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Effects of monoaminergic drugs on training-induced motor cortex plasticity in older adults

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Abstract

Primary motor cortex (M1) plasticity is involved in motor learning and stroke motor recovery, and enhanced by increasing monoaminergic transmission. Age impacts these processes but there is a paucity of systematic studies on the effects of monoaminergic drugs in older adults. Here, in ten older adults (age 61 + 4 years, 4 males), we determine the effects of a single oral dose of carbidopa/levodopa (DOPA), D-amphetamine (AMPH), methylphenidate (MEPH) and placebo (PLAC) on M1 excitability and motor training-induced M1 plasticity. M1 plasticity is defined as training related long lasting changes in M1 excitability and kinematics of the trained movement. At peak plasma level of the drugs, subjects trained wrist extension movements for 30 min. Outcome measures were motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation at increasing intensity (stimulus response curve, SRC) and peak acceleration of the trained wrist extension movements. Measures were obtained before and after completion of training. The curve parameters plateau (MEPmax), inflection point, and slope were extracted from SRC. At baseline drugs had a differential effect on curve parameters, while kinematics remained unchanged. Training alone (PLAC) increased MEPmax but did not improve kinematics. Drugs affected training-related changes of the curve parameters differently, but did not enhance them or kinematics when compared to PLAC. The results demonstrate that in the older adults, MEPH, DOPA, or AMPH have differential effects on baseline M1 excitability and training-related M1 plasticity but fail to enhance them above the naïve level.

Keywords

Motor cortex; Monoaminergic drugs; Transcranial magnetic stimulation; Motor evoked potential

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1. Introduction

Motor training-induced plasticity in the primary motor cortex (M1) is a process involved in motor learning and in recovery of motor function after stroke (Bütefisch et al., 2014; Bütefisch et al., 2000; Dancause and Nudo, 2011; Karni et al., 1995; Nudo et al., 1996b; Pascual-Leone et al., 1994). Mechanisms that modify synaptic efficacy, such as long-term potentiation (LTP), are thought to be involved in training-induced M1 plasticity (Bütefisch et al., 2000). There is considerable interest in neurorehabilitation research toward harnessing training-induced M1 plasticity through brain stimulation and pharmacologic agents (Cramer et al., 2011). We have previously reported that non-invasive stimulation of M1 by means of repetitive transcranial magnetic stimulation (rTMS) enhances motor training induced M1 plasticity above the naïve level in older healthy adults (Bütefisch et al., 2015). Specifically, older subjects failed to demonstrate behaviorally assessed motor learning following a single training session. However, when rTMS was applied during the training in a very specific temporal relationship to the training movement, subjects demonstrated long lasting improvement (>60 min) of the trained movement.

D-Amphetamine (AMPH) facilitates the induction and retention of training-related M1 plasticity and reduces the amount of training required for the induction of these changes (Bütefisch et al., 2002; Sawaki et al., 2002). These findings are in line with reports of the facilitatory effect of AMPH on memory storage (Doty and Doty, 1966; Evangelista and Izquierdo, 1971; Krivanek and McGaugh, 1969; Soetens et al., 1993, 1995) and induction of LTP in-vitro (Delanoy et al., 1983; Gold et al., 1984). AMPH is a drug that promotes the presynaptic release of the monoamines noradrenaline, dopamine, and serotonin (Iversen and Iversen, 1981; Rothman and Baumann, 2003). Because training-dependent M1 plasticity after injury to M1 is thought to occur through similar mechanisms (Bütefisch et al., 1995b; Dancause and Nudo, 2011; Nudo et al., 1996a), the facilitatory effect of AMPH on M1 plasticity may contribute to the reported beneficial effect of AMPH on functional outcomes after brain injury (Walker-Batson et al., 1992, 1995). However, there are previous studies on stroke patients that did not find such beneficial effects of AMPH on M1 plasticity (Sonde et al., 2001; Treig et al., 2003).

AMPH exerts its effects through involvement of different monoamines. When co-administrated with haloperidol, a drug that blocks dopaminergic and noradrenergic receptors, the effect of AMPH on locomotor recovery in rats was blocked (Feeney et al., 1982). These findings suggest a role for dopaminergic or noradrenergic transmission in recovery. The beneficial effect of dopamine on cognition and memory formation has been demonstrated in human and animal studies (Floel et al., 2005a, b; Floresco and Phillips, 2001; Knecht et al., 2004; Meintzschel and Ziemann, 2006).

Dopamine also improved outcomes in stroke patients undergoing physical therapy (Scheidtmann et al., 2001). While there is no evidence that these behavioral improvement was mediated by effects of dopamine on M1 plasticity, there are reports of noradrenergic transmission related enhancement on M1 plasticity (Meintzschel and Ziemann, 2006). Taken together, these reports suggest that monoaminergic drugs enhance training-induced M1 plasticity. Although age impacts neuroplasticity and motor control processes (Mattay et al.,

2002; Talelli et al., 2008; Ward and Frackowiak, 2003), the effects of monoaminergic drugs on training-induced M1 plasticity in older adults have not been systematically investigated. Understanding the mechanisms of action of monoaminergic drugs in older adults and individuals poststroke is an important prerequisite to the development of pharmacologic interventions for enhancing M1 plasticity.

In the present study, in an older population of healthy adults, we tested the effects of carbidopa/levodopa (DOPA), D-amphetamine (AMPH), and the indirect noradrenergic agonist methylphenidate (MEPH) on training-related M1 plasticity using a randomized, double-blind, placebo-controlled, cross-over study design (Fig. 1). We hypothesized that drug-related increases of noradrenergic and dopaminergic transmission would enhance training-related M1 plasticity. M1 plasticity was defined as long lasting (>60 min) increases in M1 excitability and improved training kinematics of the trained movement. Enhancement of training-related M1 plasticity was defined as a larger training-related increase in M1 excitability and movement kinematics.

2. Results

Ten subjects (mean age 61 ± 4 years, 4 males) met all inclusion criteria and completed all experiments (Fig. 1). Data from one subject were excluded from analysis due to TMS-related saturation of the amplifier, resulting in inaccurate EMG measures in two of the four experimental sessions.

2.1. Effects of drugs on baseline M1 excitability and motor performance

Stimulus Response Curve (SRC) Data: The measured and fitted SRC curves are plotted in Fig. 2. SRC parameter estimates for all drug conditions and time points are listed in Table 1. At baseline, compared to placebo (PLAC), pairwise comparisons of SRC parameters revealed a significant reduction in MEPmax for AMPH ($p < 0.01$, effect size = -0.27 ± 0.05 , Fig. 2A). In contrast, DOPA resulted in an increase in MEPmax compared to PLAC ($p = 0.036$, effect size = 0.81 ± 0.28 , Fig. 2B). AMPH and DOPA had no significant effect on S50 and K. Compared to PLAC, MEPH had no significant effect on any SRC parameters (Fig. 2C).

The repeated-measures ANOVA with drugs as the independent variable showed no significant effect of the drugs on motor threshold (MT) at baseline. The repeated-measures ANOVA revealed no significant effect of the drugs on peak acceleration or reaction time at baseline (Fig. 4).

2.2. Effects of drugs on training-induced M1 plasticity and motor performance

2.2.1. SRC data—For each drug condition, SRC parameters immediately after training (post 1) were compared to SRC parameters at baseline (Fig. 3). For the PLAC condition, training resulted in an increase in MEPmax ($p < 0.001$, effect size = 0.62 ± 0.11) at post 1 compared to baseline (Fig. 3A). Training in the AMPH condition resulted in an increase of MEPmax ($p < 0.01$, effect size = 0.49 ± 0.08), slope ($p = 0.036$, effect size = -3.7 ± 1.22),

and S50 ($p = 0.052$, effect size = 4.77 ± 1.68) (Fig. 3B). Training in the MEPH and DOPA condition induced no change in SRCs (Fig. 3C and D).

Comparisons of training-related change in SRC from baseline to post1 between PLAC and each of the 3 other drug conditions revealed a smaller change in MEP amplitudes from baseline to post1 for DOPA compared to PLAC ($p = 0.009$). No significant differences in change in SRC curve from baseline to post1 were observed between PLAC and AMPH or MEPH.

2.2.2. Peak acceleration and reaction time—Training had no significant effect on the peak acceleration of wrist extension movements or reaction time in any of the drug conditions when post1 measures were compared to baseline using paired t -tests (Fig. 4). Further, the ANOVA detected no significant effect of drugs on training related change in peak acceleration (peak acceleration post1) or reaction time (reaction time post1).

2.3. Effects of drugs on longevity of training-induced M1 plasticity and motor performance

SRC data: For each drug, comparisons of SRC parameters at 60-min after completion of training (post2) versus the baseline measures were performed. In the PLAC, AMPH, MEPH, and DOPA conditions, none of the SRC parameters were different at post2 compared to baseline (Fig. 3B and C). Comparison of training related change in SRC curve parameters from baseline to post2 showed no differences between PLAC versus AMPH, MEPH, or DOPA.

Peak acceleration and reaction time: Training had no effect on the peak acceleration of wrist extension movements or reaction time in any of the drug conditions when post2 measures were compared to baseline (Fig. 4). The repeated measures ANOVA showed no overall effect of drug on change in peak acceleration (peak acceleration post2) or reaction time (reaction time post2).

2.4. Effects of drug and duration of training on quality of training movements

For each drug condition, the peak acceleration of wrist extension movements executed during training was evaluated as a function of time (Fig. 5). Demonstration of an increase in peak acceleration as a function of time would indicate an online improvement in motor performance (related to motor learning) while decreases in peak acceleration may suggest fatigue or absence of motor learning. Inspection of averaged peak acceleration (normalized to the acceleration for the first training movement) of subjects across time demonstrate a trend for smaller values (<1) for AMPH and MEPH toward end of training, suggesting decrement in motor performance with training (Fig. 5A), with less evidence for decrement in performance in the PLAC and DOPA conditions (values close to 1 or >1) (Fig. 5A). The 2-way repeated measures ANOVA demonstrated no effect of drug or time, but an interaction approaching significance ($p = 0.06$) (Fig. 5B).

3. Discussion

Our main findings are the differential effects of the AMPH, DOPA, and MEPH on the input-output relationship of the M1 corticospinal pathway, at baseline and after motor training. In extending previous reports of drug effects on TMS measures of corticospinal excitability, we demonstrate the differential effect of 3 monoaminergic drugs on the three SRC curve parameters (S50, k, and MEPmax). Despite statistically significant drug and training associated changes in the input-output relationship of the M1 corticospinal pathway, there was no significant effect on motor performance, motor learning and psychophysics.

3.1. Training-induced M1 plasticity in the naïve (PLAC) condition

The effect of repetitive movements of the elbow, wrist or finger/thumb on movement kinematics and M1 excitability has been studied previously in healthy individuals (Bütefisch et al., 2000; Classen et al., 1998; Muellbacher et al., 2001, 2002b; Ziemann et al., 2001, 2004) and stroke patients (Bütefisch et al., 1995a; Muellbacher et al., 2002a), and show comparable results regardless of the body part executing the movements. M1 excitability increases, as measured by training-induced increases in MEP amplitude, accompanied by increases in either peak acceleration or change in the direction of the trained movement, pointing to common mechanisms underlying these training induced changes. LTP-like mechanisms are thought to be involved in this form of plasticity (Bütefisch et al., 2000; Ziemann et al., 2004). In the present study, motor training in the PLAC condition resulted in a significant increase of corticospinal excitability as indicated by increases in MEPmax extracted from the Boltzmann function. When measured at a constant level of motor activity (at rest in the current experiment), the 3 curve parameters (S50, k, and MEPmax) completely characterize the input-output relationship of the M1 corticospinal pathway in a given training/drug condition. Therefore, a demonstration of a motor training-dependent or drug-dependent statistically significant change in one or more of the curve parameters would indicate a long lasting change in the input-output relationship in that condition. Although there is no direct evidence in the present study that increases in MEPmax were related to changes in excitability at the cortical level, previous studies demonstrated that practice dependent plasticity and the early phase consolidation of motor learning occurs at the level of the motor cortex (Classen et al., 1998; Muellbacher et al., 2002b). The current findings are somewhat consistent with our previous report of long-lasting increases in MEP amplitudes following training (Bütefisch et al., 2015). While in previous studies SRC characteristics were expressed as area under the curve or MEP_{sum} , results of the present study extend these findings by demonstrating where along the SRC curve these changes occur, suggesting that indeed the analysis of different SRC curve parameters may be a more sensitive and comprehensive method to capture changes in corticospinal excitability. For the PLAC condition, training related increases in MEPmax indicate a shift in the balance between the activity of excitatory and inhibitory components of the corticospinal volley, including changes in recurrent inhibition of late recruited motoneurons by those recruited earlier (Devanne et al., 1997). The lack of significant changes in S50 and k in the PLAC condition would indicate that the threshold and gain of the most excitable elements at the cortical, subcortical, and spinal level remained unchanged (Devanne et al., 1997).

Increases in MEPmax following training in the PLAC condition were not associated with significant improvement in the kinematics of the trained movement. Reduced motor training-related improvement in behavior in the elderly population has been reported by us and other investigators (Bueteftisch et al., 2015; Cirillo et al., 2010, 2011; Floel et al., 2005a; Rogasch et al., 2009; Sawaki et al., 2003). Specifically, in our recent study of elderly subjects, practicing wrist extension movements did not result in any improvement of motor kinematics, suggesting lack of motor learning (Bueteftisch et al., 2015). However, when training was combined with rTMS, motor performance improved significantly (Bueteftisch et al., 2015). These recent findings would argue that the employed training paradigm is appropriate to test drugs for their ability to enhance motor learning above the naïve level of learning.

3.2. Effects of DOPA on M1 excitability

In the present study, DOPA induced an increase in MEPmax at baseline. There were no other effects of DOPA on MT or other curve parameters. These findings are similar to studies where similar dosage of dopamine did not change the MT or MEP amplitudes evoked at mean intensities of about 45 to 50% MSO, or at intensities that evoke MEP amplitudes of 1 mV (Fig. 2B) (Floel et al., 2005a; Kuo et al., 2008; Monte-Silva et al., 2010; Thirugnanasambandam et al., 2011). The comparison of DOPA-related increase in MEPmax with the results of other studies is limited by the difference in the age of the subject and lack of SRC curves or MEPmax in previous investigations (Floel et al., 2005a; Kuo et al., 2008; Monte-Silva et al., 2010; Thirugnanasambandam et al., 2011).

As indicated above, changes in MEPmax suggest a shift in the balance between the activity of excitatory and inhibitory components of the corticospinal volley. DOPA related increases in MEPmax could be explained by its modulatory effect on GABA-A neuronal activity (Seamans and Yang, 2004). Specifically, the size of the MEP amplitude is related to the generation of TMS induced I-waves (Di Lazzaro et al., 2004). At low intensity, TMS applied with a figure of eight coil in an orientation to induce a posterior to anterior (PA) current in the brain activates the corticospinal cells indirectly and produces a descending wave which is termed I1 wave. At higher stimulus intensities, later volleys appear (late Iwaves), which are thought to originate from a more complex neuronal circuitry. These later I-waves are under the control of GABA A-ergic inhibition (Di Lazzaro et al., 2012). However, as indicated above, changes in the curve parameters do not allow discrimination of the location of the change in excitability along the M1 corticospinal projections. Given the complexity of dopaminergic action on neuronal activity, excitability, and plasticity (Seamans and Yang, 2004), isolating mechanisms involved in mediating the observed effects of DOPA is difficult and beyond the scope of the current experiments.

3.3. Effects of DOPA on training-induced M1 plasticity

In the present study, motor training under the effect of DOPA had no statistically significant effect on curve parameters. However, when compared to PLAC, the training-related change in SRC between baseline and post1 was smaller in the DOPA condition (Fig. 3). Considering the DOPA-related increases of MEPmax at baseline, an explanation for the lack of training-induced increases in SRC and MEPmax could be that DOPA-related increases in MEPmax

at baseline prevented further induction of training related synaptic plasticity i.e. formation of motor memory. In support of this notion is the similar magnitude of training-induced increase in MEPmax in the placebo condition and the DOPA-related increase in MEPmax at baseline. This interpretation is further supported by reports that intervention-related increases in MEP amplitude prevent additional increases of MEP amplitude with subsequent interventions (Ziemann et al., 2004).

Our finding of smaller training related change in SRC at post1 in the DOPA condition is consistent with the results from Monte-Silva and colleagues (Monte-Silva et al., 2010), where L-dopa reduced the magnitude of tDCS-related M1 excitability increase. Specifically, in studies of cortical network plasticity using anodal or cathodal tDCS to either increase or decrease cortical excitability for about an hour after the end of stimulation, L-dopa reduced the anodal tDCS-related increases in M1 excitability and prolonged the inhibitory after-effects of cathodal tDCS in a dose-dependent manner (Kuo et al., 2008; Monte-Silva et al., 2010). The tDCS-related effects on M1 excitability are thought to depend on neuronal calcium influx, because they are inhibited by blocking NMDA receptors and calcium channels (Liebetanz et al., 2002). However, in contrast to the training-related muscle representation specific excitability changes of the present study, the primary mechanism underlying the tDCS related changes in cortical network excitability are thought to be a modulation of resting membrane potential of a broad range of cortical neurons (Purpura and McMurtry, 1965).

The effect of L-dopa in a paradigm of LTP-like plasticity of M1, referred to as paired associative stimulation (PAS) protocol, were also dependent on the dosage. In this paradigm, repetitive peripheral nerve stimulation is paired with TMS of M1 (Stefan et al., 2000) to elicit input specific M1 excitability increases that depend on NMDA receptor activation and related influx of calcium (Stefan et al., 2002). L-Dopa at a dosage used in the present study (100 mg) had no effect on this PAS-induced form of M1, while higher dosages such as 200 mg L-dopa resulted in suppression of PAS-induced plasticity (Thirugnanasambandam et al., 2011), which is similar to our results.

In the present study, motor training under the effect of DOPA had no effect on motor kinematics. Our results are in contrast to the reported training-enhancing effect of a single dose of L-dopa in elderly subjects (Floel et al., 2005a). Specifically, in this previous study of young and elderly healthy subjects, there was no training-related effect on behavior in the placebo condition in elderly subjects, while younger subjects demonstrated training-related effects (Floel et al., 2005a). Further, similar to our results, L-dopa had no effect on behavior, corticospinal excitability, or training kinematics at baseline. However, training under the effect of L-dopa enhanced training-related effect on behavior, with younger subjects having a greater L-dopa related gain. As mentioned above, these behavioral changes were not reflected in drug-related effect on the measures of MEP amplitude of the muscle supporting the training.

In sum, we show that administering of 100 mg L-dopa increases corticospinal excitability when tested with higher intensity TMS, possibly via reduced GABAergic inhibition, but prevents any additional motor training-induced increases in corticospinal excitability. These

findings are consistent with the results of L-dopa dosing studies showing a clear non-linear, dosage-dependent effect on both facilitatory and inhibitory plasticity in different paradigms (Monte-Silva et al., 2010; Thirugnanasambandam et al., 2011). In contrast to the study in older adults by Floel et al. (2008) we did not find an effect on behaviorally assessed motor learning.

3.4. Effect of AMPH on M1 excitability

Our results showed that at baseline, AMPH resulted in a decrease in MEPmax when compared to PLAC (Fig. 2), while other curve parameters and MT remained unchanged. This would indicate that the threshold and gain of the most excitable elements at the cortical, subcortical and spinal level remained unchanged following AMPH administration (Devanne et al., 1997). Comparison to the results of other studies is limited by the younger age of the subjects, differences in intensities of TMS-evoked MEPs, and target muscles. Specifically, the majority of other previous studies, including those from our lab, demonstrated no effect of AMPH on MEP amplitude when measured at medium intensities of about 50–65% MSO for distal hand muscles (abductor pollicis) (Bütefisch et al., 2002; Sawaki et al., 2002; Tegenthoff et al., 2004). As indicated above, decreases in MEPmax indicate an AMPH-related shift in the balance between the activity of excitatory and inhibitory components of the corticospinal volley. As DOPA has the opposite effect on MEPmax, the AMPH-related shift in excitability may be mediated through different mechanisms.

3.5. Effects of AMPH on training-induced M1 plasticity

Training under the effect of AMPH resulted in an increase in all three curve parameters (MEPmax, S50, and slope) indicating training-induced enhancement of cortical excitability in the AMPH condition. However, the change in SRC curve parameters, i.e. SRCpost1 or SRCpost2 were not different between the AMPH and PLAC conditions, suggesting that AMPH did not increase training-related enhancement of cortical excitability beyond the effect of training alone (PLAC). This is in contrast to our previous findings in young individuals practicing thumb movements in a motor learning paradigm, where AMPH enhanced motor learning and retention beyond the naïve or placebo training related gains (Butefisch, 2003; Bütefisch et al., 2002; Sawaki et al., 2002).

As a group, the individuals tested in the present study did not demonstrate behavioral correlates of motor learning in the naïve (PLAC) condition. One possible explanation for the difference in the AMPH enhancing effect on motor learning could be that in previous studies, only subjects with evidence of motor learning in the naïve condition were included. The lack of AMPH-related gains in motor learning was previously reported for a small group of young subjects who did not show motor learning in the naïve condition (Sawaki et al., 2002). This may indicate that AMPH may play an important role in the enhancement of formation of a motor memory, but not the initiation of motor memory formation. This notion is supported by the finding that dextromethorphan, a drug that blocks NMDA receptor function, abolishes AMPH-related enhancement of tDCS-induced form of M1 plasticity (Nitsche et al., 2004). Previous studies on the effects of D-amphetamine on learning in older adults are limited to studies of stroke patients undergoing rehabilitation therapy, and the

results of these studies are inconsistent (Sonde et al., 2001; Treig et al., 2003; Walker-Batson et al., 1992, 1995).

3.6. Effects of MEPH on M1 excitability

Our results showed no effects of MEPH on baseline corticospinal excitability, motor performance, or training-induced motor memory formation when compared to PLAC. To our knowledge, there are no studies on the effect of MEPH on corticospinal excitability, psychophysics, and motor learning in the healthy aged population. Most previous studies were done in young adults (Ilic et al., 2003; Kratz et al., 2009; Wang et al., 2014). In young adults, inconsistent results for its effect on corticospinal excitability and GABAergic inhibition were reported. Increases in MEP amplitudes and reduced short interval cortical inhibition (SICI) were reported by some investigators (Ilic et al., 2003) while others reported no effect (Kratz et al., 2009).

3.7. Effects of MEPH on training-induced M1 plasticity

The few previous investigations on the effect of MEPH in the elderly population have been limited to its effect on post-stroke motor recovery and demonstrated inconsistent findings. In 9 subacute stroke subjects, the combination of tDCS and MEPH demonstrated greater gains in motor function compared to tDCS alone or MEPH alone, but there were no differences in TMS-derived measures of corticospinal plasticity among the 3 groups (Wang et al., 2014). Additionally, somewhat consistent with our current finding of lack of effects of MEPH on motor performance and cortical excitability, analysis of individual treatment effects showed no significant improvement in hand motor performance with MEPH alone (Wang et al., 2014). In contrast, a beneficial effect of MEPH combined with physical therapy on motor function was reported in a small clinical trial in individuals with acute stroke (Grade et al., 1998; Lolk et al., 2011) but cortical excitability was not tested.

3.8. Relevance of our findings to aging neuroscience and clinical research

Our present findings have important implications for aging neuroscience research and patients with neurological diseases such as stroke. As training-induced M1 plasticity is influenced both by the normal aging process as well as the neuropathology, the limited understanding of the effects of normal aging on neuroplasticity processes and drug mechanisms may contribute to the inconsistency in previous literature regarding the clinical effectiveness of drugs in populations with neurologic diagnoses. Several recent reviews have concluded that although drugs such as AMPH continues to be a popular ‘recovery-enhancing’ drug for neurologic disorders such as stroke, data from human studies and clinical trials are disappointing and lack consensus (Barbay and Nudo, 2009; Breceda and Dromerick, 2013; Goldstein, 2009; Martinsson et al., 2007a, b). The effects of monoaminergic drugs such as AMPH and DOPA on motor recovery depend on several factors such as lesion location, dosing and timing of drug administration, type, intensity and timing of concomitant behavioral training (Goldstein, 2009). It is, therefore, critical to control the intensity of motor training in clinical trials of amphetamine or similar drugs in humans, which is challenging to do in clinical and non-laboratory settings (Goldstein, 2009). The current findings on older individuals showcase the need for further investigations on the

effects of aging and neurologic disease on how monoaminergic drugs influence training-related plasticity.

3.8.1. Limitations—We cannot exclude the possibility of missing more subtle training-related changes in movement kinematics. In the current study, we did not measure blood plasma concentrations of the administered drugs to verify sufficient peak plasma level. However, given the differential effects of the drug on our TMS measures at baseline and the demonstrated CNS effects of previously published data using the dosages and timing of drug administration (Floel et al., 2005a; Kuo et al., 2008; Monte-Silva et al., 2010), it is reasonable to assume that the training was executed under the influence of these different drugs. Further, although the study used a within-subject design, our results may have been further strengthened by evaluating motor performance and M1 excitability prior to the drug administration at each day of study. The sample size was calculated based on our previous studies in young adults with fewer or similar number of participants and we detected statistically significant differential effects of the drugs on M1 excitability and training-related M1 plasticity. The current study results need to be substantiated in a future study with a larger sample size and inclusion of a larger age range. Additionally, in a larger sample size, compared to conventional statistical approaches such as analysis of variance, incorporation of linear mixed models (Boisgontier and Cheval, 2016) and nonlinear statistical model with mixed-effects (Park and Schweighofer, 2017) may provide more robust results that can be replicated with multiple studies. Due to the potential limitation caused by the complex interactions between fatigue, motivational factors, and motor learning (Park and Schweighofer, 2017), another interesting avenue for future research would be to evaluate the effects of aging and drugs on training-related M1 excitability and motor performance during multiple training sessions.

4. Experimental procedure

The experiments were approved by the Institutional Review Board at West Virginia University and Emory University, and conducted according to the Declaration of Helsinki. All subjects provided written informed consent.

4.1. Subjects

Fifteen healthy individuals were enrolled and assessed for eligibility for the study. Of the 15 participants, 5 subjects were excluded from participating in the main experiments because they showed abnormalities in neuropsychological testing, brain magnetic resonance imaging (MRI), or EKG (see Fig. 1 and inclusion criteria). The remaining ten individuals (mean age 61 ± 4 , 4 males) fulfilled the following inclusion criteria and completed the study: no history of neurologic or psychiatric disease, normal MRI of the brain, normal cognitive function as confirmed by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph et al., 1998), no contraindication for transcranial magnetic stimulation (TMS) or MRI, no intake of CNS active medications, normal EKG and ability to complete the criteria of the inclusion experiment (see below). All subjects were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971) and had a normal neurological examination.

4.2. Overview of study design and experiments

In a double-blind, randomized, placebo-controlled study design the effects of single oral doses of D-amphetamine (AMPH, 10 mg), methylphenidate (MEPH, 20 mg), carbidopa/levodopa (DOPA, 25/100 mg), and placebo (PLAC) on training-induced M1 plasticity were tested (Fig. 1). M1 plasticity was defined as training-related increases of motor evoked potential (MEP) amplitudes in a muscle supporting the training movement (extensor carpi ulnaris muscle, ECU) and increase in peak acceleration of the trained movement (Bütefisch et al., 2000; Muellbacher et al., 2001; Ziemann et al., 2004). Prior to subject recruitment, the randomized order of drug administration was determined for 15 subjects. The primary outcome measures of this study were the motor training-related increases in the mean MEP amplitudes of the stimulus response curve for the ECU muscle and the peak acceleration of wrist extension movements. Reaction time, defined as time interval between the auditory cue and movement onset, was a secondary outcome measure. All measures were obtained prior to the training (baseline), immediately after training (post1), and again 60 min (post2) after completion of training (see Section 4.6 for details).

Each subject completed one screening session, one MRI session, and 4 experimental sessions. After completing initial screening and obtaining a brain MRI, the subjects were allocated in a randomized pre-determined order for the 4 experimental drug conditions, one for each of the 3 drugs and one for placebo, with a 1-week wash out period between consecutive sessions. The order of drug administration was randomized and balanced across subjects. All subjects and all investigators involved with the experiments, data acquisition, and data analysis were blinded to the drug conditions. Statistical analysis was performed by a statistician not involved in any other aspects of the study.

4.3. Subject screening and inclusion experiment

During the screening visit, all procedures were explained to the subject, and informed consent was obtained. Subjects were screened for the inclusion criteria listed above and received a complete neurological examination. Additionally, we determined the ability of TMS to elicit a measurable motor evoked potential (MEP) of $>100 \mu\text{V}$ amplitude, and confirmed that the MEP amplitude increased with increasing stimulus intensity (up to 100% of maximum stimulator output (MSO)) to at least 20% over the MEP amplitude at motor threshold (MT) (Wittenberg et al., 2003), as well as the ability to perform the training movements. The experimental set-up was similar to the main experiment, and is described in detail below.

4.4. Brain MRI acquisition and 3D reconstruction

High-resolution T1 weighted anatomical images were collected on a 3T GE scanner with the following parameters: Spoiled gradient recalled (SPGR) acquisition; FOV = 240; matrix 256×256 ; slice = 1.5 mm; 124 slices (West Virginia University) or a high resolution 1 mm isovoxel MPRAGE on a Siemens 3T scanner (Emory University). Brain MRIs were reviewed by a board-certified neurologist to exclude structural abnormalities. After anatomic normality of the brain was established, the MRI was reconstructed in Brainsight (Rogue Research, Montreal, Canada) and served as each subjects' reference for the TMS coil position within and across testing sessions.

4.5. Neuropsychological testing

The RBANS (Randolph et al., 1998) was administered to all subjects to establish normality of cognitive function (see inclusion criteria). The test is composed of 5 subtests and a total score, each with a mean of 100 and a standard deviation of 15. We determined that any score of 85 or lower on any of the subtests or the total score would suggest abnormal cognitive function and would exclude a subject. All subjects included in this study scored above 85 on all the subtests and the total score. The average RBANS total score was 107 ± 12.54 (range 94–133).

4.6. Drug administration, motor training, TMS, and kinematic measures

4.6.1. Drug administration—At the beginning of each experimental session, a single oral dose of one of four different drugs was administered according to each subject's assigned pre-determined order. Subjects were blinded to the drug condition. The drug was administered by a research nurse who was not involved in any aspects of data acquisition and analysis. DOPA (25/100 mg) and MEPH (20 mg) were administered 1 h prior to training, while AMPH (10 mg) and PLAC were administered 2 h prior to training. Timing of the drug administration was determined such that motor training could be performed when drugs had reached its peak plasma concentration. Plasma levels of MEPH have been shown to peak at 2-h following oral drug administration (Kimko et al., 1999). For AMPH, the dose and timing of administration were selected based on previous studies in healthy subjects (Angrist et al., 1987; Bütefisch et al., 2002; Ziemann et al., 2002), and stroke patients (Walker-Batson, 2013). Previous studies show that DOPA has a nonlinear dosage-dependent effect on cognition and neuroplasticity. In animal studies, an inverted U-shaped dose–response curve of dopamine on cognition was demonstrated (Seamans and Yang, 2004). In the present study, we chose a medium dosage of 100 mg DOPA because it has been shown to affect tDCS-induced plasticity in former experiments (Kuo et al., 2008; Monte-Silva et al., 2010) and had a positive effect on motor memory formation (Floel et al., 2005a). Further, higher dosages of DOPA were reported to result in nausea of the participants in previous experiments (Monte-Silva et al., 2010; Thirugnanasambandam et al., 2011). After 1 h, 100 mg DOPA results in a blood plasma level of about $1.3 \pm 0.6 \text{ mg l}^{-1}$ (Dingemanse et al., 1995). In the current study we administer 25 mg carbidopa along with 100 mg of L-dopa to inhibit L-dopa decarboxylation. Administration of 25 mg carbidopa with 100 mg L-dopa has demonstrated CNS effects in other studies (Floel et al., 2005a) and is also the ratio (1:4) in clinical doses for treatment of Parkinson's disease. In other studies a similar 1:4 ratio of a decarboxylase inhibitor benzeraside and L-dopa was used (Monte-Silva et al., 2010). A sufficient availability of L-dopa in the CNS would be also supported by the results of one study demonstrating clear CNS effects for 100 mg of L-dopa without any blockade of the conversion of L-dopa. In this study a D2-receptor antagonist domperidone was used (Kuo et al., 2008). For the purpose of blinding, the research nurse communicated to the investigators the time of the start of the TMS measures. The time of arrival of the subject and the time of drug administration remained unknown to the other investigators. The research nurse worked with the subject to administer the drug according to the protocol in a room located in a different part of the building.

After drug administration, subjects were monitored for vital signs and possible side effects by the research nurse until the beginning of the experiment. Throughout the experiment, the investigators (Drs. Pergami MD PhD and Bueteftisch MD PhD) continued to monitor the subjects for side effects and checked the vital signs prior to discharging the subject to home or earlier, and more frequently if clinically indicated.

4.6.2. Subject setup—The details of the subject setup were published before (Bueteftisch et al., 2015). Briefly, subjects were seated in a dental chair with their right arm positioned in a molded armrest. The forearm was immobilized. The hand was supported by the armrest in a fully pronated and mildly flexed (30 degrees) position but freely movable in extension. Hand movements were recorded in the two primary movement planes (extension/flexion; abduction/adduction) with a 2D accelerometer mounted on the dorsum of the hand (Bueteftisch et al., 2015).

Baseline TMS stimulus response curve (SRC): Surface electromyographic (EMG) activity (bandpass 1 Hz–1 kHz) was recorded with surface electrodes (11-mm diameter) mounted in a belly-tendon montage on the skin overlaying the extensor carpi ulnaris (ECU). The ECU muscle is a muscle supporting the movements targeted during motor training. For data acquisition a LabVIEW system was used (LabVIEW, National Instruments, CA, USA). The raw EMG was sampled and digitized at a rate of 5 kHz and stored on a PC for off-line analysis. TMS was delivered through a figure of eight shaped coil (7-cm wing diameter) using two Magstim 200 stimulators connected via a Bistim module (Magstim Company, UK). The coil was placed tangentially to the scalp and rotated 45 degrees away from the midline (Kaneko et al., 1996; Werhahn et al., 1994). Stimuli were delivered to the optimal site for the contralateral ECU muscle, and the site was marked on the subject's reconstructed MRI of the brain (see above for details). At this ECU hot spot site, the resting motor threshold (MT), defined as the minimum stimulus intensity to evoke an MEP of $>50 \mu\text{V}$ for at least five of ten trials (Rossini et al., 1994), was determined to the nearest 1% of the maximum stimulator output (MSO) for ECU. Brain MRIs were reconstructed as 3D images to be used as reference for targeting of the "hot spot" during TMS application. Using the Brainsight Navigational system, the 3D trajectories of the initial coil position were marked on the subject's 3D brain MRI images. Using these trajectories as reference, the stimulating coil was kept at a constant position with respect to the subject's head for the entire duration of each experiment. The neuro-navigation system was also utilized to maintain consistency of coil position within and across the experimental sessions. After identifying the resting MT, MEPs were elicited by single TMS pulses at increasing stimulus intensities (Devanne et al., 1997; Ridding and Rothwell, 1997). Stimulation started at the next lower 5% level of subjects MT and the absence of a measurable MEP was confirmed. If any measurable MEPs were seen at that level, intensity was decreased by 5% MSO to confirm complete absence of MEP responses. Intensities were then increased in increments of 5% of MSO up to a maximum of 80% MSO. Intensities beyond 80% of MSO are not well tolerated. Ten stimuli were delivered at each intensity with an interstimulus interval (ISI) of 5-s. EMG responses were set to zero for intensities below the intensity that demonstrated absence of measurable MEP to a level of 35% MSO. These data were used to plot the TMS stimulus response curves (SRC).

Baseline Motor Performance: Five auditory paced wrist extension movements were recorded by a custom-made two-dimensional accelerometer mounted to the dorsum of the hand, 3 cm distal to the styloid process of the ulna (Bütefisch et al., 2015). Subjects were instructed to extend the wrist as quickly as possible. The acceleration in the two main movement planes (extension/flexion; abduction/adduction) was recorded (Bütefisch et al., 2000).

Post-training measurement of SRC and motor performance: To assess training-induced changes in M1 excitability and motor performance, SRC and motor performance measures were collected (using procedures identical to those described above for baseline measurements) immediately after completion of training (post1), and again 60-min after completion of training (post2). We hypothesized greater training-related increases in MEP amplitude and peak acceleration in AMPH, DOPA and MEPH conditions compared to PLAC.

4.6.3. Motor training—Following the administration of the drug, and after obtaining baseline measurements of SRC and motor performance, subjects completed the motor training. Because the objective of the present study was to enhance motor learning, we chose a training paradigm that has been previously demonstrated to be effective in inducing motor learning related M1 plasticity in young adults (Bütefisch et al., 2000; Muellbacher et al., 2002b; Ziemann et al., 2004). Accordingly, subjects performed auditory paced ballistic wrist extension movements (no specific pre-set training angle was given) at 0.5 Hz frequency for three blocks of 10 min duration with 2–3 min of rest in between training blocks (30 min, 900 movements total). Subjects were instructed to return to the baseline position by relaxation as confirmed by EMG.

4.7. Data analysis

Analysis of MEP amplitude and kinematic measures was performed by personnel blind to the drug condition. For *SRCs*, recordings with increased EMG background activity were excluded from analysis. Peak to peak MEP amplitudes were calculated using a custom-written data-processing program (LabVIEW, National Instruments Inc.). For each subject and each drug, the average of 10 MEP amplitudes elicited at each TMS intensity was calculated, and *SRCs* were obtained at each time point (baseline, post1, post2) by plotting the stimulus intensity versus the mean MEP amplitudes. *SRCs* were modelled by a 3-parameter sigmoid function:

$$MEP(S) = \frac{MEP_{MAX}}{1 + e^{K(S50 - S)}}$$

Here, *S* represents the TMS intensity (%MSO), *MEPmax* represents the maximum MEP amplitude, *S50* represents the intensity needed to evoke 50% of *MEPmax*, and *K* represents the slope parameter. The Boltzmann equation was used to fit the averaged data points with the Levenberg-Marquardt algorithm (Capaday, 1997; Capaday et al., 1999; Devanne et al., 1997; Jensen et al., 2005). The 3 curve-fit parameters (*MEPmax*, *S50*, and *K*) were obtained for the 4 drug conditions, and the 3 time points (baseline, post1, post2) (Table 1).

The peak acceleration of ballistic wrist movements recorded during movement tests at baseline, post1, and post2 was computed as the resultant vector of the peak accelerations in the two main movement directions (Buetefisch et al., 2014, 2015; Buetefisch et al., 2000, 2002). In addition, movement reaction time was computed as the latency between the auditory tone and onset of ballistic wrist movements (Buetefisch et al., 2015).

4.8. Statistical analysis

To determine the effects of the drugs on baseline SRC parameters, MT, movement kinematics, and reaction time, separate one-way repeated-measures ANOVA were performed for each variable. Additionally, for baseline, pair-wise comparisons (Bonferroni correction for multiple comparisons) were performed to detect differences between PLAC versus each of the 3 drugs (AMPH, DOPA, MEPH).

To test our hypothesis regarding training-induced M1 plasticity, for each drug, separate paired *t*-tests (with Bonferroni correction) were conducted to determine whether motor training in the presence of AMPH, MEPH, DOPA and PLAC resulted in changes in SRC parameters (*S*, MEPmax, *k*) at post1 and post2 compared to baseline. To determine whether training related changes in SRC were different in the AMPH, MEPH, and DOPA condition when compared to PLAC, 2-way ANOVAs were performed with drug and intensity as independent variables and the difference in MEP amplitudes between PLAC and each drug condition as dependent variables. Planned pair-wise (Bonferroni correction) comparisons were performed to assess differences between PLAC and each of the other 3 drugs (AMPH, MEPH, DOPA).

To evaluate training-induced changes in motor performance, the following change scores were calculated: peak acceleration post1 = peak acceleration post1 – peak acceleration baseline, peak acceleration post2 = peak acceleration post2 – peak acceleration baseline, reaction time post1 = reaction time post1 – reaction time baseline, reaction time post2 = reaction time post2 – reaction time baseline. These change scores were compared across the 4 drugs using a repeated-measures ANOVA with post-hoc pairwise comparisons (Bonferroni).

Exploratory analyses were performed to determine the effects of the drugs and duration of training on movement kinematics. Peak accelerations of the training movements (40 representative movements per 10 min training block (every 7th movement) were analyzed. The average peak acceleration during the first 10 min of training (train10), 10–20 min of training (train20), and the last 10-min of training (train30) were computed. A two-way repeated measures ANOVA was performed to determine the effect of drug condition and training time (Train10, Train20 and Train30) on peak accelerations.

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Trisha M. Kesar carried out the data analysis and participated in the preparation of the manuscript, Samir R. Belagaje and Paola Pergami carried out the experiments and participated in the preparation of the manuscript, Marc

W. Haut participated in the design of the experiments and preparation of the manuscript, Gerald Hobbs carried out the statistical analysis of the data, Cathrin M. Buetefisch designed the study, carried out the experiments and prepared the manuscript.

None of the authors have potential conflicts of interest to be disclosed.

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Fig. 1.
Flow diagram showing overview of procedures for the clinical trial.

Fig. 2.

Effect of drugs (PLAC, AMPH, MEPH, DOPA) on TMS-derived stimulus response curves (SRC) at baseline. (A–C) In the three panels, MEP amplitudes obtained after administration of a single oral dose of either AMPH, MEPH or DOPA (filled symbols) are plotted on the same graph as measures taken in the placebo condition (PLAC, open diamonds). For each SRC, the best fit line (computed using Boltzmann curve fit parameter estimates) is superimposed on the mean MEP amplitudes to demonstrate the goodness of the curve fit. (A) AMPH resulted in a significant reduction in MEPmax when compared to PLAC ($p = 0.004$). (B) MEPH had no effect on SRC. (C) DOPA resulted in a significant increase in MEPmax when compared to PLAC ($p = 0.036$). Amplitude of MEP are expressed in mV, intensity of TMS is expressed as percentage of maximum stimulator output (MSO). $N = 9$, Mean $\pm$ SE.

Fig. 3.

Effect of drugs (PLAC, AMPH, MEPH, DOPA) on training related changes in SRC. (A–D) For each drug – PLAC (open diamond), (AMPH (filled circle), MEPH (filled square), and DOPA (filled triangle), training related changes in SRC are demonstrated. Measurements were taken at baseline (thin solid line), immediately after the training (post1, thick solid line), and again 60 min after completion of the training (post2, dotted line). SRC curve parameters (MEPmax, S50, slope, see methods for details) were compared as a function of time (baseline, post1, post2; data not shown). (A) Training in the PLAC condition resulted in a significant increase in MEPmax (post1, $p = 0.0002$). (B) Training under the effect of AMPH resulted in a significant increase in all 3 curve parameters (MEPmax ($p = 0.00006$), slope ($p = 0.036$), and S50 ($p = 0.052$)) immediately after training (post1). Measures at post 2 were not statistically significant different from baseline. (C) Training under the effect of MEPH resulted in no significant change in SRC. (D) Training under the effect of DOPA had no statistically significant effect on SRC. $N = 9$, Mean $\pm$ SE.

Fig. 4.

Effect of drugs (PLAC, AMPH, MEPH, and DOPA) and motor training on movement kinematics and psychophysics. Averaged peak acceleration (A) and reaction time (B) values for each of the 4 drug conditions (PLAC, AMPH, MEPH, and DOPA) are shown.

Measurements were taken during 5 ballistic wrist extension movements at baseline (baseline), immediately after the training (post1), and again 60 min after completion of the training (post2). At baseline, there was no statistically significant difference between PLAC and any of the other 3 drug conditions for peak acceleration and reaction time. There was no statistically significant effect of training on peak acceleration or reaction time in any of the drug conditions. $N = 9$, Means $\pm$ SE.

Fig. 5.

Effect of drugs on training kinematics. Subjects performed auditory paced ballistic wrist extension movements at 0.5 Hz frequency for three blocks of 10 min duration (Train 10, Train 20, Train 30) with 2–3 min of rest (vertical lines) in between training blocks. For each training block, 40 representative movements were extracted and explored for the effects of drugs or time. (A) For each drug condition, the mean peak acceleration of wrist extension movements is plotted as a function of time. Peak acceleration was normalized to the acceleration of the first movement. For AMPH and MEPH there is a trend for smaller values of peak acceleration (<1) as a function of time. This suggests that subjects' performance showed a decrement with training due to fatigue or other factors. There was less evidence for a decrement in motor performance in the PLAC and DOPA condition (values close to 1 or >1). (B) Bar plots showing the effect of time and drug on average peak accelerations generated in each training block. The 2-way repeated measures ANOVA with drugs and time as independent variable and peak acceleration as dependent variable demonstrated no effect of drug or time, but an interaction approaching significance ($p = 0.06$). $N = 9$, Mean $\pm$ SE.

Table 1

Parameter value estimates derived from the Boltzmann curve fitting analysis of SRC curves. For the 3 curve parameters (S50, K, and MEPmax) the estimates and approximate standard error (SE) are listed for each drug (PLAC, AMPH, MEPH, DOPA) and time point (baseline, post1, post2).

Drug	Curve Parameter	baseline		post1		post2	
		Estimate	S.E.	Estimate	S.E.	Estimate	S.E.
PLAC	S50	55.27	3.04	52.69	5.63	51.19	1.08
PLAC	k	-5.83	2.45	-8.40	4.50	-5.33	0.91
PLAC	Rmax	1.01	0.13	1.63	0.33	1.29	0.05
AMPH	S50	54.07	2.92	58.84	5.16	57.67	0.87
AMPH	k	-4.34	2.46	-8.04	3.47	-5.27	0.70
AMPH	Rmax	0.73	0.10	1.22	0.26	1.08	0.04
MEPH	S50	54.65	2.96	50.75	2.65	55.64	1.16
MEPH	k	-5.85	2.39	-4.81	2.26	-6.92	0.90
MEPH	Rmax	1.04	0.13	1.10	0.12	1.22	0.06
DOPA	S50	62.24	9.08	53.48	4.79	55.63	2.19
DOPA	k	-10.11	4.84	-7.42	3.82	-7.91	1.62
DOPA	Rmax	1.81	0.62	1.41	0.26	1.53	0.13