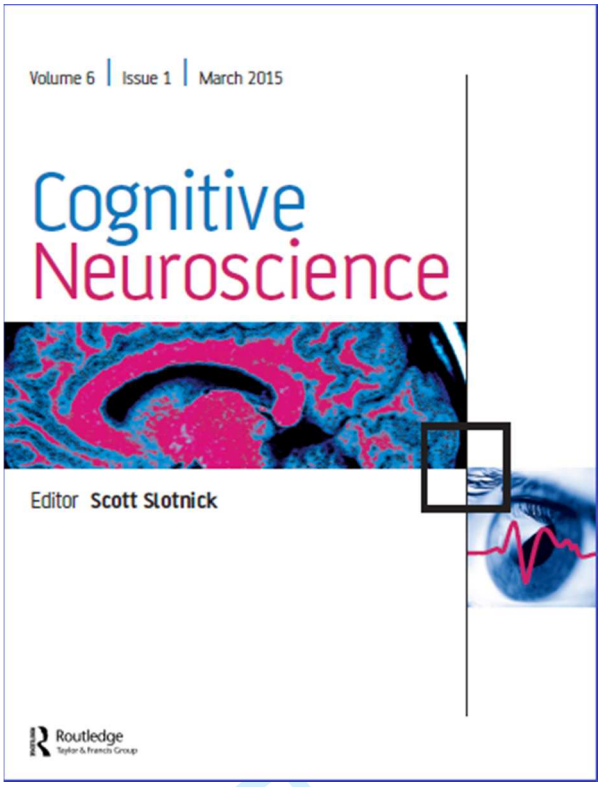

Epigenetic influence on practice induced performance change during cognitive tasks

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Epigenetic Influence on Practice Induced Performance Change During Cognitive Tasks [1]

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Abstract

Methylation has been shown to be a mechanism allowing experience to influence genes and behavior. We found that 7 year old children homozygous for the C allele in interaction with the COMT gene showed greater improvement in reaction time (RT) with practice on the Attention Network Test (ANT). This finding indicates that epigenetic effects may operate on or through genes that influence executive network operation. However, T present allele carriers showed faster overall RT and conflict resolution. Some children showed an initial improvement in ANT RT followed by a decline in performance, and we found that alleles of the DBH gene were related to this performance decline. These results suggest a genetic dissociation between improvement while learning a skill and reduction in performance with continued practice.

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Introduction

Most skills improve in speed and accuracy with practice (Fitts & Posner 1967). What role does gene expression play in these changes? According to a recent paper:

" Emerging evidence suggests that epigenetic mechanisms including DNA methylation are essential regulators of synaptic plasticity and experience dependent behavioral change"….(Day et al 2013).

Based on this idea we hypothesized that improvements in reaction time (RT) over successive sessions in a cognitive task might depend upon the presence of an enzyme important for methylation. A key enzyme in this process, methylene tetrahydrofolate reductase (MTHFR), catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which subsequently donates a methyl group to homocysteine. This methyl group is ultimately used in cellular methylation reactions, including epigenetic modification. Individuals homozygous for the T variant (677C>T) of MTHFR have a significantly reduced level of enzymatic activity that translates to lower general methylation levels in the genome of peripheral leukocytes and lower red blood cell folate levels (Stern, Mason et al. 2000). Studies of adult schizophrenic patients and healthy individuals have shown that the presence of this polymorphism blunts the activity of the prefrontal cortex, reduces the response to errors and reduces activity in the dorsal anterior cingulate (Roffman et al, 2008a,b, 2011).

In this research we examined 70, 7-8 year old children as they practiced a child appropriate version of the Attention Network Test (ANT) (Rueda et al 2004). The test is described in the methods section, but it uses the flanker task (Eriksen & Eriksen, 1974) as a measure of the time to resolve conflict and also measures orienting and alerting as other aspects of attention. Some of these children were part of an ongoing longitudinal study of the development of attention networks (Posner, Rothbart, Sheese & Voelker,2014). At age 7 the children were able to carry out three sessions of the ANT within a two week period. The conflict score of the ANT is obtained by subtracting RTs in congruent flanker trials from those in incongruent trials and is associated with activation of the anterior cingulate (ACC). Conflict scores improve with development up to ages 7-8 (Rueda et al 2004). A study of children and young adults from 4 to 21 has shown that up to age 7 resolution of conflict correlated with the size of the ACC (Fjell et al 2012). Beyond age 7 improvements in reaction times in the ANT were largely due to changes in white matter efficiency connecting the anterior cingulate to other brain areas.

Improved reaction time, such as that occurring during repeated measurement of the ANT has long been thought to involve selection of the most appropriate action to improve the speed of response (Fitts & Posner, 1967). Thus we expected to find an initial

improvement in reaction time in our children. We hypothesized that if this improvement involved methylation, it would be lower for those children who had the T allele of MTHFR in comparison to those with the CC genotype because the T allele would be associated with less efficient gene regulation during the learning process.

Methylation has been associated with the regulation of some genes facilitating neural function that are expressed in the brain areas being studied (Ursini et al, 2011; Zhao et al, 2013; Swift-Scanlan et al, 2014). In particular, performance on the flanker and other conflict related tasks have been shown to involve the COMT gene (Blasi et al 2005, Diamond et al, 2004). In some studies that effect was specific to the executive attention network (Fossella et al 2002). In our longitudinal study we have shown that the COMT Val^{158}Met genetic polymorphism, and haplotypes of the COMT gene related to pain reduction (Diatchenko, et al 2005,2006), influenced executive attention in childhood (Voelker, et al 2009). Thus our second hypothesis was that genetic variation in the MTHFR gene would interact with COMT in influencing overall reaction time in the ANT.

Based on our previous studies with infants and children we expected a decrease in maintaining attention during repetitive practice (Kieras, 2006). Thus an increase in RT after repeated practice might reflect a difficulty in maintaining motivation and attention over time. Difficulty in maintenance of attention has been associated with the alerting network of the ANT. This network is modulated mainly from the locus coeruleus norepinephrine system. The dopamine β-hydroxylase (DBH) gene product converts dopamine to norepinephrine, and one variation of this gene has been found to be related to maintaining attention (Greene, Bellgrove, Gill and Robertson; 2009). A different polymorphism is related toworking memory performance (Parasuraman, Greenwood, Kuman & Fossella, 2005). For these reasons we hypothesized that increases in reaction time with long continued practice might be influenced by variation in the DBH gene.

In short we hypothesized that improved performance over time would be associated with more efficient methylation and increased neurotransmitter activity (interaction of MTHFR and COMT) and that reduced performance after longer periods of practice would be related to modulation by norepinephrine (DBH). The ANT has a measure of conflict resolution that has been shown to relate specifically to the executive network. We use the conflict measure and a child friendly behavioral task to examine these genetic influences on the executive attention network.

Materials and Methods

Subjects: Seventy children, 44 from our ongoing longitudinal study (Rothbart et al 2011), were recruited at 7-8 years of age (*M*=93.4 months, *SD*=13.1 months) (63% male). 36 were newly recruited to the study. Three children were re-recruited after an absence in participation in the study, and the remaining 31 had attended the previous year's session. 75.7% of the children were white, 10% Hispanic, 2.9% African American, 1.4% asian, 1.4% with Native American heritage and the remaining 8.6% were of mixed ethnicity. Genetic information was collected from 68 subjects.

Behavioral measures:

ANT training: Three training sessions were attended within a two week period, separated by at least one day. 68 of the subjects attended all 3 sessions, two attended only one session. Two thirds of the data was missing for one child's session, and the remaining one third was used to represent this time point. The child version of the ANT was administered as a computer game. Each session began with a brief practice before the testing process. Three sets of 32 targets weredisplayed as different animals, half pointing left and half right, with flankers either congruent or incongruent with the target. Prior to the target one of four cue conditions were presented in randomized order: center cue , double cue , spatial cue at target location, and no cue. The flankers were incongruent to the target for half of the trials and congruent for the other half.. The child pressed one of two buttons to designate the head of the target animal, RT and errors were recorded. Median reaction times for correct trials longer than 100 millisec were computed for each person and the overall mean of these medians are presented in the Tables and Figures.

HTKS Task

The head-toes-knees-shoulder (HTKS) task (Pontiz et al 2008, McClelland et al, 2014) is a modification of a 'Simon Says' game, where points are earned for correct movement towards a body part. This is a conflict task since the rules direct the child to touch a specific body part different than the one named. (ie. in response to the command 'touch your knees', the child touches their shoulders during a correct trial). The HTKS also challenges attention, inhibitory control and working memory. The HTKS version used in this study had 3 blocks of 10 trials, where the first block had two rules, the second block added two more rules (total =4), and the final block had 4 rules, where the previous rules are switched to a new arrangement. This sequence places increasing cognitive demand on the child by block. The task was presented over two sessions, where the first block was performed in session 2 and the remaining blocks in session 3. In a trial, two points were earned if the child touches the correct body part directly or after pausing. One point was earned if a child touched the correct body part but moves their hands first toward a different body part . The HTKS score is the total point number out of a possible total of 20 points per block.

Genotyping: Saliva was collected from 67 subjects using Oragene DNA collection kits (DNA Genotek Inc, Ottawa, Canada) and one buccal sample was taken using a swab. Two subjects did not contribute to the genetic analysis. The samples were processed following the Oragene protocol. The MTHFR locus was amplified using 10μM each of the following primers, 5'-CGAAGCAGGGAGCTTTGAGG and 5'-AGGACGGTGCGGTGAGAGTG, and the following conditions: 2mM each deoxynucleotide, 1.5mM $MgCl_2$, 1.25 units Taq DNA polymerase (recombinant, Thermo

Scientific, USA) with its 1x $(NH_4)SO_4$ buffer, and approximately 10ng of DNA. The amplification conditions were as follows: 94°C 3 min, 40x(94°C 30 sec, 56°C 30 sec, 72°C 30 sec), 72°C 3 min. The resultant products were digested with HinfI (NEB, USA) at 37°C and size-separated on a 1.5% agarose gel to reveal 233 bp (C allele) and 57/176 bp (T allele) products. DBH was amplified with 10μM each primer (Cubells et al 1998), with the following differences from the MTHFR amplification, 3mM $MgCl_2$ and a 60°C annealing temperature. The products were digested with EcoNI (NEB, USA) and gel-separated to identify 207 bp (A allele) and 38/169 bp (G allele) fragments. The COMT haplotype was determined following Voelker et al, (2009).

Genes, allele freqs,

Gene*	snp	minor allele frequency
MTHFR	rs1801133	33.1% T
DBH	rs1108580	45.6% A
COMT	rs4680	44.1% G

- The frequency of alleles did differ significantly from the global MAF but not from the North American MAF

Results

Behavioral Effects

Adult studies have shown clear improvement over several sessions in both ANT reaction times and the measures of conflict obtained by subtracting congruent RT from incongruent RT (Ishigami & Klein,2010; 2011). Table 1 shows that in our study average reaction times declined from Day 1 to Day 2 and slightly increased from Day 2 to 3. An Analysis of Variance showed a difference in reaction time between sessions (F (2, 203) = 4.65, MSE = 64435.05, p = .011) and significant change in reaction time over sessions within subjects (F (2, 134) =16.08, MSE = 65332.61, p < .001), where we see a significant change in reaction time between sessions 1 and 2 (F (1, 67) = 47.15, MSE = 232420.59, p < .001). There is no significant difference between sessions 2 and 3 (F (1, 67) =1.23, MSE = 8798.56) but below we show a significant interaction between the upswing and a genetic effect

INSERT Table 1 About here

In previous studies with young children we found that repeating a task over many trials often led first to a decrease in RT due to practice but later there was an increase as the children began to find the task very boring and tiresome (Kieras, 19.). We did not assume that positive practice effects ended, but that they were not sufficient to overcome

slowing due to reduced motivation. In support of this general idea we found no significant correlation between RT change from Day 1 to 2 and the change from Day 2 to 3 (r (68) = .019). In the present study we could view the scores over the three days as reflecting an unknown combination of improvement in practice and reduction in performance with lost motivation. Below we discuss genetic effects that may support this separation.

Conflict

The mean conflict score for each session of the ANT is shown in Table 1. Lower scores represent better performance in resolving conflict. In a repeated measures analysis, conflict scores improved significantly (F (2, 134) = 3.21, MSE = 5498.54, p = .043). The contrast between sessions 1 and 2, showed a significant improvement (F (1, 67) = 5.30, MSE = 17713.33, p = .024), while between sessions 2 and 3 there was little change (F (1, 67) =.036, MSE = 97.68).

Genetic effects

Table 2 shows the relation of the major behavioral findings to alleles of the three genes that we measured and hypothesized to be related to performance, DBH, MTHFR, and COMT.

INSERT TABLE 2 and Figure 1 ABOUT HERE

MTHFR X COMT

As shown in Table 2 individuals with the CC genotype of MTHFR showed more improvement over days than those with a genotype that included the T allele. Over the three sessions, the improvement was marginally significant (F (2, 128) = 2.85, MSE = 10855.69, p = .062). A within-subjects contrast shows that the difference in reaction times between sessions 2 and 3 interacted significantly with MTHFR (F (1, 64) = 4.15, MSE = 25210.97, p = .046). However, as shown in Figure 1 the differential improvement in RT for the CC group with practice was only found for those children with the AA genotype of COMT. A repeated measures ANOVA including both COMT and MTHFR genotypes for the 3 sessions showed a main effect of MTHFR (F (2, 124) = 6.59, MSE = 23528.26, p = .002) and a significant interaction between MTFHR and COMT (F (2, 124) = 5.72, MSE = 20410.02, p = .004).

However, reaction times were faster during Day 1 and 2 for the carriers of the T allele. The superior RT for those with the T allele was surprising because a lowered level of methylation found with the T allele has been related to mental and physical illness (Roffman, et al 2008 a,b) and reduced performance (Hoffstetter et al 2013). In support of this idea the T group had slightly higher error rates on Day 1 and 2 than the CC group (see Table 2) which was non-significant (F (1, 64) = 1.22, MSE = .001). The children with a T allele of MTHFR and AA genotype of COMT also had a slightly lower overall error rate than the other groups.

INSERT FIGURE 2 ABOUT HERE

Role of DBH

Another feature of the RT data was the overall longer RTs found on Day 3 than Day 2. While overall the upswing was not significant, there was a significant difference between session 2 and 3 RTs for the two allelic groups of DBH (F (1, 64) = 4.74, MSE = 14272.94, p = .03). As shown in Figure 2 the increase in RT from day 2 to 3 occurred only with individuals homozygous for the G allele. There was also a significant interaction between DBH and COMT on the RTs between Day 2 and 3 (F (1, 62) = 4.21, MSE = 11899.01, p = .04) where a strong upswing in RT occured only for the COMT AA group.

In addition, the strong practice effect from Day 1 to Day 2 produced an interaction between DBH and MTHFR (F (1, 62) = 5.77, MSE = 13482.99, p = .02); the GG genotype of DBH showed a reduced practice effect when combined with the MTHFR high methylation allele (CC) but not otherwise. This suggests that the influence of waning attention is found from the start of practice and not only during the upswing in RT on day 3.

In agreement with the RT data the ANT conflict scores show more improvement for those homozygous for the high methylation (C) allele of MTHFR when also homozygous for the A allele of COMT. However, overall better conflict resolution is shown by the T present allelic group of MTHFR. This results in a significant interaction between MTHFR and COMT over the 3 sessions (F (2, 124) = 4.57, MSE = 7537.75, p = .01), similar to what is shown in Figure 1 for RT.

INSERT FIGURE 3 ABOUT HERE

HTKS

There was no main effect of MTHFR on HTKS score nor any interaction between MTHFR and COMT as found for ANT reaction time. However, there was a significant within-subjects effect between COMT genotype and HTKS score, where the performance of AG/GG individuals declined significantly from blocks 2 to 3 and that of the AA group remained high (F (2, 130) = 3.79, MSE = 45.34, p = .03). As shown in Figure 4, the AA group better maintained scores in the face of higher levels of conflict. In addition, there is a significant main effect of DBH (F(1,63) = 4.07, MSE = 64.21, p=.048) and interaction between DBH and MTHFR (F(1,63) = 5.66, MSE = 89.33, p = .02) on the change in HTKS score between blocks 2 and 3. The DBH GG individuals had a decreased score, and the MTHFR CC by DBH AG/AA individuals maintained their performance with complexity change, while those with other genotypes showed a decline.

Discussion

Skills generally improve in reaction time and accuracy with practice (Fitts & Posner, 1967). For many skills the extent of improvement slows down as practice continues yielding a power function of reaction time with amount of practice (Anderson, Fincham & Douglass, 1999; Fitts & Posner, 1967; Newell & Rosenbloom, 1981). The power function suggests a single underlying process of improvement (Delaney, Reder, Staszewski &Ritter 1998). Newell & Rosenbloom (1981) proposed that a single process of chunking together responses was responsible for the improvement from the start of learning. In the ACT theory (Anderson, 2007) the power function is thought to emerge from a uniform increase in strength with repetition of procedures. Although some have argued that an exponential function fits better than the power function (e.g Heathcote, Brown, & Mewhort 2000) there is general agreement on a monotonic function relating RT and practice.

However, in some cases the improvement in reaction time may be followed by an increase in RT with further practice. The increase in RT with practice is often attributed to reduced motivation or attention as interest in the task declines. We have found this upswing in RT to be particularly strong in children (Kieras, 2006). Based largely on the performance of rats in mazes, Hull (1943) proposed performance of a task might lead to the build up of a reactive inhibition which would work to reduce habit strength and lead to a temporary increase in reaction time. Consistent with Hull's idea we found a significant increase in RT from Day 2 to Day 3 for children with an allele of the DBH gene that leads to reduced attention. That allele also reduced the practice effect from Day 1 to Day 2 suggesting that, like reactive inhibition, it is present even when overall improvement occurs due to practice.

Individuals differ in both the rate of improvement and in the likelihood of showing an increase as practice continues. We have found that the improvement in RT in 7 year old children is related to a gene that influences executive attention (COMT) in interaction with a gene that effects the efficiency of the process of methylation. We find that the CC genotype of MTHFR, which provides better overall methylation, shows improvement in RT over the three sessions. This learning effect occurs in interaction with COMT, suggesting that methylation works upon genes associated with cognitive performance. Similarly, the MTHFR CC genotype was associated with better HTKS performance under increased cognitive demand in interaction with DBH.

There have been several recent studies relating MTHFR and COMT to performance in cognitive tasks in normal adults and schizophrenic patients. In one study (Kontis et al 2013) the MTHFR T allele reduced the negative effects of the AA version of COMT on performance and improved the performance of G carriers. On the other hand Roffman and associates (2008a,b) showed that for those with the AA genotype of COMT performance was worse if they also had the T allele of MTHFR In our view such discrepancies may arise because participants are at very different levels of prior exposure to tasks and the findings confound learning in the task with their performance at a given

moment. In accord with theories of how epigenetic effects work (Day et al 2013) we attempted to examine specific influences of genetic variation on learning with practice over three days.

Methylation and Behavior

In accord with our hypothesis individuals with the T mutation of MTHFR showed less improvement over the sessions than those homozygous for the C allele. The T mutation presumably reduced learning by providing reduced opportunity for methylation. As shown in Figure 1 this effect was driven by the AA genotype of the COMT gene. Thus children with low methylation efficiency and less efficient dopamine degradation showed little evidence of improved performance with practice. We also examined a haplotype of the COMT gene related to high and low pain levels (Diatchenko et al, 2005,2006) which we reported earlier is related to performance during infancy (Voelker et al 2009). In the current study the genotype and haplotype showed similar results so we reported only the genotype in this paper.

However, the clear advantage of the MTHFR CC genotype in the presence of the AA genotype of COMT for learning is reversed if one looks at RT performance on Day 1 alone. In this case the CC genotype is much worse overall than for those children who have a T mutation present and are in the COMT AA allelic group (See Figure 1).

The lack of a practice effect when the T allele is present might be due to a floor effect on RT. However, in Table 1 RT for other groups are faster than for the T present group on Day 1 and all groups show improvement on Day 2.

Another possible explanation for the faster RTs for children with the T allele may occur because of a tendency toward impulsivity, since it has been reported that children who have the T mutation have elevated levels of ADHD (Gokcen, Kocak, & Pekgor 2011). Children with ADHD often show impulsivity as a trait. The somewhat higher error rates for carriers of the T allele on Days 1 and 2 (see Table 2) provides some support. However, those children with the MTHFR T allele and the COMT AA genotype who showed fast RTs also show a slightly lower overall error rate. Their combination of fast RTs with reduced error is clearly inconsistent with a general impulsivity of those with the T allele.

Studies showing poorer performance of participants with the T allele involve adults. It is possible that the difference in age between our study and other studies may account for the advantage of those with the T allele in overall reaction time and conflict resolution.

Attention and Persistence

A second feature of the ANT data was the upswing in RT between Day 2 and 3. It is common for children to show a performance to peak at some time and then to show a reduction, probably due to reduced attention and motivation **(**Kieras, 2006**).** The DBH GG genotype shows a significant increase in RT between Day 2 and Day 3. The GG

genotype was also associated with a decline in performance of the HTKS with increased challenge. Other studies have implicated polymorphisms in this gene in the lack of persistence during RT tasks (Greene, et al 2009). In fact we found that this DBH polymorphism is most related to slower responding when no cue is given, a condition that has been associated with lower tonic alertness (Posner, 2008). Since the brain mechanisms of tonic alertness have been associated with the locus coeruleus brain's norepinephrine system, studies linking motivation to continue the task and attention networks might be useful in understanding the neural basis of motivation.

The finding of no significant correlation between the improvement in RT from Day 1 to Day 2 and the increase between Day 2 and 3 provides some support for separating these two features of practice based on their opposite effect on overall RT. However, it seems unlikely that the factors of improved performance with practice and diminished performance with reduced motivation are occurring at completely separate times. In one common theory of learning effective performance at any time is a combination of habit strength from practice and reactive inhibition based on repeated trials (Hull, 1943). Our finding of an influence of DBH in conjunction with MTHFR on improved performance (day 1 to day 2) as well as the upswing in RT (Day 2 to Day 3) generally supports the idea of both improvement due to practice and reduction due to attention throughout performance and suggests that no single factor can account for the power function often found in RT with number of trials.

Mechanisms of change

Fjell and colleagues (2012) have shown that reaction time of children and young adults in the flanker task depends heavily on the functional connectivity between the ACC and other areas. Recent work in mice shows that learning motor skills depends upon the activation of ogliodendrocytes leading to improved myelination (McKenzie et al 2014). A recent study in rats demonstrated changes in gene expression related to gene methylation status in the ventral tegmental area during reward related learning. Learning was inhibited in the presence of a DNA methyltransferase inhibitor (Day et al 2013). Hypermethylation within the gene body was shown to be associated with increased gene expression in a neuronal activity-dependent manner. This hypermethylation was required for learning and not subsequent memory retrieval. MTHFR may be playing a key role facilitating this learning-based mechanism and one possibility is that it may be regulating COMT and other genes in the dopaminergic pathway in a similar manner as shown for plasticity genes of the VTA. Thus, more efficient MTHFR activity better supports learning by facilitating gene body methylation in genes relevant to the learning process.

Why is MTHFR working for COMT AA and not for COMT G carriers? Individuals homozygous for the lower activity allele (AA) have been associated with better cognitive performance, presumably because higher synaptic DA levels would have greater opportunity for DA signaling and thus enhance neuronal activity. If learning requires gene methylation in an activity-dependent manner, and COMT AA individuals have more activity, there would be more potential for gene modification. This, combined with

MTHFR CC makes it possible for maximal gene methylation to occur and support the strongest response to learning. This mechanism would suggest that MTHFR and COMT work in concert to provide the optimal environment for learning-related gene regulation, and would not necessarily implicate MTHFR in the modification of COMT expression.

Our study shows important differences between MTHFR groups in the effectiveness of practice in improving reaction time within two hours. It appears that the efficiency of white matter may be changed rapidly by spatial training (Hofstetter et al, 2013), working memory (Takeuchi et al 2010) or meditation (Tang et al 2010). The role of glia in the production of myelin is well documented and activated axons transmit signals to neighboring glial cells, thereby promoting myelination (Hofstetter et al 2013). The studies of mice show clearly that potentiation of ogliodendrocytes is one necessary condition for skill learning (McKenzie et al 2014). We have hypothesized that a similar mechanism may operate in improved white matter following brief meditation training in humans (Posner, Tang & Lynch, 2014). These studies suggest that improved reaction time with practice found in our study could arise by improving the efficiency of white matter between the ACC and motor regions.

The one-carbon folate cycle, of which MTHFR plays a major role, is tightly regulated and supports many crucial processes that play a role in learning, including neurotransmitter function and epigenetic regulation. Changes in DNA methylation coincides with the maturation of neural progenitors and methylation factors have been shown to control the timing of astrogliogenesis (Teter et al, 1996; Fan et al 2005). Diseases resulting in demyelination, such as Alzheimer's disease and multiple sclerosis, show differences in DNA methylation patterns in the brain (Bakulski et al, 2012; Huynh et al 2014). We propose that differences in MTHR activity influences individual differences associated with DA signaling, through changes in the expression of genes that support learning, and that these changes ultimately result in possible differences in neural myelination. Since the one-carbon folate cycle influences many cellular functions, future research should address the specific mechanism(s) of methylation responsible for differences in learning.

We believe our findings indicate that practice on a task involves both improvements in reaction time, due in part to improved myelination of relevant pathways and decrements in performance due to lowered levels of alertness to the task. Individual differences in these practice effects are partly due to the efficiency of epigenetic methylation leading to differences in the rate at which practice changes performance. However, our current methods do not allow elimination of the possibility that MTHFR works via a different mechanism than modulation of the genome or that a correlated genetic influence might be responsible for these effects. Animal studies may be able to show more directly the exact mechanism involved in these findings. Moreover, future studies will be needed to examine the generality of these findings to different ages and types of performance.

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Table 1.
Mean of median RT (msec), conflict scores (msec) and error rate by session of the ANT

session	RT mean (conflict)		RT SD	error rate
1	824	(59)	123	.03
2	766	(43)	107	.03
3	777	(45)	123	.03

Table 2.
ANT RT by session and genotype (Number of participants)

	session	DBH GG	DBH GA/AA	MTHFR CC	MTHFR CT/TT	COMT AA	COMT AG/GG
RT	1	814 (20)	826 (48)	841 (31)	807 (37)	802 (20)	831 (48)
	2	747 (20)	773 (46)	778 (31)	755 (35)	743 (20)	775 (46)
	3	787 (20)	768 (46)	765 (31)	781 (35)	736 (20)	790 (46)
error rate	1	.023 (20)	.030 (48)	.023 (31)	.032 (37)	.032 (20)	.026 (48)
	2	.027 (20)	.030 (46)	.027 (31)	.031 (35)	.028 (20)	.030 (46)
	3	.022 (20)	.029 (46)	.027 (31)	.027 (35)	.030 (20)	.026 (46)

Figure captions

Figure 1 ANT RT by MTHFR x COMT genotype for each session of training for (A) COMT AA individuals and for (B) COMT AG/GG individuals

Figure 2 ANT RT by DBH genotype for each session

Figure 3 HTKS score by MTHFR genotype for each level of complexity (level 3 represents the highest conflict)

Figure 1 panel A

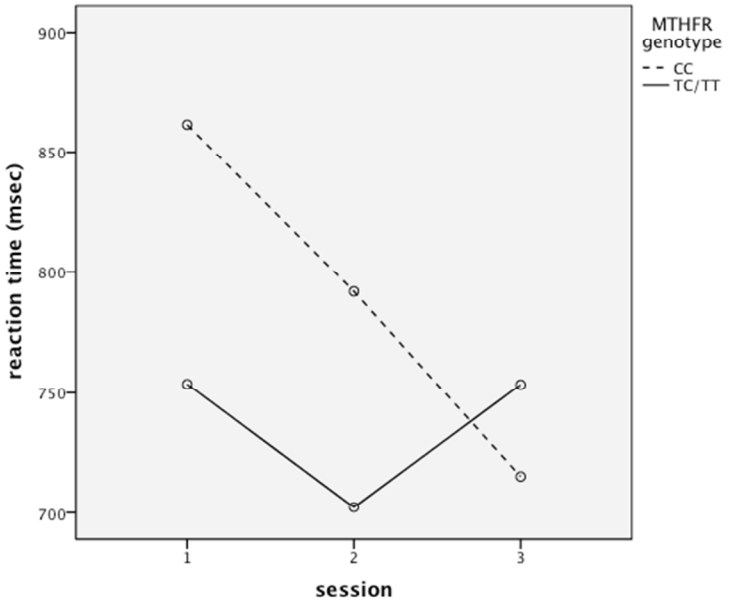

Figure 1 panel B

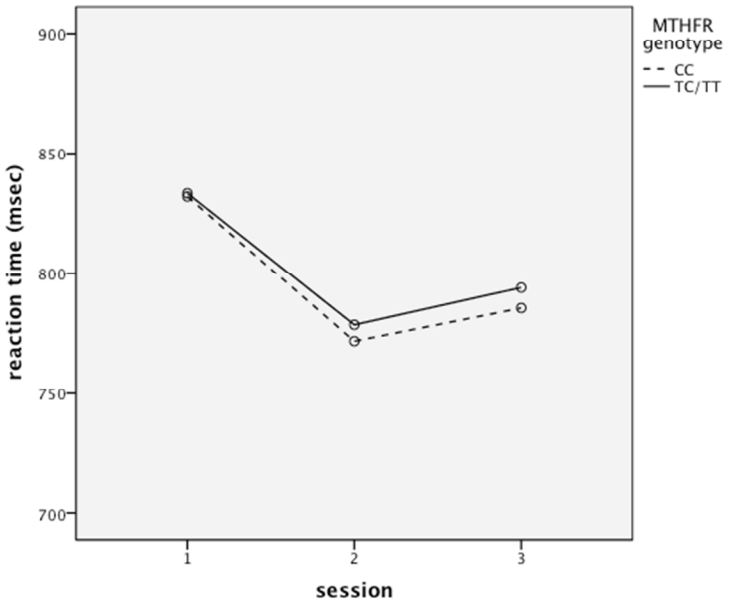

Figure 2

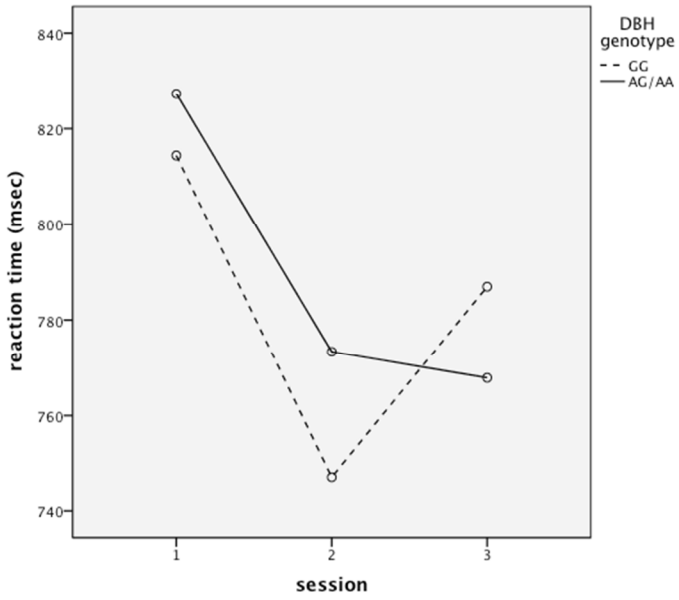

Figure 3