trials — and a Filter Cost — where the effect of ignoring irrelevant items was calculated by comparing performance on Low Load trials to Filter trials.

Based on previous work, and the results of the experiment in chapter 1, it was hypothesized that a mixture of pre- and postsynaptic drug effects would be found. The predicted presynaptic drug effects on ACC of a detriment to Load Cost and an improvement to Filter Cost were not found. However, the hypothesized effect of postsynaptic drug binding resulting in faster RTs for the two costs was found. In trying to isolate the pre- and postsynaptic effects participants were divided by WM span, and the two DA SNPs. While Low WM span participants were expected to have a benefit to performance due to drug administration, it was actually High WM span participants who surprisingly improved on Low Load trials. Contrary to previous results, participants with the Val allele for the COMT SNP, who are thought to have the lowest PFC DA levels, displayed the best abilities in the load subcomponent. The superior load performance of Val participants was ameliorated by bromocriptine administration. Interestingly for the DRD2 SNP, the hypothesized decreases in RT costs for the two subcomponents was found, especially in male A1+ participants, however, the effect was actually driven by a slowing in RT for the easiest, Low Load trials.

The results presented here demonstrate the possibility of isolating individual differences in pre- and postsynaptic drug effects. While previous studies had examined the interaction of WM performance and DA drug manipulations, to our knowledge the current study is the first to look at the effect on isolated Filter and Load WM subcomponents, and their interaction with DA genetics.

Main drug effect:

What is the effect of administration of a DA agonist on Load and Filter WM subcomponents? Administration of bromocriptine resulted in a reduction in RT Load and Filter Costs, without a measurable change in ACC for either subcomponent. Importantly, and contrary to predictions, the improvement in RT costs was not due to faster responding on the harder Filter and High Load trials, but actually due to slower responding in Low Load trials. The faster response on more difficult trials suggests the presence of a shift in the speed accuracy tradeoff.

The results reported here do not entirely fit with initial predictions made from previous research (Frank & O'Reilly, 2006). Our hypothesis was that presynaptic binding

would lead to impaired ACC in Load conditions and enhanced ACC in Filter conditions. However, no discernible changes in ACC were found. Further, to the extent that postsynaptic effects were found, predictions were that faster RTs would be found. Overall RTs slowed down after Bromo administration, as was mentioned in Chapter 1, this was likely due to a main effect of arousal after Bromo administration. Relative to Low Load trials there were faster RTs for both Load and Filter conditions, which would align with postsynaptic D2 effects, however, ACC effects would also have been expected. This suggests the possibility of combined pre- and postsynaptic drug effects, where ACC effects in the current task were not discernible. Notably though many experiments have found clearer results when participants were separated by WM span (Frank & O'Reilly, 2006; Gibbs & D'Esposito, 2005; Kimberg et al., 1997).

Working memory span-drug interaction

Individual differences in WM performance are thought to arise from variation in DA signaling (Granon et al., 2000). Previous work has shown that when participants are separated by WM span, DA drug administration results in contrasting effects. Individuals with a lower WM span show improved performance after DA drug administration, while High span individuals perform more poorly (Gibbs & D'Esposito, 2005; Kimberg et al., 1997). The effect on Load and Filter cost relative to WM span had not been previously explored. In contrast to previous studies, when participants were divided by span, bromocriptine administration resulted in improved ACC for High Span individuals.

Interestingly, the improved ACC was on the easiest, Low Load trials, where the displays contained the fewest items. This did lead to a change in the two cost measures, with High Span participants displaying the impaired ACC costs expected for postsynaptic drug binding. However, it was due to an improvement in performance on the less taxing Low Load trials, rather than the expected drop in performance for the more difficult Load and Filter trials. It is possible that this unpredicted result is due to the relatively small sample size, and that with additional participants the pattern of results would change. Additionally, given the weak statistical evidence for the above stated results no strong conclusions would be warranted.

COMT-drug interactions

Consider next the analysis of results when participants are divided based on the COMT Val158Met (RS4680) SNP. As the COMT enzyme helps to break down DA in the PFC, and WM load maintenance appears to be reliant on the PFC, COMT status was expected to be mainly related to the Load Cost measure. Met participants, who have the less stable form of the COMT enzyme, resulting in higher PFC DA, were expected to demonstrate superior load performance at baseline. The opposite result was found here, at baseline Val participants had superior load performance. Also contrary to predictions, drug administration resulted in impaired performance in Val participants, and performance in Met participants that rose up to near that of the other allele groups. This is surprising given that hypothesized higher DA levels at baseline for Met participants.

There are three possible reasons for this unexpected result. First, the sample size is relatively small for the current study, so it is possible that with a much larger data set results consistent with initial predictions would be found. The relatively small sample size also contributes to the two other possible explanations. A second possibility is that gender may have been a source of additional variance. Estrogen levels in females interact with DA signaling, resulting in increased DA release from higher estrogen levels (Becker, 1990). More recently, the COMT gene itself has been shown to interact with varying estrogen levels depending on the females endocrine state. When estrogen levels were high in females with the lower PFC DA Val allele, they had an improvement in DA function, while the opposite was true for individuals with the higher PFC DA Met allele (Jacobs & D'Esposito, 2011). Thus, depending on their estrogen cycle, females would be adding additional variance to the data. Third, it is likely not just COMT that determines DA levels in the PFC, but likely an interaction of multiple genes. It has been shown that performance on WM tasks was not predicted well by COMT Val158Met allele status alone, but by the interaction of the COMT SNP and the DRD2/ANKK1-Taqla SNP (Stelzel et al., 2009). While both SNPs were collected from the current participants, a much larger sample size would be needed in order to look at interactions between the SNP alleles.

DRD2-drug interactions

Lastly, we review the analysis when participants are divided based on the DRD2/ANKK1-Taqla (RS1800497) SNP. DRD2 is thought to mainly relate to striatal DA levels, where the mixture of pre- and postsynaptic effects was expected to predominantly occur. Due to the lower number of D2 receptors in A1+ allele carriers they were predicted to be more susceptible to postsynaptic drug binding. Presynaptic binding in the striatum was expected to lead to a drop in ACC in the Load component, while improving ACC in the Filter component. With more postsynaptic drug binding, increased tonic DA levels were predicted to lead to faster RTs. When examining participants based on the DRD2 SNP a trial type X drug X gender X DRD2 interaction was present. The hypothesized effects on ACC were not found to be present, but there was an effect on RT. After administration of bromocriptine Male A1+ (less receptors) carriers had reduced RT costs for both the load and filter components, consistent with predictions of postsynaptic RT effects. Importantly though, the apparent reduction in RT costs, was actually due to a slowing of RT on the easier, Low Load trials, which was not expected.

Although no interaction involving the DRD2 SNP and gender was predicted, given the previously noted effect of estrogen levels on DA transmission, it is perhaps not surprising to find one present. Previous studies, although few, have noted a gender influence on the same DRD2 SNP (Lee et al., 2002; Swan et al., 2005). The most parsimonious interpretation of this pattern results involves one main assumption: the current task was not sensitive enough in detecting changes in ACC due to drug administration. The lack of a univariate result for the RT measures does imply that the multivariate interaction is due to a combination of changes in both ACC and RT dependent measures. The changes in RT that were found imply that males with lower

D2 receptor density were more likely to have postsynaptic drug action, resulting in decreases for both Load and Filter Cost RTs. It should be stressed though that caution must be taken in making these interpretations due to the statistically weak nature of the results.

Conclusions

We have shown here that after administration of a low dose of Bromocriptine participants were actually faster to respond to High Load and Filter trials, then the easier Low Load trials. This effect resulted from an unexpected slowing in Low Load trial RTs, rather than a decrease in RTs for High Load and Filter trials. This change in RTs for the three trial types resulted in reduced Load and Filter costs. While the COMT Val158Met SNP did predict baseline differences in WM load performance, the effect was in the opposite direction than predicted, with Val participants, who are theorized to have lower PFC DA levels, displaying superior performance. Drug administration then actually resulted in improved load ACC for the higher PFC DA group, Met participants, rather than hurting performance. When examining the DRD2/ANKK1-Taq-IA SNP drug administration resulted in participants with lower receptor density (A1+) having the predicted RT effects of postsynaptic drug binding, especially in male participants, perhaps to due differences in estrogen cycles in females adding additional variance.

We have presented a pattern of results giving evidence, although admittedly statistically weak, supporting initial predictions of an involvement in D2 signaling in mediating WM component processing, with a SNP associated with striatal D2 receptor density involved in both Filter and Load WM component processing, specifically in RT, and a SNP associated with PFC DA involved in ACC Load WM component processing. Further studies will be needed, however, to bolster evidence given the strength of the results presented here. It will be especially important for future studies to explore the effect of administration of different DA dosage levels, in order to explore the putative inverted-u shaped curve for DA function. Until a study is completed showing that at intermediate DA levels performance is improved, while at higher levels performance is degraded, it will continue to be difficult to interpret the results of studies involving DA manipulations, making the exploration of individual differences in DA function even more difficult.

Chapter 3 Motor learning from reward and errors

3.1 Introduction

When we are learning a new motor skill are we learning from success or failure? Or more specifically, is the motor system relying on errors or reward to adjust behavior? Historically motor learning has been seen as error driven, with the cerebellum being a key component in adjusting performance after an error is committed (Ito, 2001; Wolpert et al., 1998). Computational models of motor learning tend to be error based, with successful movement execution resulting in minimal learning.

Intuition suggests that successful performance is important for learning: for example, elite basketball players spend countless hours of practice, presumably benefitting from all of their successful shots. It is likely that motor learning occurs not just through a single mechanism or process, but by the combination of many processes (Huang et al., 2011). Evidence suggests that reward based learning may play an integral role in acquiring new motor skills (Hosp et al., 2011), and further that seemingly forgotten motor learning will return when reward feedback is reintroduced (Shmuelof et al., 2012). Additionally, when participants are only given information about success or failure in reaching for a target, but no vectorial information about the direction or magnitude of their endpoint, a visuomotor adaptation can still be learned (Izawa & Shadmehr, 2011). It is not just over a longer time scale that reward can affect motor learning, but also on a trial by trial basis with successful motor execution causing a reduction in variability when the same action is immediately repeated, despite no error occurring (Verstynen & Sabes, 2011). This subsequent reduction in variability may not be due to reinforcement learning, but due to use-dependent plasticity. Further showing the independence of adaptation and use-dependent plasticity is that learning from both mechanisms can happen simultaneously, and in opposing directions (Diedrichsen et al., 2010). More recent evidence has suggested that more explicit processes, such as a verbalizable strategy, are also an important element of the interplay resulting in motor learning (Taylor et al., 2014).

Motor learning provides an interesting alternative paradigm to cognitive learning for investigating reward and error systems in the brain. In cognitive learning reward based systems have been well established in the form of dopaminergic signaling in the basal ganglia (Keri, 2003), however, little work has focused on the involvement of errors in learning in the cognitive domain. In motor learning, on the other hand, the cortical regions involved in learning from errors have been well established (Gilbert & Thach, 1977; Horn et al., 2004), with much less focus on the involvement of reward (Doya, 2000).

The cerebellum is thought to contribute to motor learning by predicting the future sensory consequences of motor actions, in the form of a forward model (Wolpert et al., 1998). This internal model is then updated with an error signal representing the

difference between expected and actual outcomes (Tseng et al., 2007). Consistent with this role of the cerebellum in motor learning neuroimaging has found activation in the cerebellum to errors while reaching (Diedrichsen et al., 2005), and more specifically the presence of sensory prediction errors when either an unexpected sensory consequence of a movement was present, or when an expected sensory consequence was absent (Schlerf et al., 2012b). Studies with patients who have cerebellar damage provide further evidence showing that damage leads to deficits in sensorimotor adaptation (Morton & Bastian, 2004; Smith & Shadmehr, 2005; Tseng et al., 2007).

A common technique for studying visuomotor adaptation is to introduce a mismatch between vision and proprioception, for example, by using prism goggles that shift the visual input. Normal participants and patients with cerebellar lesions produced large errors when first making a reaching movement in the shifted environment. Over time the unimpaired normal participants are able to reduce their errors and become accurate at hitting a target, while the patients with lesions are not (Martin et al., 1996). Importantly, when the visual perturbation is removed, and vision is returned to normal, an error is produced in the opposite direction of the initial shift. This continued shift after returning back to baseline, or aftereffect, is used as measure of the degree of adaptation. These aftereffects of learning are presented as evidence that a sensory motor map has been adjusted, with the length of time that the aftereffect persists, or the degree to which it interacts with learning a new rotation, providing a measure of the retention of this learning (Criscimagna-hemminger & Shadmehr, 2008; Krakauer, 2009).

Recent advances in neural stimulation techniques have allowed for more direct probing of the location and timing of brain regions involved in motor learning. Participants view a virtual cursor on a screen and are asked to make reaching movements, with vision of their hand occluded. A perturbation is then added which participants must learn to compensate for. The perturbation can either be introduced gradually, where the full extent of the manipulation is slowly introduced, or abruptly where participants experience the entire perturbation the first time it is introduced. While performing tasks such as this measures of the motor cortex's excitability can be done by using single pulse Transcranial Magnetic Stimulation (TMS) over the cortex, and measuring the magnitude of Motor Evoked Potentials (MEPs) using electrodes placed on the muscles. Recent evidence using this technique has suggested that the cerebellum is most active during the early stages of an abrupt perturbation, when large errors are corrected (Schlerf et al., 2012a).

TMS can also be applied repetitively (rTMS) to an area of the brain, which then reduces the excitability of the target area — a virtual lesion — that lasts for a period of time after the stimulation is completed (Pascual-leone et al., 2000). Using this virtual lesion technique combined with functional Magnetic Resonance Imaging to target stimulation it was found that rTMS applied to the left cerebellum reduces a subject's ability to correct for large supraliminal errors (Bijsterbosch et al., 2011). Another popular brain stimulation technique is transcranial Direct Current Stimulation (tDCS), where electrodes placed on the skull pass a constant current through the brain. In one study

using tDCS, anodal stimulation of the cerebellum resulted in faster adaptation when an abrupt 30° visuomotor rotation was introduced (Galea et al., 2011).

The cerebellum is not the only area of the brain to have been shown to play a significant role in motor learning, brain stimulation studies have also revealed a possible role for primary motor cortex (M1) in the consolidation and retention of motor learning. When a visuomotor rotation was introduced while anodal stimulation was applied over M1 increased retention was found in the form of a slower decay of the after effect (Galea et al., 2011). Additional studies using tDCS have found enhanced learning and retention when anodal tDCS is applied over M1, both in the course of a single session (Galea & Celnik, 2009), and over the course of multiple sessions possibly interacting with sleep dependent consolidation (Reis et al., 2009). These results, combined with those studying the cerebellum, suggest that the M1 may play a complimentary role to the cerebellum, with M1 important motor learning consolidation.

What is the motor cortex consolidating? In a comparison of abrupt and gradual introductions of a perturbation, in this case a force field, rTMS was applied to M1 before subjects completed the task. During the task participants experienced one of three perturbation schedules, an abrupt introduction of the full perturbation in one trial, a gradual introduction of the force field over 240 trials, or an intermediate introduction where the field was introduced over 45 trials. rTMS to M1 did not affect early adaptation in any of the conditions, but in both the gradual and intermediate schedules adaptation was disrupted at the plateau where the same movement was repeated (Xivry et al., 2011). The evidence presented here suggests an intriguing dichotomy where the cerebellum may be important for adjusting to errors through sensory prediction errors, while M1 may be consolidating the learning of a new sensorimotor map in response to repeated successful movements, perhaps through a combination of use-dependent plasticity and reinforcement learning.

3.2 Focus of current project

What might be the mechanism that results in learning in M1? If M1 is learning from error-based information, then more learning should occur from trials that were not successful, i.e. where the target is missed. On the other hand, use-dependent plasticity would result in learning in M1 when a target is successfully hit. Finally, if learning in M1 is occurring through reinforcement learning, then both success and failure in motor execution would result in a change in behavior. However, when looking at the results from previous studies we are not able to tell whether the motor system is learning from reward or lack of error, as reward and size of error are inversely correlated. For example, in many motor learning studies feedback about the endpoint of a participants' reach is given, providing vectorial information about how far off they were from the target. There are two types of feedback being given here, whether the target was hit or missed, and how large the error was. This paradigm makes it difficult to manipulate the reward and error feedback, as a small error will always mean you were near the target. How could we still dissociate learning from success and failure in motor learning?

In visuomotor adaptation paradigms participants perform reaching movements and after a baseline period must compensate for a perturbation. After an adaptation phase sensorimotor adaptation is tested by removing the perturbation and comparing the participants' reaches to under the original baseline. While it might seem that the amount of a perturbation that is learned would be directly correlated with the magnitude of the aftereffect, one study suggests they may not be directly related (Hadipour-Niktarash et al., 2007). In their study TMS pulses are delivered over M1 at the end of reach reaching movement. This procedure results in reduced retention of the learning in a motor task (the aftereffect), without affecting the participants' ability to adjust to the perturbation when it is still present. Thus there is no difference in performance in adjusting to the perturbation when TMS pulses are delivered, but the aftereffect decays much faster. Our goal in the current experiment was to use single pulse TMS to target the feedback to M1 immediately after successful or unsuccessful reaches for a target. More specifically, to explore the role of reward and error feedback to M1 in both adjusting to a perturbation and in consolidation of the new sensorimotor map. Making the presence or absence of TMS contingent on the outcome of each trial has not previously been used in studies of sensorimotor adaptation.

3.3 General material and methods

Participants

One hundred and ten right-handed participants (mean age, 21.9 ± 3.9 years, SD; 72 females) were recruited from the UC Berkeley community. Thirty three participants were used in experiment 3A and seventy seven in experiment 3B. Exclusionary criteria included any history of psychiatric or neurological disorders, an episode of loss of consciousness and use of psychotropic drugs. Prior to participation in the study participants were asked to get a normal nights rest and to refrain from drinking caffeine. The UC Berkeley Committee for the Protection of Human Subjects approved all study procedures. All volunteers gave written informed consent and were either paid or received course credit for their participation.

Experimental timeline

Each participant completed a single experimental session. On the day of the testing session and prior to participation in the study, participants gave informed consent, filled out a screening form to rule out any exclusionary criteria and were confirmed to be right handed by the Edinburgh handedness inventory (Oldfield, 1971).

Apparatus and general task procedures

Participants were seated in front of a horizontally oriented monitor and made 8cm out and back reaching movements to visually displayed targets. Participants held on to a digitizing pen and made the reaching movements on a Wacom digitizing tablet (Intuous 3, Wacom, Vancouver, WA, USA). The targets and other task stimuli were displayed on a 15-inch, 1280 X 1024 pixel resolution LCD monitor, mounted 25.4 cm above the tablet. The monitor was mounted in the same plane as the tablet. With this arrangement, participants were unable to see their hand while performing the task. To minimize head movement during the TMS session, participants rested their head in a chin rest. The experimental task was implemented in custom software implemented in Python (open source).

Data analysis

All initial data analyses were performed using Matlab (MathWorks, Natick, MA). The data were then analyzed with a repeated measures ANOVA using the general linear model framework of SPSS (IBM, 2011). Error on each trial was calculated using the angular difference between the target location and the hand position when the hand intersected the plane of the target. For averaging across trials, each movement trajectory, regardless of the actual target location, was rotated to a common axis, resetting as though the target was located at 0° . A straight line was computed between the starting position and the actual hand position. The angle between this line and the 0° reference line was then calculated. With this convention, a positive angle indicates an

error in the clockwise direction and a negative deviation indicates an error in the counter clockwise direction.

A one-way repeated measures ANOVA was performed comparing the mean error for each participant averaged over each of: the last 16 trials of the baseline period (prior to the introduction of the perturbation), the last 16 trials of the adaptation period, the first 16 trials of the washout period and the last 16 trials of the washout period. Thus, each participant contributed 4 values, the mean of their errors for each of the four time points. All reported ttest values are for a two-tailed test unless otherwise specified.

3.4 Experiment 3A: Success only feedback

3.4.1 Methods

On each trial, the participant made an out and back reaching movement towards a virtual target presented at 1 of 7 possible locations, with polar angles of 0°, 10°, 20° 30°, -10°, -20° and -30° (**Figure 21**). If the participant’s movement intersected the target, the target turned green and a pleasant “ding” was played. If the movement missed the target, the target turned red and an aversive “buzz” was played. A financial bonus system was used to increase motivation due to the repetitive nature of the task.

Participants were assigned to one of four TMS groups. In the miss-TMS group, a pulse was delivered from the TMS coil every time the participant missed the target, timed at 0ms from when they crossed the plane of the target. In the hit-TMS group, the pulse was delivered every time the participant successfully hit the target, timed at 0ms from when they crossed the plane of the target. In the hit(delay)-TMS control group a pulse was also delivered every time they hit the target, but was delayed 700ms after the

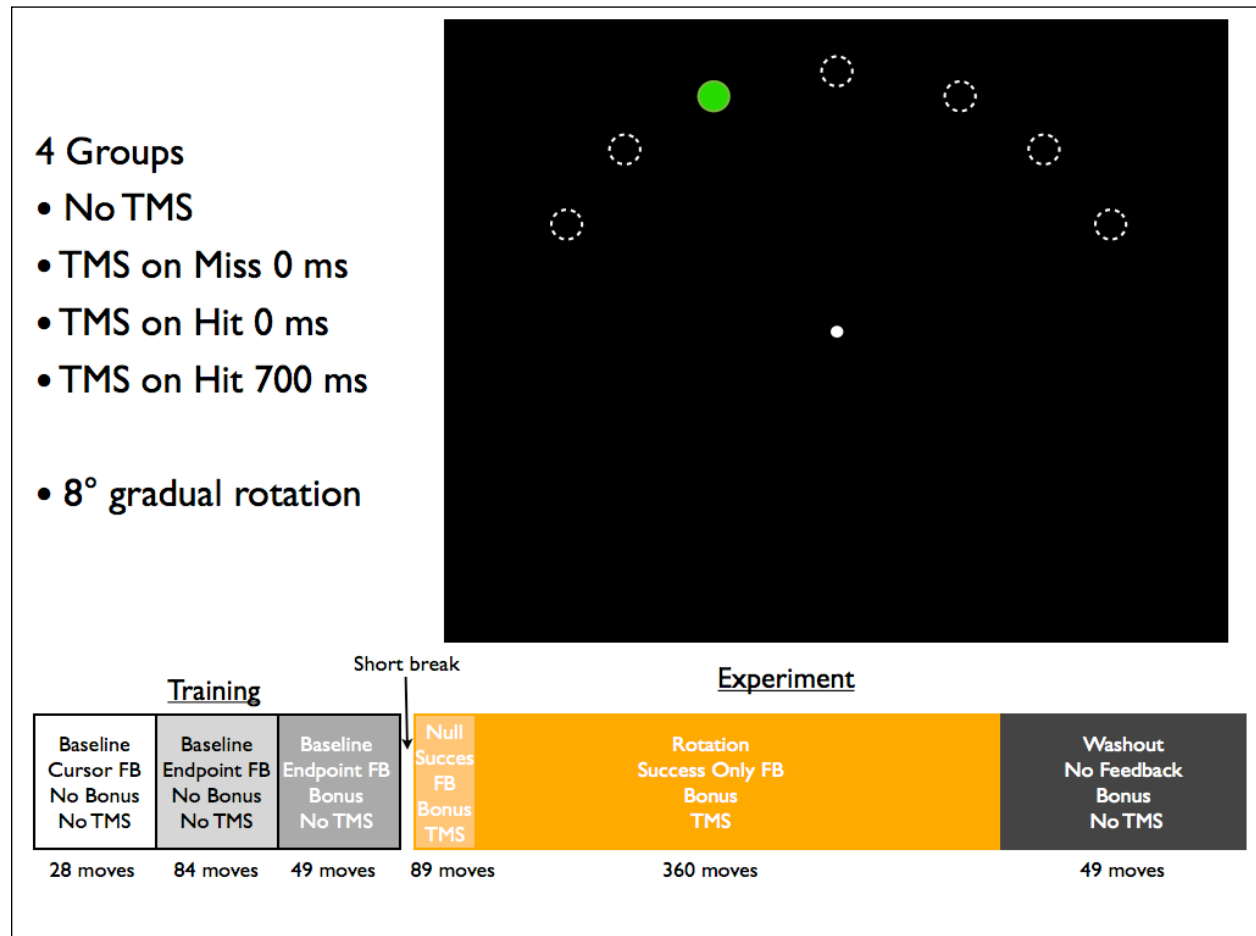


Figure 21: Reward only feedback task
Experiment 3A

participant crossed the plane of the target. Delayed delivery of TMS stimulation has been shown to be an effective control for the aversive nature of TMS stimulation (Hadipour-Niktarash et al., 2007). Finally, in the no-TMS control group, no TMS pulse was delivered.

The session began with 161-trial training phase, composed of 23 movements to each target. During the first 28 trials of the training phase participants received online feedback, limited to a small 3.5mm diameter cursor, to allow them to become accustomed to making reaches in the novel environment. For the subsequent 84 movements participants only received endpoint cursor feedback with the location of the feedback depicting where their movement crossed the plane of the target. For the final 49 movements of the training phase the financial incentive structure was implemented while the participants continued to receive endpoint feedback.

In the test session, the participant completed 498 trials, with an average of 71 movements to each target. During the entire test session, participants only received feedback about the success or non-success of reaching the target, without receiving any feedback about where their hand position was relative to the cursor (Izawa & Shadmehr, 2011). After the first 89 baseline trials of the experimental session a counterclockwise visuomotor rotation was gradually introduced between the “virtual” cursor location and the hand location, with 1° being added every 30 trials and reaching a maximum of 8° displacement. A gradual perturbation was used so that participants could reasonably learn to adjust to the perturbation without vectorial error information. After the 360 trials with the rotation, the participants performed a final set of 49 movements where no feedback was given (visual or auditory). This washout phase allowed us to measure the temporal decay of the newly acquired sensorimotor mapping. TMS was not applied during this epoch.

TMS and localization of stimulation sites

Before the experimental session Delsys Electromyography (EMG) electrodes were placed on: the First Dorsal Interosseous (FDI) muscle of the hand, the lateral head of the triceps brachii, and over the anterior portion of the deltoid. A reference electrode consisting of an alligator clip attached to a 3M Red Dot conductive electrode was additionally attached on the skin directly above the lateral epicondyle of the elbow. A Magstim Rapid stimulator (Magstim, Whitland, UK) and a figure-of-eight coil with 70 mm wing diameter were used. The coil was placed tangentially to the scalp with the handle initially pointed backwards at a 45° angle with respect to the anterior-posterior axis.

Thresholding was done for all participants, even those in a group that did not receive TMS pulses during the experimental session. Single pulses of TMS were applied to the left M1 to localize the motor “hotspot” for the right FDI muscle. The motor hotspot was defined as the site that elicited maximal motor evoked potentials (MEPs) at FDI. For some participants the angle of the hand relative to the anterior-posterior axis was rotated to achieve maximal MEPs at minimal stimulation intensity. Resting motor

threshold (RMT) was determined at the FDI hotspot and was defined as the minimum stimulation intensity that elicited MEPs of $\geq 50 \mu\text{V}$ amplitude at the FDI in ≥ 5 of 10 consecutive pulses. The mean FDI RMT measured at the M1 hotspot for experiment 3A for the miss-TMS, hit-TMS and hit(delay)-TMS was 68.70 ± 8.92 , 61.90 ± 6.72 and $58.00 \pm 11.49\%$ (mean \pm SD) of the maximum stimulator output respectively. Subsequently, single pulses were delivered to the left M to localize the hotspot for the lateral head of the triceps brachii and anterior portion of the deltoid for the right arm. During the experimental session, TMS was applied at 120% of RMT at the midpoint between biceps and deltoid hotspots to stimulate the representation of the muscles chiefly involved in the task (Hadipour-Niktarash et al., 2007; Thoroughman & Shadmehr, 1999). On trials where TMS was delivered the stimulation was timed relative to when the participant's reach crossed the plane of the target.

Predictions

Based on earlier work, M1 is hypothesized to play a role in the consolidation of motor learning, through use-dependent plasticity. This process should be most relevant on trials where execution was successful. Thus, when TMS pulses are delivered immediately at the completion of a movement where the target was successfully hit (hit-TMS), the perturbation of use-dependent mechanisms should result in a reduction in the retention of learning. This will be evident in the form of a faster decay of the aftereffect relative to no-TMS controls. No effect is expected for the hit-TMS(delayed) group where pulses are given on successful movements, but delayed by 700ms, nor when delivered immediately at the completion of trials where the target was missed (miss-TMS).

3.4.2 Results

We first examine the performance of the no-TMS group to verify that an aftereffect and subsequent decay was present, implying the presence of sensorimotor adaptation (**Figure 22**). A *t* test was performed comparing the mean hand angle of the last 16 trials of the baseline period to the last 16 trials of the adaptation block. The mean hand angle during the last 16 trials of baseline was -0.5 deg ($\pm 0.9^\circ$, SEM), indicating that participants tended to reach close to the target. In the last 16 trials of adaptation, the mean was 3.6° ($\pm 1.2^\circ$, SEM), a significant shift in hand angle ($t_7 = 2.98$, $p = 0.02$). The shift in hand angle of 4.1° is much less than the 8° perturbation that was introduced. However, it is similar to the effect observed in visuomotor adaptation studies. The mean of the first 16 trials in the washout phase was 3.9° ($\pm 1.3^\circ$, SEM). When compared to the last 16 trials of the baseline phase (prior to the introduction of the perturbation), there was a reliable difference ($t_7 = 2.66$, $p = 0.03$), indicating

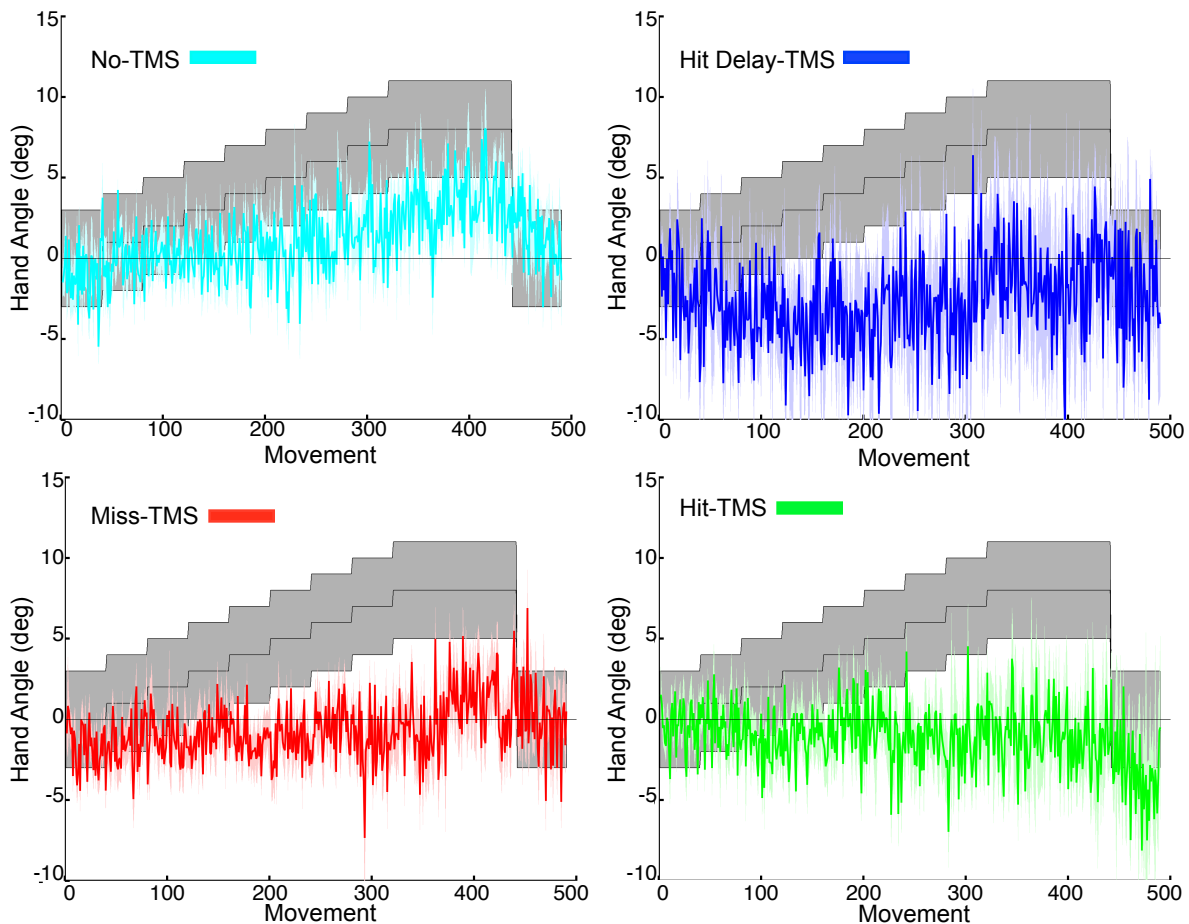


Figure 22: Clockwise from top left. a) No-TMS, b) Hit(delay) - TMS, c) Miss-TMS, d) Hit-TMS
Average hand-target angle
(shaded region represent SEM)

significant adaptation. There was no measurable decay of the aftereffect ($t_7 = 1.74$, $p = 0.13$) as measured by a comparison of the first and last 16 trials of the washout phase (**Figure 23**).

The same procedure was used to assess learning for the three other groups. All three TMS groups did not show adaptation to the same extent that was seen in the no-TMS group (**Figure 22**). The miss-TMS group ($N = 10$) had small errors during the last 16 trials of the baseline phase and did not shift their reach angles to compensate for the perturbation for the last 16 trials of the adaptation phase (mean of last 16 trials of the adaptation phase: $-0.35 \pm 1.6^\circ$, SEM). Interestingly though, there was a significant aftereffect ($t_9 = 2.98$, $p = 0.02$) when comparing the first 16

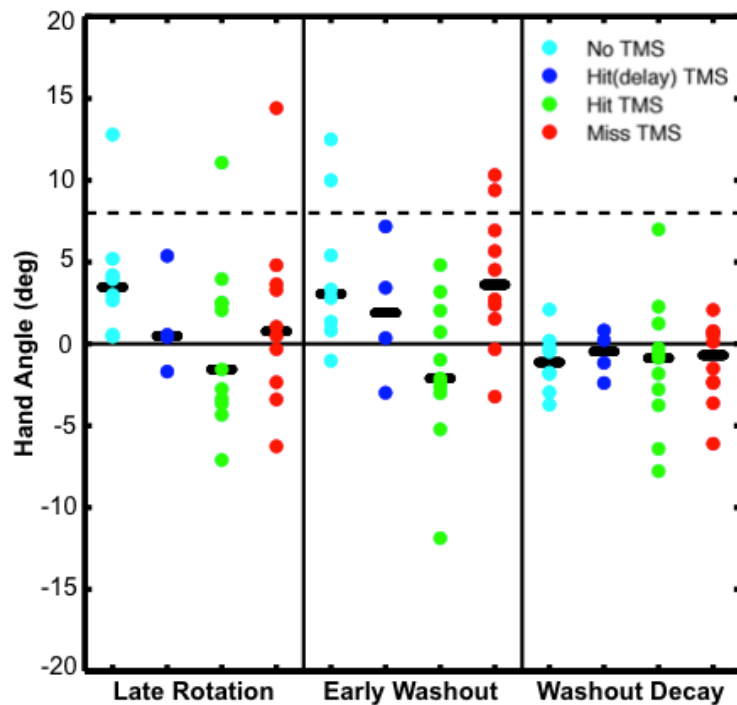


Figure 23: Success Only Feedback Learning Metrics - bias from baseline removed, dots represent individuals, black line is median of group

trials of the washout phase to the last 16 trials of the baseline phase. A significant decay in the aftereffect was also present ($t_9 = 2.52$, $p = 0.03$) when comparing the last 16 trials of washout to the first 16 trials of the washout period (**Figure 23**), although given that no measurable adaptation occurred caution must be noted in interpreting these results. The hit-TMS group ($N = 11$) showed a similar pattern of results, performing well at baseline, but did not shift their reach angles to compensate for the perturbation. Additionally the hit-TMS group additionally did not have a significant aftereffect. Despite no initial aftereffect being present, by the end of the washout period there was a marginal trend in a one-tailed ttest ($t_{10} = 1.35$, $p = 0.102$) for hit-TMS participants to move away from the direction of compensating for the perturbation (**Figure 22**). Finally, the hit(delay)-TMS control group ($N = 4$) had fairly large errors during the baseline phase and also did not shift their reach angles to compensate for the perturbation, nor did they have a significant aftereffect. As with the hit-TMS group a trend was present in a one-tailed test for a decay in the washout period ($t_3 = 1.89$, $p = 0.08$) (**Figure 23**). It should be noted that the number of subjects ($N = 4$) in the hit(delay)-TMS group was small.

To compare groups, a mixed-effects repeated measures ANOVA was used with the factors Group (hit-TMS, miss-TMS, hit(delay)-TMS and no-TMS) and Timepoint

(mean: last 16 baseline, last 16 adaptation, first 16 washout and last 16 washout). There was a main effect of Timepoint ($F_{3,27} = 3.12$, $p = 0.04$) with the effect being driven mainly by the No-TMS group having a shift at the end of the adaptation phase to compensate for the perturbation. There was no effect of Group ($F_3 = 1.10$, $p = 0.37$) nor a Timepoint X Group interaction ($F_{9,87} = 1.68$, $p = 0.11$). As there were only four participants in the hit(delay) group, we repeated the ANOVA with just the three other groups. In this revised analysis, the interaction was marginally reliable ($F_{6,50} = 2.27$, $p = 0.051$), but the main effect of Timepoint is no longer significant ($F_{3,24} = 1.58$, $p = 0.22$). As before, this trend for an interaction was driven by the no-TMS group, the only group that significantly shifted their hand angles to compensate for the perturbation.

In summary, weak, but significant learning was seen in the no-TMS participants. In contrast, no learning was seen for any of the miss-TMS, hit-TMS or hit(delay)-TMS groups, although a significant aftereffect was present in the miss-TMS group. Additionally, in the hit-TMS and hit(delay)-TMS groups, there was weak evidence for a decay in the opposite direction of the perturbation by the end of the washout phase. Importantly, given the number of tests that were ran, it is possible that some of the significant results may be spurious.

3.5 Experiment 3B: Error feedback

3.5.1 Methods

No significant learning was found in experiment 3A for any of the 3 TMS groups. One possibility is that endpoint error feedback must be provided in order for robust learning to occur. As can be seen from the results of Experiment 3A there was large variability between participants' performance when only success/non-success feedback was provided. Learning is much more robust with endpoint feedback. Additionally, by using endpoint feedback a larger perturbation can be used, allowing for the detection of a smaller effect on consolidation.

For experiment 3B participants received endpoint cursor feedback with the location of the feedback depicting where their movement crossed the plane of the target. On each trial participants made an out and back reaching movement towards a virtual target presented at 1 of 3 possible locations, with polar angles of -7.5° , -15° and

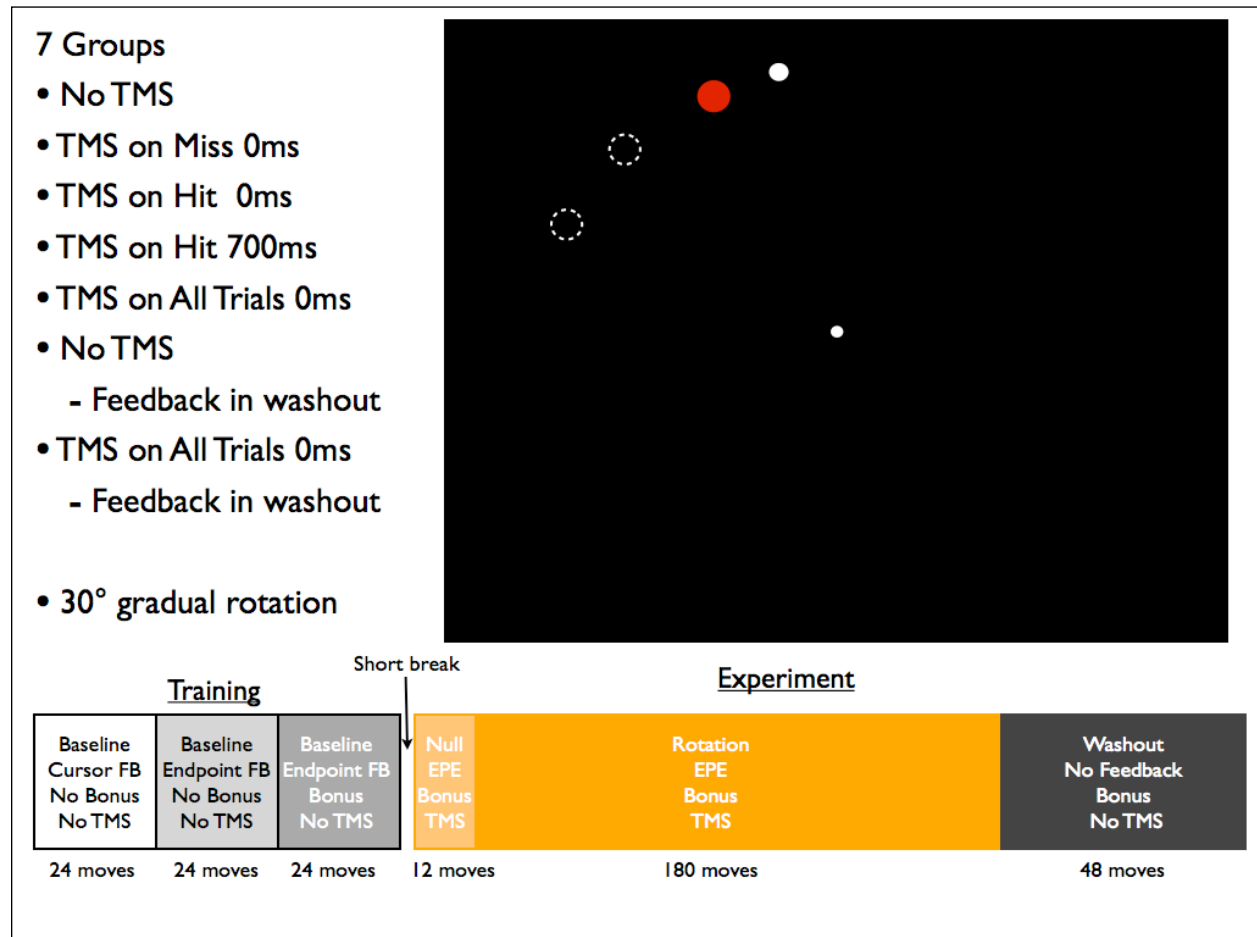


Figure 24: Endpoint error feedback task
Experiment 3B
 (hit-TMS washout feedback and all-TMS washout feedback received endpoint feedback during washout)

-22.5° (**Figure 24**). As in Experiment 3A, visual and auditory feedback was provided as to success/non-success in hitting the target. During a training phase participants completed 72 trials, with 24 movements to each target. For the first 24 movements, participants received online cursor feedback to allow them to become accustomed to making reaches in the novel environment. For the middle 24 trials only endpoint feedback was provided, with the location of the feedback depicting where their cursor had crossed the plane of the target. For the final 24 trials of the training phase, the financial incentive structure was implemented while participants continued to receive endpoint feedback.

After a short break, participants completed 240 trials of the test phase, with 80 movements to each target. During the experimental session all participants continued to receive endpoint feedback until the washout phase. After the first 12 trials a counterclockwise visuomotor rotation was gradually implemented between the “virtual” cursor and the hand position, with 1° added every 6 trials until a maximum displacement of 30° was reached (Hadipour-Niktarash et al., 2007). After completion of the adaption phase the subsequent 48 trials were a washout phase, where no TMS pulses were delivered, to allow for measurement of the decay of the newly acquired sensorimotor mapping.

Participants were assigned to one of seven groups. The same four groups were repeated from experiment 3A (No-TMS, Hit-TMS, Hit(delay)-TMS, and Miss-TMS) with the addition of an All-TMS group for whom a pulse was delivered at the endpoint of their reach, regardless of whether the target was hit or missed. The All-TMS group was included in order to replicate the original result in (Hadipour-Niktarash et al., 2007). As before, for all five of these groups no feedback was given during the washout period. Due to a failure to replicate the All-TMS result, two additional groups were included after the fact. In the original Hadipour-Niktarash et al paper feedback had been given during the washout phase, so in a further attempt to replicate their original result a No-TMS (feedback in washout) and an All-TMS (feedback in washout) groups were added. The two feedback in washout groups received endpoint feedback during the washout period. For the other four groups, the target disappeared at the completion of the reach and no visual nor auditory feedback was given.

TMS and localization of stimulation sites

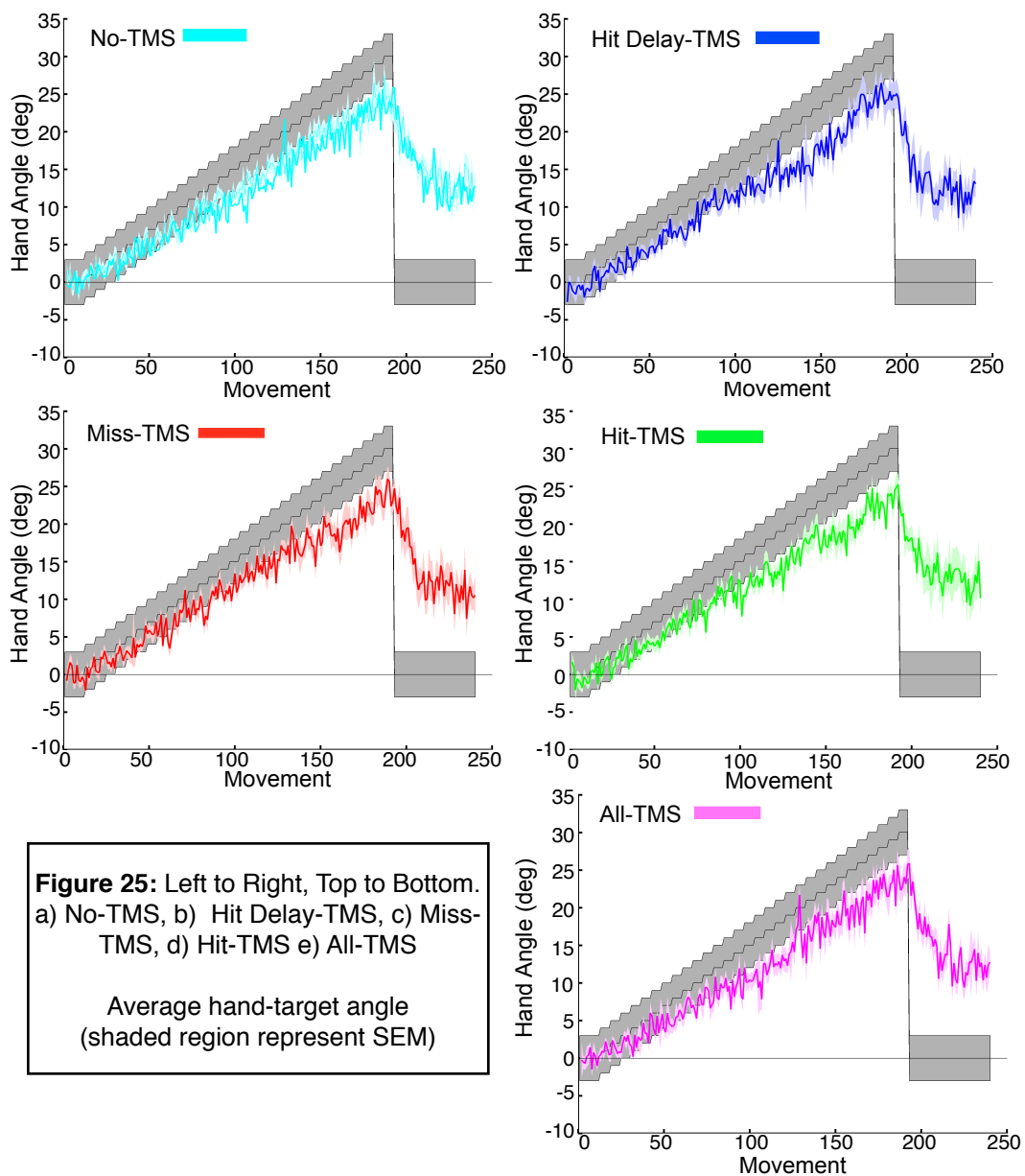
The TMS procedures for experiment 3B were exactly the same as in experiment 3A. For experiment 3B the Resting motor threshold (RMT) measured at M1 for the miss-TMS, hit-TMS, hit(delay)-TMS, all-TMS and all-TMS feedback in washout were respectively 63.17 ± 9.65 , 67.33 ± 11.84 , 63.42 ± 10.63 , 59.50 ± 10.92 and $58.40 \pm 11.23\%$ (mean \pm SD) of the maximum stimulator output. As in experiment 3A, during the experimental session, TMS was applied at 120% of RMT at the midpoint between biceps and deltoid hotspots to stimulate the representation of the muscles chiefly involved in the task (Hadipour-Niktarash et al., 2007; Thoroughman & Shadmehr, 1999). On trials where TMS was delivered the stimulation was timed relative to when the participant’s reach crossed the plane of the target.

Predictions

TMS pulses delivered immediately at the completion of a movement where the target was hit (hit-TMS and hit-TMS feedback during washout) or delivered on every trial (all-TMS) will reduce the retention of learning, in the form a faster decay of the aftereffect relative to controls (no-TMS and no-TMS feedback during washout). No effect is expected for hit(delay)-TMS participants, where pulses are delivered after a successful reach, but delayed 700ms, nor when delivered immediately after trials where the target was missed (miss-TMS).

3.5.2 Results

To examine whether participants had sufficiently adapted to the perturbation a ttest was performed comparing the mean hand angle of the last 16 trials of baseline (before the perturbation was introduced) to the last 16 trials in the adaptation block. To confirm learning occurred in the absence of TMS we focused on the no-TMS group first. During the last 16 trials of the baseline phase no-TMS participants (N = 12) generally reached directly for the target (mean, $1.7 \pm 0.2^\circ$) while during the last 16 trials of the adaptation phase participants had larger hand angles (mean, $23.0 \pm 1.4^\circ$, SEM) resulting in a significant shift ($t_{11} = 16.92$, $p < 0.0001$) to compensate for the perturbation (**Figure 25**). An aftereffect of the learning was confirmed by comparing the mean hand



angle in the first 16 trials of the adaptation phase to the first 16 trials in the washout period (mean, $18.4 \pm 0.9^\circ$, SEM), which resulted in a significant aftereffect for the no-TMS group ($t_{11} = 18.00$, $p < 0.0001$) (**Figure 26**). Finally, to confirm that the aftereffect decayed the first 16 trials in the washout period were compared to the last 16 trials of the washout period (mean, $12.2 \pm 1.4^\circ$, SEM) resulting in a significant decay of the aftereffect by the end of washout for the no-TMS group ($t_{11} = 4.53$, $p < 0.001$). For the remaining experimental groups the presence of learning and a decaying aftereffect were confirmed in the same manner.

The miss-TMS group ($N = 12$) performed well during the baseline period and had larger hand angles by the end of the adaptation phase resulting in a significant shift ($t_{11} = 20.00$, $p < 0.0001$) to compensate for the perturbation. A significant aftereffect ($t_{11} = 16.42$, $p < 0.0001$) with significant decay was also present ($t_{11} = 4.62$, $p < 0.001$) (**Figure 26**). For the hit-TMS group ($N = 12$) there was a significant shift ($t_{11} = 46.57$, $p < 0.0001$) to compensate for the perturbation, as well as an

aftereffect ($t_{11} = 13.20$, $p < 0.0001$) with significant decay ($t_{11} = 5.20$, $p < 0.001$). Participants in the hit(delay)-TMS control group ($N = 12$) also showed larger hand angles by the end of the adaptation phase resulting in a significant shift ($t_{11} = 19.25$, $p < 0.0001$) to compensate for the perturbation, as well as a significant aftereffect ($t_{11} = 11.48$, $p < 0.0001$) that decayed ($t_{11} = 6.49$, $p < 0.0001$). Finally, in the all-TMS control group ($N = 12$) the same pattern was present, with a significant shift ($t_{11} = 16.50$, $p < 0.0001$) to counter the perturbation, and an aftereffect ($t_{11} = 14.88$, $p < 0.0001$) with significant decay ($t_{11} = 9.12$, $p < 0.001$).

A mixed-effects repeated measures ANOVA using Timepoint (mean: first 16 adaptation, last 16 adaptation, first 16 washout and last 16 washout) as the within-subject factor and Group (hit-TMS, miss-TMS, hit(delay)-TMS, all-TMS and no-TMS) as the between-subjects factor revealed a main effect of Timepoint ($F_{3, 53} = 699$, $p < 0.0001$), but did not contain a main effect of Group ($F_4 = 0.06$, $p = 0.99$), nor a Timepoint X Group interaction ($F_{12, 165} = 1.68$, $p = 0.98$). The lack of a difference in group means

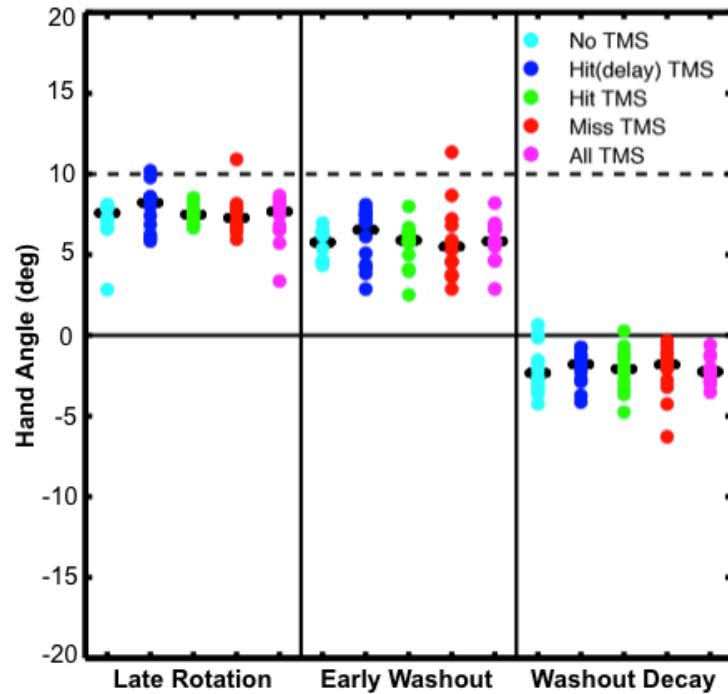


Figure 26: Endpoint Feedback Learning Metrics - bias from baseline removed, dots represent individuals, black line is median of group

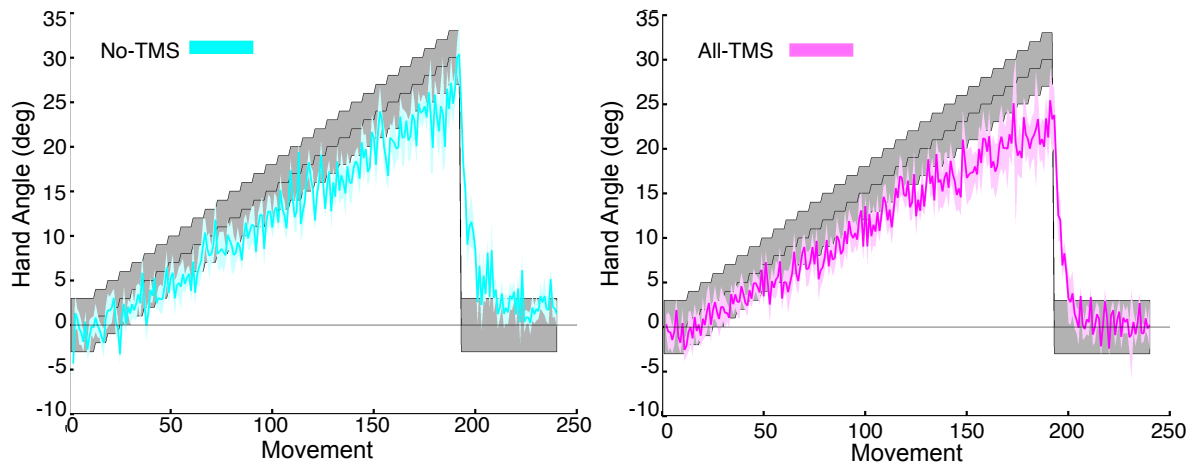


Figure 27: No-TMS (left) and All-TMS (right) Endpoint feedback in washout
Average hand-target angle
(shaded region represent SEM)

was confirmed by performing ttests between the groups comparing the mean hand angle for each of: the first and last 16 trials of the adaptation phase, the first and last 16 trials of the washout period, and the whole washout period, none of which were significant.

The results presented above were surprising. The original Hadipour study (Hadipour-Niktarash et al., 2007) had found when TMS pulses were delivered on every trial the aftereffect decayed more quickly than when compared to no TMS and a 700ms delay TMS condition. An important difference, however, is that in the original study participants continued to receive endpoint feedback during the washout period. We therefore ran two additional groups of subjects, with feedback given during the washout period, one with no TMS and one where TMS was delivered on every trial during the adaptation phase. These groups provide a closer match to the two main groups of the original study.

Both the no-TMS (feedback in washout) and all-TMS (feedback in washout) groups performed well during the baseline phase (**Figure 27**). The no-TMS (feedback in washout) participants ($N = 7$) shifted their reach angles ($t_6 = 10.95$, $p < 0.0001$) to compensate for the perturbation, as well as showing a significant aftereffect ($t_6 = 5.77$, $p < 0.01$) which decayed ($t_6 = 5.41$, $p < 0.01$) by the end of the washout period (**Figure 28**). In the all-TMS (feedback in washout) group ($N = 10$) the same pattern of results was present. By the end of the adaptation phase a significant shift ($t_9 = 10.53$, $p < 0.0001$) to compensate for the perturbation was present, as well as an aftereffect ($t_9 = 3.85$, $p < 0.01$) that had significant decay by the end of washout ($t_9 = 7.97$, $p < 0.0001$) (**Figure 28**).

A mixed-effects repeated measures ANOVA using Timepoint as the within-subject factor and Group (no-TMS and all-TMS) as the between-subjects factor was performed and revealed a main effect of Timepoint ($F_{3, 13} = 147, p < 0.0001$), but did not contain a main effect of Group ($F_1 = 1.88, p = 0.19$), nor a Timepoint X Group interaction ($F_{3,13} = 0.65, p = 0.60$). From inspection the main effect of Timepoint is present due to participants adjusting their hand angle in order to compensate for the perturbation.

The difference in group means was assessed by performing ttests between the groups comparing the mean hand angle for each of: the last 16 baseline trials and last 16 trials of the adaptation phase, and the first and last 16 trials of the washout period, as well as a comparison of the means for all washout trials. The comparison of the means for all washout trials was the only ttest to reach the level

of a statistical trend with a one-tailed test ($t_{14} = 1.49, p = 0.08$): the mean for the no-TMS group was larger than the all-TMS group (**Figure 28**). However, if the drop from the point of adaptation is calculated for each subject by subtracting the mean of the washout trials from the mean of the last 16 trials of adaptation, the comparison of the means is not significant ($t_{14} = 0.25, p = 0.80$). That the drop from the end of adaptation to beginning of washout is not significant suggests that any differences that might be present, although none reach significance, are due to a difference in the amount that hand angles were shifted to compensate for the perturbation, rather than a difference in washout itself.

In summary, while all groups showed significant learning to compensate for the perturbation, as well as a significant aftereffect that decayed over time, no statistical differences were found between the groups. This demonstrates a failure to replicate the result from the original Hadipour-Niktrash et al paper, and at the very least, suggests a weak effect.

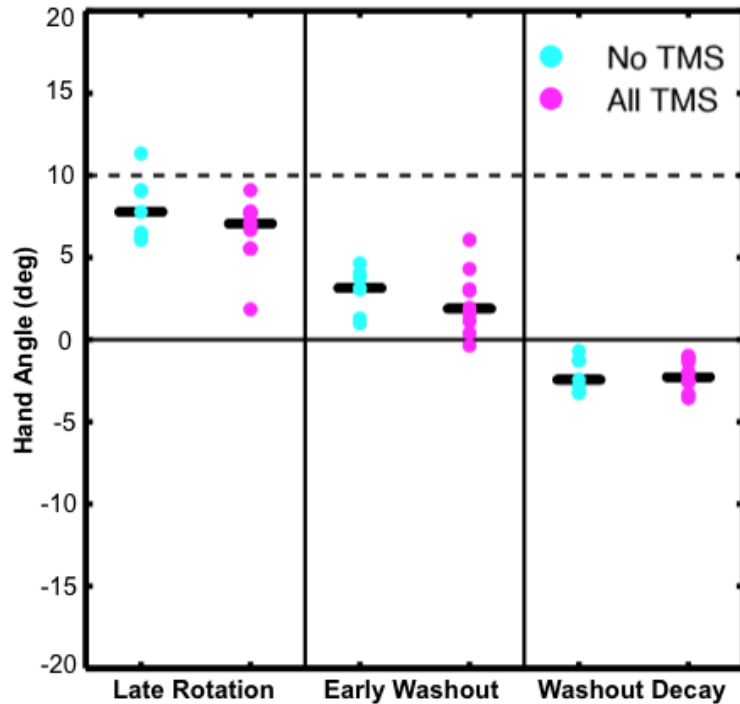


Figure 28: Endpoint Feedback In Washout Learning Metrics - bias from baseline removed, dots represent individuals, black line is median of group

3.6 Discussion

Transcranial magnetic stimulation (TMS) was used to explore the role of M1 in learning and retention of a new sensorimotor map during a visuomotor adaptation paradigm. Previous work had suggested that the cerebellum is playing a role in learning a new visuomotor transformation, while M1 is important for the retention of this new information. What had not been explored previously was the role that reward - hitting the target - and error - missing the target - are playing in the consolidation of sensorimotor learning in M1. A success only (experiment 3A) task and an endpoint error (experiment 3B) feedback task were used on two separate sets of subjects while TMS was applied timed to the endpoint of participants' reach. In experiment 3A a small gradual visuomotor rotation was applied to a virtual cursor while feedback was only given about success (hit) or non-success (miss) of reaching the target. Four experimental groups completed experiment 3A. In experiment 3B a larger gradual visuomotor rotation was applied to a virtual cursor while participants received endpoint cursor feedback. Five initial groups completed experiment 3B. After failing to replicate the previous Hadipour-Niktarash et al result two additional groups completed experiment 3B with the modification that endpoint feedback was delivered during the washout period.

For both experiment 3A and experiment 3B, we hypothesized that feedback from successful motor execution would be more important for M1 consolidation. Therefore, TMS pulses delivered immediately at the endpoint of the subject's reach would reduce the strength of the aftereffect, but only when those pulses were delivered on either successful reaches, or on all trials regardless of success in hitting the target.

Experiment 3A

The task used in experiment 3A was adapted from Izawa and Shadmehr 2011 (Izawa & Shadmehr, 2011). In the original experiment an aftereffect in changed reach angles was not measured, so the first goal in the current study was explore the presence of adaptation and of an aftereffect in the control group, no-TMS participants.

In the absence of TMS (No-TMS group), participants were able to learn to compensate for a visuomotor rotation, even though feedback only provided information about the success or failure in reaching for the target. There was also the presence of a significant aftereffect. In contrast, when TMS was applied over M1, none of the miss-TMS, hit-TMS or hit(delay) TMS control group showing significant adjustment for the perturbation. This highlights one important issue with the use of TMS: the difficulty for a control for the effect of applying TMS, especially in M1 stimulation, regardless of any neural effects. Stimulation of M1 causes a significant twitch of the targeted muscles, which may serve as a mildly aversive stimulus. At the completion of both experiment 3A and experiment 3B participants who received TMS were asked to give a rating of 1-10 of how large a twitch they had when the pulse was delivered, as well as a rating of 1-10 of how aversive they found the twitch. Neither the twitch (mean, 5.6 ± 0.2) nor aversive (mean, 4.5 ± 0.3) ratings were particularly high. However, a highly significant Pearson

correlation was present between participants' twitch and aversiveness ratings ($r = 0.48$, $p < 0.0001$). The standard control of using a different site for TMS stimulation would not be sufficient as any other site than M1 would result in little or no (depending on the distance from M1) resulting twitch. This problem may be especially present when pairing stimulation with a subset of trials, as an aversive stimulus is also being paired with that trial.

In the current experiment if participants were finding the TMS aversive, one would expect the pairing of the TMS pulse to reduce learning when it is paired with the subject successfully hitting the target, perhaps even causing reaches to move away from the target. This was the case in the hit-TMS and hit(delay)-TMS groups. However, it should also have caused subjects to avoid missing the target in the miss-TMS group, where no adaptation was measured. It may be that stimulation in general impairs learning due to a drop in motivation for participants, although a possible interesting effect was seen with aftereffects. In the hit-TMS group, one possible marker of the aversive nature of TMS was found, where despite no initial aftereffect being present, participants tended to decay away from the direction of compensating for the visuomotor rotation. This suggests that hit-TMS participants were actually learning to move away from the direction necessary to successfully hit the target. It should be emphasized that this interpretation is entirely post hoc. This possible effect does not appear to be specific to the TMS being timed to immediately at the endpoint of the reach as the hit(delay)-TMS control group also had a trend to shift away from the direction of compensating for the perturbation, despite no measurable adaptation or initial aftereffect being present. The initial hypothesis was that TMS applied immediately at the endpoint of a successful reach (Hit-TMS) would result in a faster decay in the aftereffect. However, this hypothesis was not able to be explored due to the lack of sufficient learning in all the TMS groups.

Two possible explanations for this lack of a finding led to experiment 3B. One possibility is that the learning that occurs during success only feedback is not through the same cerebellar mechanisms used in learning from endpoint feedback. Second, in order to have participants learn a perturbation with success only feedback, it is necessary to use a small rotation. Perhaps this made it difficult to detect an effect.

Experiment 3B

Experiment B used the task introduced by Hadipour-Niktarash et al 2007 (Hadipour-Niktarash et al., 2007), but modified to allow for the selective disruption of success and error trials. In the original study the retention of the adaptation was found to be lower, in the form of a more quickly decaying aftereffect, when TMS pulses were timed exactly to the endpoint of a participant's reach, compared to when the pulse was delayed 700ms, or no TMS pulse was delivered. A key difference between the original task and the one used in experiment 3B was that in the current experiment no feedback was given during the washout period, to allow for a measure of pure decay of the aftereffect, rather than interference in learning to compensate for the perturbation being removed.

All five initial groups (hit-TMS, hit(delay)-TMS, miss-TMS, all-TMS and no-TMS) significantly adapted to the perturbation, as well as having a significant aftereffect that decayed by the end of the washout period. Importantly, no differences were found in performance between the five groups, in either of the baseline, adaptation or washout phases.

The results reported for the all-TMS and no-TMS groups fail to show the same effect on consolidation seen in the original paper. Two additional groups were tested in an attempt to replicate their results. In the current study feedback was originally withheld during the washout phase in order to get a more pure measure of the decay, which was uncontaminated by learning to go back to baseline. In order to provide a pure replication a second no-TMS group and a second all-TMS group participated, however, this time participants continued to receive endpoint feedback during the washout period. The no-TMS (feedback in washout) and all-TMS (feedback in washout) groups both had significant adaptation and an aftereffect that significantly decayed. Importantly, while weak statistical evidence was found for a difference in washout between the groups, it was likely present only due to differences in the amount of adaptation between the two groups, as when the drop in washout is calculated by subtracting the mean of the first 16 washout trials from the mean of the last 16 adaptation trials, there is no difference between the groups. It is possible that with the collection of additional data the possible presence of a weak effect would be strengthened. However, it does appear that the result of Hadipour-Niktarash et al 2007 may have been contributed to by a non-significant difference in learning before the washout phase began, and most importantly, the effect is certainly only present when feedback is given in the washout phase.

General discussion

Drawing from previous work showing that the cerebellum was important in learning a new sensorimotor transformation, while M1 retained this new information, we hypothesized that M1 was consolidating a successful movement plan. By expanding on this theory, and applying other recent stimulation studies, we hypothesized that when TMS was applied over M1 only on trials where the target was successfully hit, the retention of the motor learning would be degraded. The expected result of this weaker retention was a faster decay of the aftereffect during a washout period. Importantly it was thought that the reduced retention would only be present when the pulses were delivered immediately timed to the completion of the reach on hit trials, but not when the pulse was delayed. An alternative possibility is that M1 is consolidating information received from an error signal. If this was the case then the reduced retention effect would only be found on miss trials.

In experiment 3A success only feedback was given. Participants who received a TMS pulse only on trials where the target was hit, not only appeared to not adjust their reach angles to compensate for the perturbation, but if anything, learned to reach in the opposite direction, regardless of whether the pulse is timed immediately to the endpoint of a participants reach, or delayed 700ms. This is exemplified by an aftereffect that

decays away from straight ahead in both the hit-TMS and hit(delay)-TMS groups. It should be noted though that the miss-TMS group did also have a milder decay away from countering the perturbation. In contrast, in the no-TMS group significant adaptation with a decaying aftereffect was found to be present. Previously, using the same success only feedback paradigm, it was found to lead to changes only in action selection, but not in perceived hand position (Izawa & Shadmehr, 2011). That action selection can also show a gradual decay brings about the possibility that adaptation effects seen in previous studies may not entirely be due to sensorimotor adaptation, but also to changes in aftereffect. Thus, just showing a decay of the aftereffect may not be sufficient to rule out an influence of changes in action selection.

In experiment 3B when subjects received endpoint feedback during the adaptation phase and no feedback during washout, all groups learned to counter the rotation, but no measurable effect of TMS was found on either learning nor retention. Finally, endpoint feedback was added during the washout phase, in order to provide a pure replication of a faster decaying aftereffect when TMS was delivered timed to the end of each reach (Hadipour-Niktarash et al., 2007). While very weak evidence for replication was found, however, if reliable, it appears largely due to a difference in asymptotic learning, rather than differences at washout. The results of the attempt to replicate combined with a failure to extend the faster decay to specific trial types, suggests that the original result is more specifically related to interference from turning off the perturbation, than a specific impairment in retention of the newly sensorimotor transformation.

In conclusion while no evidence was found for our intended hypotheses we have instead shown here that when TMS over M1 is used to target specific trials during a visuomotor adaptation task the aversive nature of the TMS manipulation itself can have profound effects on subject behavior. Interestingly, in comparing the results in experiment 3A to those in experiment 3B, there is a suggestion that the mildly aversive nature of the TMS stimulation itself might be overcome by giving a salient visual error. Finally, the importance of properly controlling for the aversive nature of TMS stimulation itself, as compared to any resulting neural effects, should be stressed. This is especially true when applying TMS to target specific trials, as both the aversive sensation and resultant neural disruption, are necessarily paired together for that trial. Without proper controls it would be easy to misinterpret a result directly stemming from the peripheral results of TMS, as being driven by the intended neural effect of TMS.

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