Dopaminergic Influences on Formation of a Motor Memory

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The ability of the central nervous system to form motor memories, a process contributing to motor learning and skill acquisition, decreases with age. Dopaminergic activity, one of the mechanisms implicated in memory formation, experiences a similar decline with aging. It is possible that restoring dopaminergic function in elderly adults could lead to improved formation of motor memories with training. We studied the influence of a single oral dose of levodopa (100mg) administered preceding training on the ability to encode an elementary motor memory in the primary motor cortex of elderly and young healthy volunteers in a randomized, double-blind, placebo-controlled design. Attention to the task and motor training kinematics were comparable across age groups and sessions. In young subjects, encoding a motor memory under placebo was more prominent than in older subjects, and the encoding process was accelerated by intake of levodopa. In the elderly group, diminished motor memory encoding under placebo was enhanced by intake of levodopa to levels present in younger subjects. Therefore, upregulation of dopaminergic activity accelerated memory formation in young subjects and restored the ability to form a motor memory in elderly subjects; possible mechanisms underlying the beneficial effects of dopaminergic agents on motor learning in neurorehabilitation.

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Formation of new memories in the central nervous system can be accomplished through different mechanisms, including activity-dependent changes in synaptic strength,[1,2] modification in firing patterns of individual neurons,[3] and increased synchronization between neuronal ensembles.[3,4] Changes in synaptic strength induced by activity-dependent coincident firing of presynaptic and postsynaptic neurons, most often referred to as long-term potentiation (LTP) and long-term depression, are modulated by heterosynaptic input.[5] One of the main heterosynaptic input systems, affecting the strength, specificity, and duration of encoded memory traces, is dopaminergic neurotransmission.[5–9]

Normal aging, associated with decreased ability to encode novel memories[10,11] and to express LTP,[8,12] is accompanied by diminished brain dopamine activity.[8,10,12] For example, neuropathological and imaging studies have shown decreased dopamine receptors, dopamine transporters, and dopamine metabolism with normal aging.[13,14] Thus, it is conceivable that age-dependent decline in brain dopamine function might contribute to the decreased ability to encode new memories in elderly adults.[8,9,14]

Motor training[4,15,16] leads to formation of motor memories, which are crucial for skill acquisition[15–17] and are influential in the process of functional recovery after stroke.[17–19] Formation of motor memories, similar to other forms of encoding,[10,11] declines with normal aging.[20] Dopamine has been shown to enhance LTP in a task-dependent manner,[5,8,9] not only in the hippocampus, but also in the cerebral cortex[5,21] and striatum.[22] It is then possible that upregulation of dopaminergic activity could result in beneficial effects on formation of motor memories, particularly in elderly adults. This is a hypothesis that has not yet been tested and that may have important implications for human neurorehabilitation. In this study, we assessed the effects of L-dopa premedication on training-dependent encoding an elementary motor memory in young and elderly healthy volunteers.

Subjects and Methods

Subjects

Twenty healthy volunteers (10 young subjects: age range, 24–38 years, mean 31 ± 4.5 years, 4 women; 10 elderly subjects: age range, 52–87 years,[20] mean 63 ± 10.4 years, 3 women) gave written informed consent and participated in this double-blind, placebo-controlled, randomized crossover

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study. The study was approved by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke.

Experimental Protocol

All healthy volunteers participated in two separate sessions, spaced at least 24 hours apart, testing the effects of L-dopa (100mg L-dopa + 25mg carbidopa, orally) + training and placebo (identical capsule, orally) + training on encoding a motor memory. Mean interval between the two drug conditions was 3.1 ± 3.7 (mean ± standard deviation) days in the young and 2.7 ± 1.4 days in the elderly. In each session, subjects fasted for at least 2 hours preceding L-dopa/placebo intake and avoided other medications to prevent interference with drug absorption.[23,24] Testing started 60 minutes after oral intake of L-dopa/placebo, a time that shows peak plasma concentrations of the drug.[24] Measurement of systolic and diastolic blood pressure and heart rate, and subjects' rating of attention to the task and fatigue levels, were taken four times during each session (see Fig 1A, experimental timeline). Motor training kinematics were monitored along the experiment.

Experimental Setup

Subjects were seated in a chair firmly connected to a frame that kept the head steady and the stimulating coil in a constant position with respect to the head. Head and coil stability were monitored with a three-dimensional laser system. Each subject's right forearm was immobilized in a molded armrest in a semipronated position with the four long fingers supported and thumb freely movable. Electromyographic (EMG) activity was recorded in the flexor pollicis brevis and the extensor pollicis brevis muscles using Ag/AgCl surface electrodes in a belly–tendon montage. Signals were amplified, band-pass filtered between 10 and 3,000Hz, and fed into a laboratory computer for off-line analysis. Thumb movements were recorded with a three-dimensional accelerometer mounted on the proximal phalanx of the thumb (Kistler Instrument, Amherst, NY). The direction of transcranial magnetic stimulation (TMS)–evoked and voluntary thumb movements was calculated from the first-peak acceleration vector. Acceleration signals were recorded in the vertical (extension and flexion) and horizontal (adduction and abduction) axes and digitized at 3,000Hz. Data were analyzed using a data collection-analysis program written in LabView (National Instruments, Austin, TX). TMS was delivered from a custom-built magnetoelectric stimulator (Cadwell Laboratories, Kennewick, WA) through a figure-of-eight magnetic coil (diameter of each wing = 4.5cm, symmetric bipolar pulse) held on the scalp overlying the left motor cortex, at the optimal scalp position for eliciting mild and isolated thumb movements. Movement threshold was defined as the minimum stimulation intensity able to elicit consistent thumb movements. Resting motor thresholds (MTs) and motor-evoked potentials (MEPs) amplitude to TMS[25] were determined before and after training using a Counterpoint Electromyograph (Dantec Electronics, Skovlunde, Denmark).

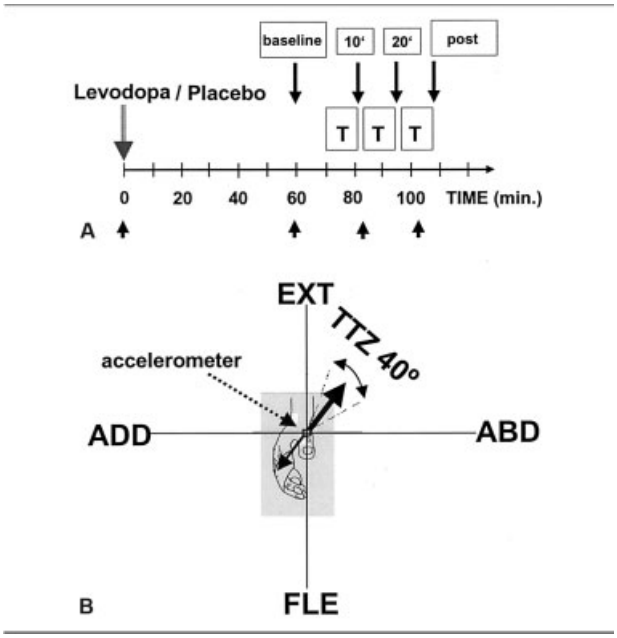

Fig 1. (A) Experimental design. L-*Dopa/placebo was administered at time 0 in each session, followed by determination of transcranial magnetic stimulation (TMS)–evoked thumb movement directions at baseline (60 minutes after drug intake) and after 10, 20, and 30 (post) minutes of training* (downward arrows). *Training consisted of three blocks of brisk thumb movements performed at 1Hz in the direction opposite to the baseline TMS-evoked thumb movement direction* (T). *Fatigue, attention toward the training task, blood pressure, and heart rate were assessed four times during the experiment* (upward arrows). *(B) Diagram showing measurement of thumb movements with an accelerometer positioned on the distal interphalangeal joint (rectangle on the thumb). Baseline TMS-evoked thumb movements in this example fell in a flexion–adduction direction* (thin solid arrow). *Training voluntary thumb motions were performed in the opposite direction (extension–abduction,* thick solid arrow*). At the end of the training period, we measured the percentage of TMS-evoked thumb movements falling in the training target zone (TTZ), the end point measure of the study.*

Encoding of a Motor Memory

To test formation of a motor memory by motor training, we used a protocol as described previously.[26–28] Motor training, consisting of voluntary thumb movements performed at 1Hz, leads to formation of a motor memory that encodes the kinematic details of the practiced motions in young individuals[28] but decays substantially after age 50 years.[20]

BASELINE DETERMINATION. Before training, 60 TMS stimuli were delivered to the optimal scalp position to elicit thumb movements at 0.1Hz, a rate that does not affect cortical excitability.[29] Subjects occasionally realized that the thumb had moved but could not determine its direction. In these trials, the baseline direction was defined as the direction of the mean angle of TMS-evoked movements (see Fig 1B, thin solid arrow). Subjects' relaxation was closely moni-

tored by EMG and auditory feedback. Trials with background EMG activity were discarded from analysis.

MOTOR TRAINING. After identifying the baseline TMS-evoked movement direction, subjects began the training period performing voluntary brisk thumb movements at 1Hz in a direction opposite to baseline (see Fig 1B, thick solid arrow) in blocks of 10 minutes for a total of 30 minutes. After each voluntary movement, the thumb returned to the start position by relaxation, as confirmed by EMG. Direction and magnitude of each voluntary movement were monitored online, and subjects were encouraged to perform accurately and consistently by an investigator blinded to the intervention type. To monitor the consistency of training movement directions, we calculated the magnitude of the first peak acceleration of the training movements (in seconds per square meter), the angular difference between baseline TMS-evoked thumb movement direction and the training movement direction vectors (in degrees), and the dispersion of training movement direction vectors (in the length of the mean vector in a unit circle).

POSTTRAINING DETERMINATION. TMS-evoked thumb movement directions were determined again after each 10-minute block with 12 TMS stimuli to monitor the time course of directional changes in TMS-evoked movements. After completing the training period, TMS-evoked movement directions were redetermined (TMS delivered at 0.1Hz for 10 minutes for a total of 60 trials).

PRIMARY END POINT MEASURE. To describe the training effects on TMS-evoked movement directions, we defined a training target zone (TTZ) as a window of ±20 degrees centered on the training direction (see Fig 1B, "TTZ 40°"). Our end point measure was the increase in the percentage of TMS-evoked movements that fell within the TTZ after training, as a measure of the magnitude of formation of a motor memory by the training.[20,26,30,31] By design, the training was in the direction opposite to the baseline direction. Therefore, the percentage of TMS-evoked movements within the TTZ before training was small (<5%).

INCLUSION CRITERIA. All participating subjects fulfilled the following inclusion criteria: (1) ability of TMS to elicit isolated thumb movements in the absence of movements of any other digits, wrist, or arm; (2) consistent (reproducible) direction of TMS-evoked thumb movements in the baseline condition; (3) absence of any medications acting primarily on the central nervous system, including antipsychotic drugs and antidepressants, or drugs interfering with the absorption of L-dopa from the gastric tract[23]; (4) normal medical and neurological examination; and (5) right-handedness (handedness score, ≥70[32]).

Statistical Analysis

Data analysis was performed by an investigator blind to the intervention type. Normal distribution (Kolmogorow–Smirnov test of normality) was assessed for all data. Repeated-measures analysis of variance ($ANOVA_{RM}$) with a polynomial contrast analysis was used to test the influence of the repeated factors $TIME_{base,\ 10\ min,\ 20\ min,\ post}$ and $DRUG_{L\text{-}dopa,\ placebo}$, and the between-subject factor $GROUP_{young,\ elderly}$ on the percentage of TMS-evoked movements in the TTZ (primary outcome measure), systolic blood pressure, diastolic blood pressure, heart rate, attention to the task, and fatigue over the course of the training. Monitoring of motor training kinematics (magnitude of first-peak acceleration of training movement, angular difference between the training movement direction and the baseline direction vectors, dispersion of training movement directions) and movement threshold were analyzed using $ANOVA_{RM}$ with the repeated factor $DRUG_{L\text{-}dopa,\ placebo}$ and the between-subject factor $GROUP_{young,\ elderly}$. Measures of corticomotoneuronal excitability (MT of the training agonist muscle [$MT_{agonist}$], MT of the training antagonist muscle [$MT_{antagonist}$], MEP amplitude of the training agonist [$MEP_{agonist}$], and MEP amplitude of the training antagonist [$MEP_{antagonist}$]) were analyzed using $ANOVA_{RM}$ with a polynomial contrast analysis for the factor $TIME_{base,\ post}$, the repeated factor $DRUG_{L\text{-}dopa,\ placebo}$, and the between-subject factor $GROUP_{young,\ elderly}$. Data were considered significant at a level of $p < 0.05$. All data are expressed as mean ± standard error of the mean, unless stated otherwise.

Results

Autonomic Parameters, Attention, and Fatigue

Attention and fatigue (Table 1) were comparable across GROUPS during both sessions (DRUG), over the course of the experiment (TIME) (all $F_{(1,18)} < 1.5$; $p < 0.05$). In both groups, systolic and diastolic blood pressure and pulse decreased over time to a similar extent with L-dopa and placebo (main effects of TIME; linear trends, all $F_{(1,18)} > 6.9$; $p < 0.02$) in the absence of interactions or main effects involving the factor GROUP.

Motor Training Kinematics

Motor training kinematics (magnitude of first-peak acceleration of training movements, angular difference between training movement direction and baseline direction vectors, dispersion of training movement directions) were comparable in the two age groups and across the two experimental conditions ($ANOVA_{RM}$ DRUG × GROUP; magnitude of first-peak acceleration: $F_{(1,18)} = 0.004$, $p = 0.95$; angular difference: $F_{(1,18)} = 0.04$, $p = 0.54$; dispersion of training movement directions: $F_{(1,18)} = 0.048$, $p = 0.83$, Table 2).

Encoding a Motor Memory

$ANOVA_{RM}$ showed a significant interaction of TIME × DRUG × GROUP on the percentage of TMS-evoked movements falling in the TTZ (primary end point measure of the study, $F_{(1,18)} = 5.83$, $p = 0.027$; Figs 2 and 3). Two of the 10 subjects in each group did not show the same direction of TMS-evoked movements at baseline in the two DRUG conditions (L-dopa and placebo sessions). Excluding these subjects

Table 1. Blood Pressure, Heart Rate, Attention, and Fatigue in the Placebo and L-dopa Conditions in the Elderly and the Young

Group, Drug	Measurement 1	2	3	4
Elderly, L-dopa				
Fatigue	5 ± 0.2	5 ± 0.4	5 ± 0.5	5 ± 0.5
Attention	6 ± 0.2	6 ± 0.3	5 ± 0.3	6 ± 0.3
HR	69 ± 2	65 ± 2	62 ± 2	62 ± 2
BP	125/76 ± 5/3	116/71 ± 4/3	113/71 ± 3/2	112/71 ± 3/2
Elderly, placebo				
Fatigue	5 ± 0.3	5 ± 0.4	5 ± 0.4	5 ± 0.4
Attention	5 ± 0.2	5 ± 0.2	5 ± 0.3	5 ± 0.3
HR	70 ± 3	66 ± 2	68 ± 2	68 ± 2
BP	126/75 ± 3/3	118/74 ± 5/2	116/74 ± 4/2	116/74 ± 4/2
Young, L-dopa				
Fatigue	5.5 ± 0.2	5.5 ± 0.2	5.6 ± 0.3	5.6 ± 0.2
Attention	5.6 ± 0.2	5.4 ± 0.2	5.5 ± 0.3	5.6 ± 0.2
HR	62 ± 1	61 ± 1	61 ± 1.7	61 ± 1
BP	113/68 ± 4/3	104/63 ± 4/2	104/61 ± 4/2	104/61 ± 3/2
Young, placebo				
Fatigue	5.4 ± 0.2	5.2 ± 0.2	5.2 ± 0.3	5.3 ± 0.2
Attention	5.5 ± 0.2	5.2 ± 0.2	5.2 ± 0.3	5.3 ± 0.2
HR	64 ± 2	64 ± 3	64 ± 3.0	62 ± 2
BP	110/70 ± 4/3	104/66 ± 3/2	107/64 ± 4/2	107/64 ± 3/2

Attention and fatigue were self-assessed by the subjects using visual analog scales (1, lowest; 7, highest). Heart rate (HR): beats/minute. Blood pressure (BP): mm Hg (systolic/diastolic). Note that systolic BP decreased progressively over time in both groups, with both drugs in the absence of significant changes in other parameters.

Table 2. Motor Training Kinematics

Drug, Group	Peak Acceleration (m/sec^2)	Angular Dispersion (length of unit vector)	Angular Difference (degrees)
L-Dopa, elderly	4.8 ± 0.3	0.94 ± 0.06	184 ± 19
Placebo, elderly	4.5 ± 0.5	0.94 ± 0.01	190 ± 19
L-Dopa, young	5.0 ± 0.5	0.92 ± 0.02	188 ± 9
Placebo, young	4.7 ± 0.5	0.93 ± 0.02	174 ± 18

Magnitude of first peak acceleration of training movements (m/sec^2), angular difference between the training movement direction and the baseline direction vectors (degrees), and dispersion of training movement directions (length of unit vector) showed no significant interaction or main effects for DRUG or GROUP, indicating comparable overall training kinematics across L-dopa/placebo and young/elderly.

from the analysis still indicated a significant interaction ($F_{(1,14)} = 7.2$; $p = 0.018$), and thus did not alter the main result. All subsequent analyses were then conducted on the sample of n = 20.

In young subjects, $ANOVA_{RM}$ of TIME × DRUG showed a significant interaction ($F_{(1,9)} = 5.2$; $p = 0.049$). Post hoc analyses demonstrated that placebo + training elicited a progressive increase in the percentage of TMS-evoked thumb movements falling in the TTZ that became significant after 30 minutes of training (from 0.70 ± 0.5% to 19.1 ± 6.5%; $t_{(9)} = 2.79$; $p = 0.021$; see Fig 2A, 30 minutes [post], white bars; see Fig 3A). In contrast, L-dopa + training elicited a faster change. After only 10 minutes, TMS-evoked thumb movements falling in the TTZ increased with L-dopa + training (from 1.2 ± 0.7% to 19.3 ± 4.6%; $t_{(9)} = 3.69$; $p = 0.005$; see Fig 2A, 10 minutes, black bar) in the absence of changes with placebo + training (from 0.70 ± 0.5% to 5.3 ± 2.9%, not significant; see Fig 2A, 10 minutes, white bar). Direct comparison at 10 and 20 minutes between both sessions showed a significant difference in the percentage of TMS-evoked movements in the TTZ in the L-dopa + training versus the placebo + training session ($t_{(9)} = 2.63$; $p = 0.027$ after 10 minutes; $t_{(9)} = 2.61$; $p = 0.028$ after 20 minutes), in the absence of significant changes with placebo + training ($t_{(9)} = 1.46$; $p = 0.18$ after 10 minutes; $t_{(9)} = 1.69$; $p = 0.13$ after 20 minutes; see Fig 2A, 10 and 20 minutes, white bars). At the end of the 30 minutes, both L-dopa + training and placebo + training reached comparable values in TMS-evoked thumb movements falling in the TTZ (from 1.2 ± 0.67% to 27.8 ± 7.8% with L-dopa + training and from 0.70 ± 0.5% to 19.1 ± 6.7% with placebo + training, both $t_{(9)} > 2.7$; $p < 0.022$; see Fig 2A, 30 minutes [post]; see Figs 3A, B).

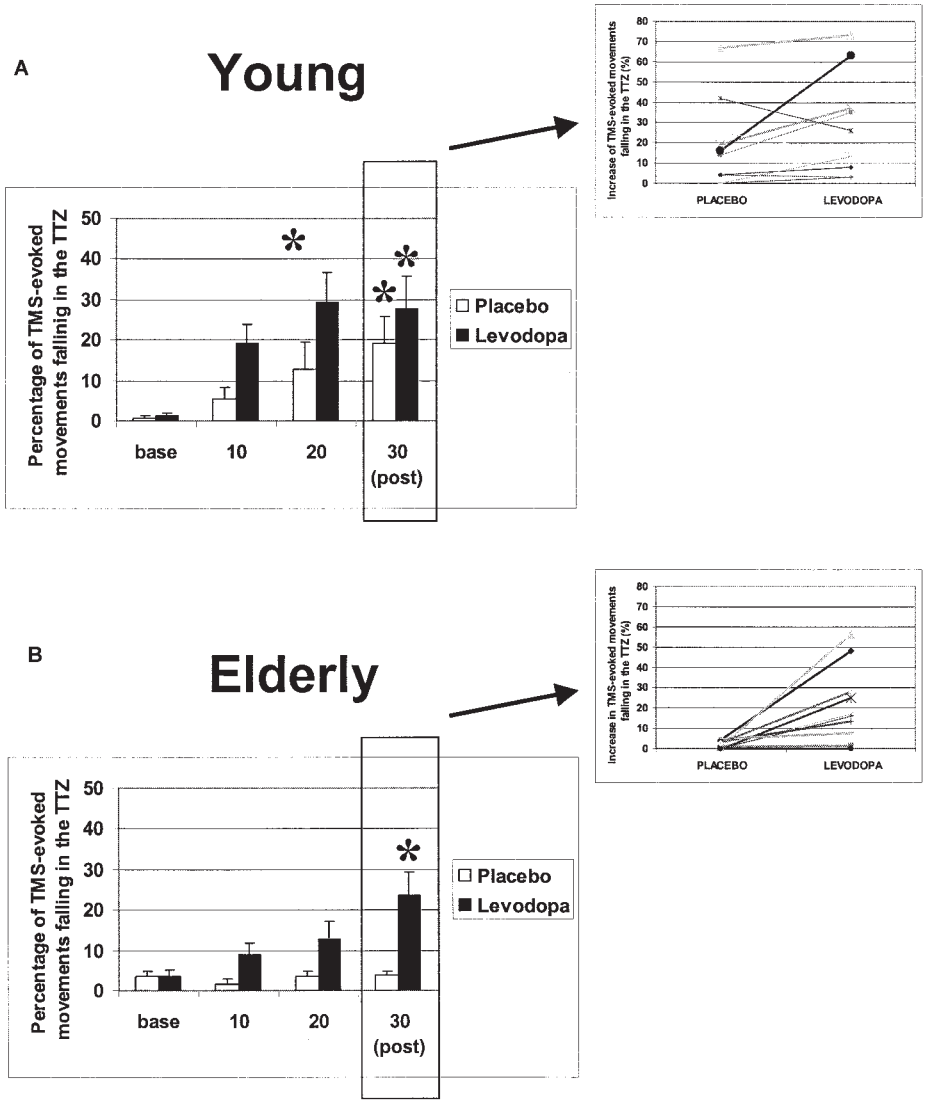

Fig 2. Percentage of transcranial magnetic stimulation (TMS)–evoked thumb movements falling in the training target zone (TTZ) in young (A) and elderly (B) healthy volunteers. In young subjects (A), training under placebo led to a progressive increase in TMS-evoked thumb movements falling in the TTZ that became significant after 30 minutes (A, 30 min [post], white bar*). L-Dopa + training accelerated the development of this form of plasticity, which became significant after only 10 minutes of training (A, 10 min,* black bar*). In elderly subjects (B), consistent with previous results,[20] training under placebo did not induce changes in TMS-evoked thumb movements falling in the TTZ (B, 30 min [post],* white bar*). L-Dopa + training substantially enhanced the response to motor training, which became significant after 30 minutes (B, 30 min [post],* black bar*), and that was comparable in magnitude with that identified in younger subjects under placebo and L-dopa (A, 30 min [post],* black *and* white bars*). Note that this effect was evident in five of the seven elderly subjects tested (inset). To illustrate the percentage change in the training + L-dopa versus the training + placebo condition, we summarize the mean change for each subject in each condition in the above insets (A, young; B, elderly).* $^{*}p < 0.05$.

In the elderly subjects, $ANOVA_{RM}$ of TIME × DRUG showed a significant interaction ($F_{(1,9)} = 8.6$; $p = 0.017$). Post hoc analyses demonstrated that training under placebo did not increase TMS-evoked thumb movements falling in the TTZ (see Figs 2B and 3C), ($t_{(9)} = 0.16$; $p = 0.87$), which is consistent with a previous report.[20] In contrast, L-dopa + training led to a significant increase in the percentage of TMS-evoked thumb movements falling in the TTZ relative to baseline at 30 minutes (from 3.6 ± 1.5% to 23.5 ± 5.8%; $t_{(9)} = 3.72$; $p = 0.005$; see Fig 2B, 30 minutes [post], black bar; see Fig 3D). This effect was clearly identifiable in 7 of the 10 subjects tested (see Fig 2B, inset; see also example in Fig 3D).

Interestingly, L-dopa + training in the elderly subjects increased the percentage of TMS-evoked thumb

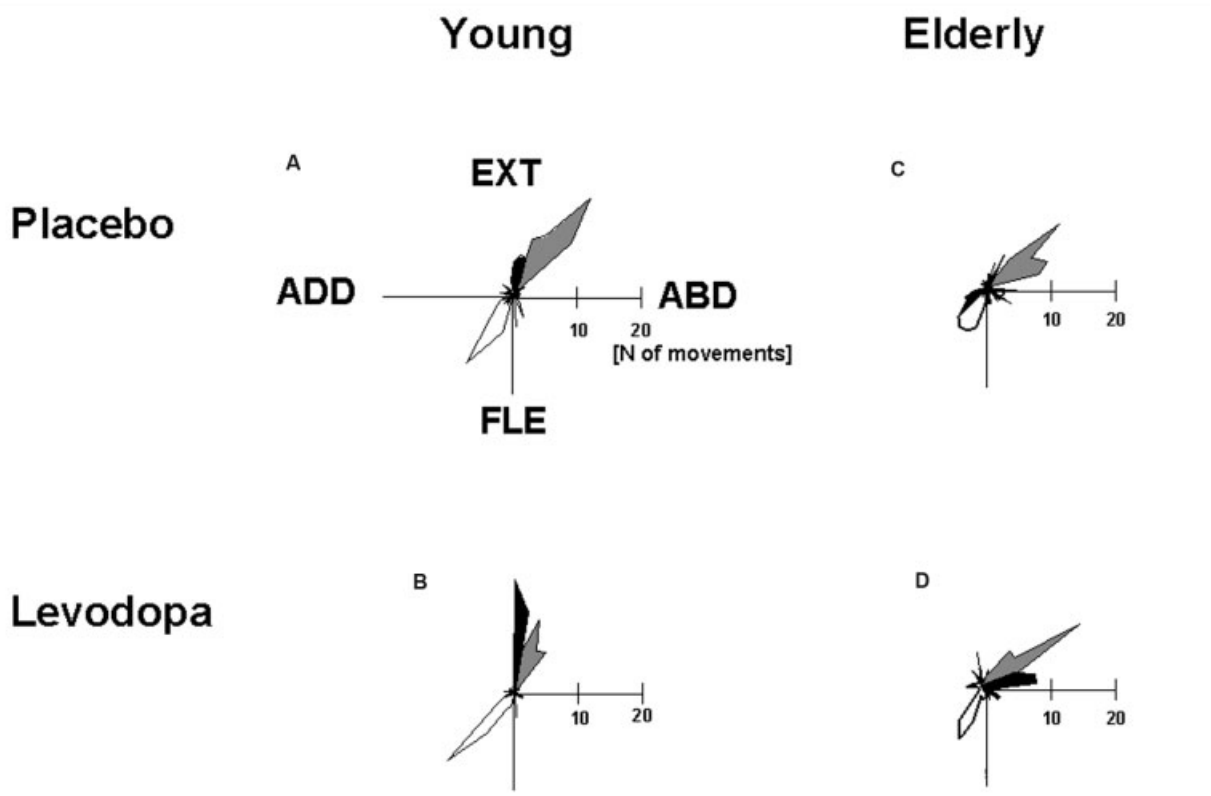

Fig 3. Transcranial magnetic stimulation (TMS)–evoked movement directions displayed as circular histograms in a representative young (A, B) and elderly (C, D) subject in the placebo (upper histograms, A, C) and L-dopa (lower histograms, B, D) sessions. TMS-evoked movement directions at baseline are displayed in white, voluntary training movements are in gray, and TMS-evoked movement directions after training are in black. In young subjects, placebo + training (A) and L-dopa + training (B) induced a substantial increase of movements falling in the training target zone (TTZ) (note the black histograms in a direction approximately opposite to the white histograms). In the elderly, only L-dopa + training (D), but not placebo + training (C), led to a substantial increase in TMS-evoked movements falling in the TTZ.

movements falling in the TTZ relative to baseline (by 19.9 ± 5.3%) to a similar extent than both interventions in the young (L-dopa + training by 26.6 ± 7.8% and placebo + training by 18.4 ± 6.9%, respectively, not significant; see Figs 2A, B, 30 minutes [post]), the main difference being the training time required to elicit this effect. With L-dopa + training, 10-minute training sufficed to elicit this effect in the young (increase by 18.1 ± 4.3%), but not in the elderly (increase by 5.5 ± 2.2%).

Motor Cortex Excitability

At baseline, movement thresholds (Table 3), MTs (Table 4), and MEP amplitudes before motor training ($MEP_{base\ agonist}$ and $MEP_{base\ antagonist}$; Table 5) were comparable across DRUG and GROUP. Motor training resulted in larger MEP amplitudes in training agonist muscles ($MEP_{antagonist\ post}$) (main factor TIME: $F_{(1,18)} = 11.4$; $p = 0.003$), in the absence of significant effects of DRUG or GROUP, or changes in resting MT (see Table 4) and $MEP_{antagonist\ post}$ (see Table 5).

Table 3. Movement Thresholds (MovT) in Muscles Mediating Movements in the Training Direction ($MovT_{agonist}$)

Drug, Group	$MovT_{agonist}$ (% StimOutput)	$MovT_{agonist}$ (% MT)
L-Dopa, elderly	69 ± 3.8	120 ± 2.2
Placebo, elderly	70 ± 3.2	121 ± 3.3
L-Dopa, young	63.2 ± 3.7	124.5 ± 3.4
Placebo, young	62.8 ± 3.6	118.8 ± 4.1

Discussion

Two main findings emerged from this study. First, L-dopa shortened the training time required to form a motor memory in young healthy volunteers. Second, L-dopa restored the ability to form a motor memory in the elderly subjects to levels similar to those seen in healthy young subjects.

Attention to the training, fatigue levels, blood pressure, heart rate (see Table 1), and especially motor training kinematics (see Table 2) were comparable across groups (young and elderly) and interventions (L-dopa and placebo). Therefore, subjects of different ages

Table 4. Motor Threshold (MT) before (MT_{base}) and after (MT_{post}) Training in Muscles Mediating Movements in the Training ($MT_{agonist}$) and Baseline (MT_{base}) Direction

Drug, Group	$MT_{agonist\ base}$ (%)	$MT_{agonist\ post}$ (%)	$MT_{antagonist\ base}$ (%)	$MT_{antagonist\ post}$ (%)
L-Dopa, elderly	56.3 ± 3.2	55 ± 3.4	55.4 ± 3.2	55.4 ± 3.2
Placebo, elderly	56.6 ± 2.8	55.8 ± 2.5	55.7 ± 2.7	55.7 ± 2.7
L-Dopa, young	50.8 ± 3.2	50.9 ± 3.3	*51.7 ± 3.2*	51.8 ± 3.2
Placebo, young	53 ± 2.8	52.2 ± 2.8	53 ± 3.1	52.4 ± 2.8

Table 5. Motor-Evoked Potentials (MEP) before (MEP_{base}) and after (MEP_{post}) Training in Muscles Mediating Movements in the Training ($MEP_{agonist}$) and Baseline Direction ($MEP_{antagonist}$)

Drug, Group	$MEP_{agonist\ base}$ (mV)	$MEP_{agonist\ post}$ (mV)	Paired *t* test, $MEP_{agonist\ base}$ (mV) vs $MEP_{agonist\ post}$ (mV) (*p*)
L-Dopa, elderly	1.18 ± 0.4	1.41 ± 0.5	<0.05
Placebo, elderly	1.017 ± 0.2	1.412 ± 0.3	<0.05
L-Dopa, young	1.49 ± 0.2	1.84 ± 0.5	<0.05
Placebo, young	1.13 ± 0.2	1.53 ± 0.2	<0.05

Drug, Group	$MEP_{antagonist\ base}$ (mV)	$MEP_{antagonist\ post}$ (mV)	Paired *t* test, $MEP_{antagonist\ base}$ [mV] vs $MEP_{antagonist\ post}$ [mV]
L-Dopa, elderly	1.16 ± 0.3	1.07 ± 0.2	NS
Placebo, elderly	1.98 ± 0.2	0.92 ± 0.1	NS
L-Dopa, young	1.23 ± 0.1	1.2 ± 0.2	NS
Placebo, young	1.7 ± 0.3	1.6 ± 0.8	NS

NS = not significant.

performed consistently in this specific motor training task under the two experimental conditions, investing comparable attentional effort,[33] a result agreeing with previous studies.[20,26,27] Preceding training, measures of corticomotoneuronal excitability, including MTs and MEP amplitudes from muscles' agonist and antagonist to the thumb movements' training direction, were comparable with both L-dopa and placebo (see Tables 3 through 5), which is also consistent with previous reports.[34]

Formation of a Motor Memory in Young and Elderly Subjects

The consistent TMS-evoked movement directions within subjects at baseline in both age groups is likely the consequence of a balance of inhibitory and excitatory influences in the neocortex regulated by mechanisms that alter synaptic efficacy.[35] In the young subjects, training under placebo led to a significant increase in the number of TMS-evoked movements falling in the TTZ (primary end point measure; see Fig 1B; see also Butefisch and colleagues,[26,27] Classen and colleagues,[28] and Sawaki and colleagues[30,31]), a type of reorganization of the neuronal network mediating thumb motions that encodes the kinematic details of the practiced movements.[28] This encoding process, influenced by LTP-like mechanisms and GABAergic, *N*-methyl-D-aspartate (NMDA), muscarinic, and adrenergic receptor function,[27,30,31,36] is likely to participate in the acquisition of procedural skills,[15,37] which also require active repetitive training.[38] In this study, frequently repeated movements probably strengthened intracortical networks mediating the trained movement directions.[35,39] This proposal is supported by the finding of a differential effect of training on motor cortical excitability: MEP amplitudes of muscles operating as training agonists increased, whereas those operating as training antagonists did not.[27,28] Overall, these findings are consistent with the view that tuning of excitatory and inhibitory influences within M1 is under way continuously, leading to strengthening of representations of the practiced movement directions.[28]

In the elderly subjects, 30-minute training under placebo did not elicit directional changes in TMS-evoked thumb movement directions, defining a differential capacity of the aging central nervous system to encode a motor memory.[20] In contrast, MEP amplitudes of muscles operating as training agonists increased to a similar extent as in younger Subjects. These findings support the view that a differential increase in excitability in training agonist and antagonist muscles may represent a prerequisite, but by itself is not sufficient to encode a motor memory.[27,28] One explanation for this finding may be that the elderly show

a more focal pattern of muscle facilitation than the young, which is insufficient to induce a change in thumb movement direction.

Effects of L-dopa on Memory Formation

In the young subjects, L-dopa accelerated memory formation relative to placebo to a similar extent as d-amphetamine did in previous reports, in the absence of overt differences in motor training kinematics, attention, arousal, and motor cortical excitability.[26,36] The net effect after 30-minute training was a trend toward a larger effect with L-dopa than placebo (an effect that was more prominent in subjects with poorer response to motor training alone; see Fig 2A, inset), a result consistent with the reported beneficial effects of L-dopa on language learning.[33] A larger number of subjects or longer training periods may be required to elicit a more marked effect.

In the elderly subjects, L-dopa restored memory formation to levels similar to those identified in young subjects in the absence of differences in motor training kinematics, attention, arousal, or motor cortical excitability. These findings suggest the hypothesis that enhancing dopaminergic transmission may constitute a possible strategy to overcome declining motor memory formation in advanced age. Because d-amphetamine, another drug proposed to influence motor memory formation, may result in severe side effects such as cardiac arrhythmias and hypertension, especially in elderly adults,[40,41] the risk/benefit ratio of interventional approaches involving L-dopa appears to be superior. It remains to be determined if L-dopa premedication may even extend the duration of motor memory formation, similar to what has been reported in young healthy subjects after d-amphetamine premedication.[26]

Patterns of formation of a motor memory differed in both age groups. Thirty-minute training under placebo successfully encoded a motor memory in the young,[26–28] but not in the elderly subjects.[20] L-dopa accelerated memory formation in the young and restored the elderly subjects' capacity to form memories with 30-minute training. Expanding the training time in the elderly group could not be implemented because training times beyond 30 minutes result in diminished attention, deteriorating training kinematics, and increased fatigue (unpublished observations). Bioavailability differences in the two age groups could not explain the decrease in memory formation in the elderly subjects, who usually have greater absorption rates and faster drug appearance in plasma than young individuals.[24]

Mechanisms Underlying Dopaminergic Influence on Motor Memory Formation

Age-dependent decline in memory formation is influenced by changes in neurotransmitters,[9,10,42,43] particularly dopamine.[8,10,43] Aging results in accumulating oxidative stress, which, in turn, downregulates dopaminergic activity[8,12] and correlates with impaired motor control in animal studies.[44] In humans, aging leads to substantially reduced D2 receptors,[14,45] as well as dopaminergic neuronal loss in the substantia nigra,[13] which also correlates with a decline in motor and cognitive function.[13,14] Altogether, these findings strongly point to a relevant role of dopaminergic function on memory formation in normal aging.

Mechanisms underlying these effects in humans are incompletely understood but may relate to the documented facilitatory effects of dopaminergic neurotransmission on NMDA dependent[5,8] and –independent LTP induction, which are involved in memory formation.[1,2] Dopamine selectively enhances active synapses and downregulates those that are inactive in a task-specific manner,[46] increasing the signal-to-noise ratio.[22,38] It is then possible that L-dopa, under our experimental conditions in both age groups, led to strengthening the synapses or intracortical connections mediating movements in the training direction, or both, whereas weakening those mediating motions in the baseline direction. Therefore, our results are consistent with the view that upregulation of cortical LTP-like processes, involved in motor memory formation under our experimental paradigm,[13,14,21,45] is one of the mechanisms underlying L-dopa–dependent effects reported here. By using specific dopamine receptor agonists, future studies could examine the differential involvement of D1/D5- and D2-like receptors in mediating the effect.

Although our experimental paradigm tested plastic changes within the primary motor cortex (M1),[27,28] it is possible that L-dopa acted at different sites. Tracer studies in macaque monkeys demonstrated that dopaminergic midbrain fibers directly modulate activity in cortical neurons in layers II and III in supplementary motor area and M1,[47] a form of cortical organization similar to that identified in humans.[48] It is possible that L-dopa's effects on M1 organization were mediated through its action on midbrain–cortical connections.[47] Alternatively, plasticity in M1 may have been influenced indirectly via the striatum, rich in dopaminergic innervation,[49] the somatosensory cortex, or the inferior parietal cortex,[50] all structures well connected to M1.[48,51]

Conclusion

Premedication with L-dopa accelerates memory formation in young adults and restores motor memory formation in elderly adults if combined with sustained training. This study demonstrates the possibility of enhancing training-driven motor memory formation in both age groups, but particularly in the elderly, who

are more often affected by conditions such as stroke.[52,53]

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