

# Mechanisms Influencing Acquisition and Recall of Motor Memories

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Received 15 January 2002; accepted in final form 3 June 2002

**Donchin, Opher, Lumy Sawaki, Ghangadar Madupu, Leonardo G. Cohen, and Reza Shadmehr.** Mechanisms influencing acquisition and recall of motor memories. *J Neurophysiol* 88: 2114–2123, 2002; 10.1152/jn.00033.2002. An internal model of the dynamics of a tool or an object is part of the motor memory acquired when learning to use the tool or to manipulate the object. Changes in synaptic efficacy may underlie acquisition and storage of memories. Here we studied the effect of pharmacological agents that interfere with synaptic plasticity on acquisition of new motor memories and on recall of a previously learned internal model. Forty-nine subjects, divided into six groups, made reaching movements while holding a robotic arm that applied forces to the hand. On day 1, all subjects learned to move in force field A. On day 2, each group of subjects was tested on their ability to recall field A and their ability to learn a new internal model in field B. Four groups participated in the experiments of day 2 under the effects of lorazepam (LZ; a GABA type A receptor-positive allosteric modulator), dextromethorphan [DM; an *N*-methyl-D-aspartate (NMDA) receptor blocker], lamotrigine (LG, a drug that blocks voltage-gated Na<sup>+</sup> and Ca<sup>2+</sup> channel), or scopolamine (SP; muscarinic receptor antagonist). Two control groups were tested in a drug-free condition: one group that was not exposed to additional experimental protocols (NP) and another group was tested under ~24 h of sleep deprivation between completion of learning on day 1 and start of testing on day 2 (SD). Recall of field A was normal in all groups. Learning of field B was reduced by LZ and DM but not by SP, LG, SD or in the NP condition. These results suggest that a 24-h sleep-deprivation period may have little or no effect on consolidation of this motor memory and that NMDA receptor activation and GABAergic inhibition are mechanisms operating in the acquisition but not recall of new motor memories in humans.

## INTRODUCTION

Studies of reaching movements have suggested that the human brain constructs motor commands based on a prediction of forces that will be experienced in the upcoming movement such that the motor commands counter the effect of the predicted forces (Ghez et al. 2000; Lackner and DiZio 1994; Shadmehr and Mussa-Ivaldi 1994). For example, when reaching movements are performed while holding the handle of a robotic arm, novel velocity-dependent forces may be imposed on the hand (called a force field). At first, no force is predicted by the motor system, but forces are experienced, and the motor commands result in the hand's trajectory deviating from a straight path. If the force field remains consistent, the motor

commands are adjusted through practice (Thoroughman and Shadmehr 1999) until the hand's trajectory becomes straight again. Studies have shown that this internal model of the experienced forces shows generalization in velocity and position space (Shadmehr and Moussavi 2000; Thoroughman and Shadmehr 2000) and from reaching to drawing movements (Conditt et al. 1997). This suggests that the internal model is learned in a way that allows it to flexibly transform desired arm motion into predictions of force. It has further been shown that this learning consolidates into long-lasting motor memories that can be used to recall the appropriate internal model after a long time without practice (Shadmehr and Brashers-Krug 1997).

Results from functional imaging experiments have suggested a role for the cerebellum in acquisition and retention of this motor memory (Nezafat et al. 2001). In agreement with this, patients with cerebellar damage were found to be dramatically impaired in their ability to learn this task (Smith 2001). On the other hand, recent neurophysiological data have demonstrated a role for the primary motor cortex in representation of the internal model of force fields (Li et al. 2001). Changes in synaptic efficacy have been implicated in memory storage in various areas of the cortex and the cerebellum (Abel and Lattal 2001; Martin et al. 2000). For example, a recent study showed that long-term potentiation was saturated in the motor cortex of rats that learned a manipulation task to retrieve food pellets (Riout-Pedotti et al. 2000). Thus it is conceivable that changes in synaptic efficacy may influence acquisition of new motor memories. If this is the case, pharmacological manipulations that interfere with synaptic plasticity would be expected to block new learning. This approach has been used before and provided insight into the mechanisms of plasticity associated with deafferentation and use-dependent plasticity (Butefisch et al. 2000; Sawaki et al. 2002; Thiel et al. 2001; Ziemann et al. 1998b). Here we test the hypothesis that drugs that have been shown to impair synaptic plasticity will influence the ability of humans to acquire a new internal model of dynamics of reaching movements.

## METHODS

### *Subjects and experimental groups*

Forty five healthy volunteers, divided into six groups, participated in this study (Table 1). Subjects were aged 18–50 (mean: 35) and included 25 men and 20 women. There was no significant difference

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TABLE 1. Treatment groups and side effects

Group	n	Manipulation	Effect	Clinical Application	Side Effects
LZ	7	Lorazepam (LZ; 0.038 mg/kg PO, 2 h before testing)	GABA <sub>A</sub> receptor agonist	Anti-anxiety; anti-insomniac	Sedation, dizziness, vertigo, weakness, and unsteadiness
DM	8	Dextromethorphan (DM; 2 mg/kg PO, 3 h before testing)	NMDA antagonist	Anti-tussive; Analgesic	Mild and infrequent drowsiness, fatigue and dizziness
SP	6	Scopolamine (SP; 1.5 mg transdermal patch behind ear, 5 h before testing)	Muscarinic antagonist	Prevention of motion sickness	At high doses may cause dizziness, restlessness, memory disturbances, locomotor difficulty
LG	8	Lamotrigine (LG; 300 mg PO, 2 h before testing)	Na <sup>+</sup> - and voltage-dependent Ca <sup>2+</sup> channel blocker	Anti-epileptic	Dizziness, ataxia, and headache
SD	8	Sleep deprived (SD; subjects did not sleep or consume caffeine between days 1 and 2 of the experiment)			
Control	8				

The table describes the six different treatment groups used in the studies and introduces the abbreviations used to refer to them throughout the paper. NMDA, *N*-methyl-D-aspartate.

in age among the groups (ANOVA,  $P > 0.3$ ) nor was there any difference in the distribution of men and women ( $\chi^2$ ,  $P > 0.9$ ). All subjects were right handed. No subject had prior experience with the robotic system. The study protocol was approved by the Institutional Review Boards of the National Institute of Neurological Disorders and Stroke. Subjects gave their written informed consent for the study.

Each subject came to the laboratory on two consecutive days termed *train day* and *test day*. On test day, subjects were tested under the influence of one of four different drugs: lorazepam (LZ, a GABA<sub>A</sub> receptor-positive allosteric modulator), dextromethorphan [DM, a *N*-methyl-D-aspartate (NMDA) receptor blocker], lamotrigine (LG, a drug that blocks voltage-gated Na<sup>+</sup> and Ca<sup>2+</sup> channels), or scopolamine (SP, muscarinic receptor antagonist) (Saucier et al. 1996). In addition, two drug-free groups were used as controls: a sleep-deprived group (SD) and one that experienced no additional experimental protocols (NP). Note that NP does not indicate that there was no protocol at all for the subjects (who went through the same pretraining and testing as the other subjects) but rather that no additional protocols, such as drugs or sleep deprivation, were used in this group. Subjects were not informed of the group to which they were assigned, except in the case of the SD group. However, placebos were not given to the NP group, so they may also have been aware of their group assignment. A careful reading of the side effects for the different drugs could also have alerted some of the subjects.

In the LZ group ( $n = 7$ ), testing was performed 2 h following intake of a single oral dose of LZ (0.038 mg/kg orally). LZ is a short-acting benzodiazepine that at this dose produces functional potentiation of GABA<sub>A</sub> receptors through positive allosteric modulation and enhancing Cl<sup>-</sup> currents through the receptor (Sybirska et al. 1993). By the time testing started, blood levels are known to be in the therapeutic range ( $>16$  nG/ml) and remain stable for 3–5 h (Greenblatt et al. 1993). A single oral dose of LZ similar to the one administered in this study attenuates intracortical excitability (Ziemann et al. 1996), use-dependent plasticity (Butefisch et al. 2000), deafferentation-induced plasticity (Ziemann et al. 1998b), and plasticity associated with adaptation to light deprivation in the visual system (Borojerdi et al. 2001) in humans.

In the DM group ( $n = 8$ ), subjects received a single oral dose of DM (2 mg/kg orally). Because DM rapidly reaches therapeutic blood levels and has a relatively short half-life (2.5 h) (Hollander et al. 1994), a single oral dose was administered 30 min preceding testing. DM at this dose results in serum and brain concentrations in humans (Hollander et al. 1994; Steinberg et al. 1996) similar to those that induce NMDA receptor block in vitro (Apland and Braitman 1990). Because DM is rapidly metabolized to dextorphan, a similarly active

compound (Hollander et al. 1994), and brain tissue DM and dextorphan concentrations are much higher than those present in blood (Steinberg et al. 1996), DM plasma levels are an imprecise indicator of CNS action (Hollander et al. 1994) and were not measured. Similar doses of DM are known to influence intracortical excitability (Ziemann et al. 1998a), use-dependent plasticity (Butefisch et al. 2000), deafferentation-induced plasticity (Ziemann et al. 1998b), and plasticity associated with light deprivation (Borojerdi et al., personal communications) in humans.

In the LG group ( $n = 6$ ), subjects received a single 200 mg oral dose of this antiepileptic drug. This drug affects voltage-gated Na<sup>+</sup> and Ca<sup>2+</sup> channels (Leach and Brodie 1995; Wang et al. 1996). At this dose, a single oral dose of LG results in clear effects on intracortical excitability (Ziemann et al. 1996) and deafferentation-induced plasticity (Ziemann et al. 1998b) in humans.

In the SP group ( $n = 8$ ), subjects had a transdermal SP patch (Transderm Scopo, belladonna alkaloid with anti-muscarinic properties; 1.5 mg) (Clissold and Heel 1985; Whiteman and Edeen 1990) placed behind the ear. At testing time, plasma concentrations reach  $>50$  pg/ml, a threshold value required for appropriate cerebrospinal fluid (CSF) levels and therefore therapeutic effects such as prevention of motion sickness (Nachum et al. 2001). At this dose, SP depresses use-dependent plasticity in humans without causing changes in intracortical excitability (Sawaki et al. 2002).

In the SD group ( $n = 8$ ), subjects were not allowed to sleep between days 1 and 2 and were monitored by nurses throughout the night. They were accommodated in a clinical ward near the laboratory where they were provided with entertainment to help them stay awake.

Drug side effects were assessed using a questionnaire. Subjects rated their condition on a scale of 1–5 (5 being worst) immediately prior to testing on test day along a number of dimensions. These included drowsiness, dizziness, jitters, fatigue, and nausea.

### Motor-learning task

The experimental setup was similar to earlier experiments (Shadmehr and Brashers-Krug 1997). Subjects held the handle of a two-link robotic manipulandum and were asked to make point-to-point reaching movements. Motion of the manipulandum was restricted to the horizontal plane. Targets appeared at 10 cm in one of six directions (45, 90, 135, 225, 270, and 315°, Fig. 1C) in a pseudo-random out-and-back pattern. The order of the target directions was the same for all subjects. The computer provided positive reinforcement in the form of a target explosion if the movement was completed within a

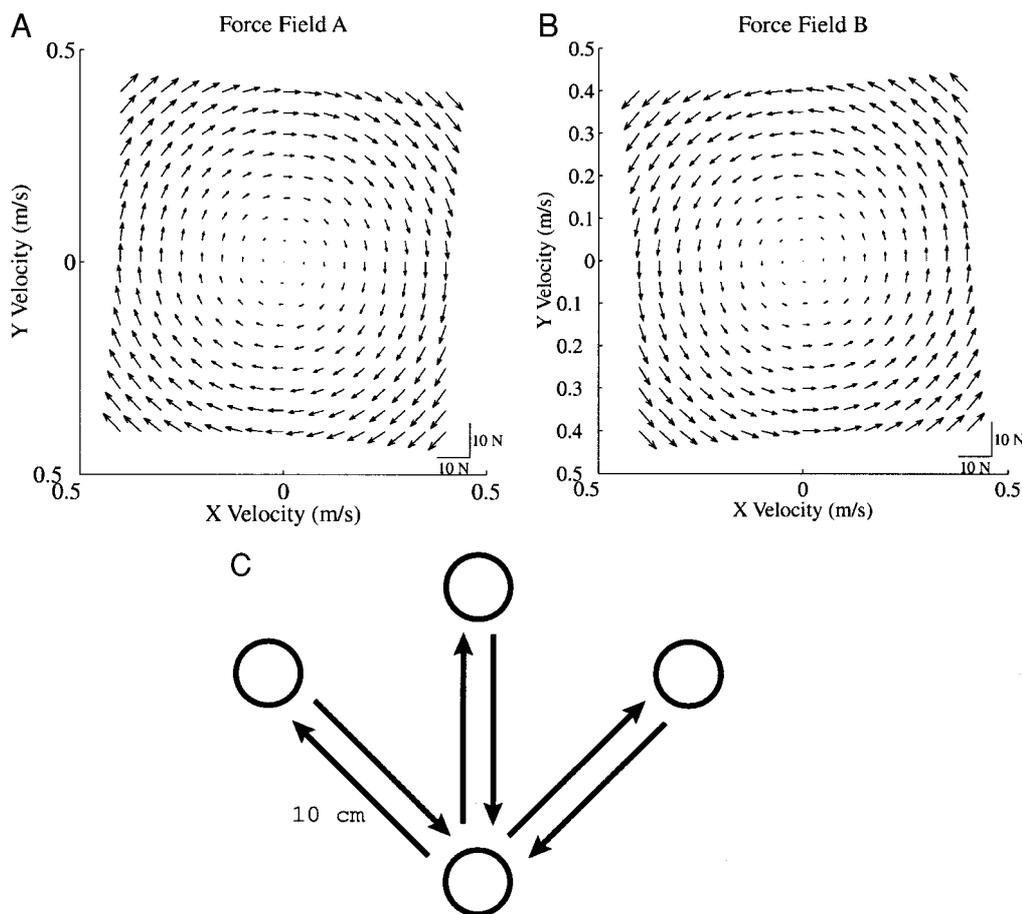


FIG. 1. Motor learning task. The figure shows the 2 velocity-dependent force fields used to perturb arm movements during training and recall sets (A) and test sets (B; see Table 2). C: the six different directions of movement are shown. Outward movements were always followed by a movement back to center.

certain window  $\sim 0.5$  s. The window was initially 140 ms and was reduced slightly after every success and enlarged slightly after every failure. The computer recorded position, velocity, and force at the handle at 100 Hz.

The robot produced forces that depended linearly on instantaneous hand velocity:  $F = \beta \dot{x}$ , where  $\beta$  was a curl matrix that resulted in forces that were perpendicular to the motion of the hand. Two different force fields were used (Fig. 1, A and B). This force field changed the dynamics of the arm, significantly distorting previously straight hand paths. With practice, the hand paths tended to become straight again. Previous studies of this simple paradigm suggested that the improvement in performance is due to the construction of an internal model of the force field by the brain (Condit and Mussa-Ivaldi 1999; Shadmehr and Mussa-Ivaldi 1994; Thoroughman and Shadmehr 2000). An important piece of evidence for this conjecture is the fact that if the force field is unexpectedly removed (i.e., returned to null), the movements exhibit aftereffects. In an aftereffect, the movement trajectory seems to be a mirror image of the distorted trials induced by initial exposure to the force field. A movement where the force field is removed is called a catch trial. Approximately one in six targets were pseudo-randomly selected to serve as catch trials.

### Experimental protocols

The purpose of the current study was to determine the effects of premedication with drugs that interfere with synaptic plasticity on the subjects' ability to learn a new motor memory. To assess the attentional level and general motor function under the effects of the different drugs and sleep deprivation, subjects were initially tested on the force field that they had learned on the previous day (recall). Therefore subjects under the influence of a drug or sleep deprivation first demonstrated their ability to recall a previously learned internal

model of a force field, then attempted to learn a new internal model, and finally demonstrated again the ability to perform in the previously learned field.

Therefore on day 1, *train day*, subjects learned a force field (field A, a clockwise curl field described by  $\beta = [0 \ 13; -13 \ 0]$  N  $\cdot$  s/m. Fig. 1A) and on day 2, *test day*, they were tested on the same field under the influence of the intervention. This was followed immediately by an attempt to learn a new force field (field B, a counter-clockwise curl field:  $\beta = [0 \ -13; 13 \ 0]$  N  $\cdot$  s/m. Fig. 1B). Finally, the subjects were asked to perform again in the presence of the initially learned field A. The protocol for the *train day* was similar for all subjects. They performed two sets of 198 movements in the null field (familiarization sets), followed by three sets of 198 movements in force field A (training sets) for most subjects. Some subjects only performed two sets of 198 movements in force field A. These subjects were from the following groups: LZ, 2; DM, 4; LG, 3; SD, 4; NP, 2. Their behavior on the test day was not noticeably different from other subjects in their respective groups and so the data were combined.

Movement trials on test day began with the null field (18 movements, re-familiarization set) followed by field A (102 movements, recall set 1). This was followed by training in field B (3 sets of 198 movements, test sets). Finally, another recall set in field A (198 movements, recall set 2) was performed. Therefore on test day we tested performance in field A both before and after learning in field B. This was to address the possibility that the drugs were more effective either at the beginning or the end of the experiment on test day. Table 2 can be consulted for a summary of the sets performed on each day.

### Measures of performance

We computed a measure of error called the perpendicular displacement (PD). This was the distance from any point in the movement to

TABLE 2. *Experimental protocol*

	Field	No. of Movements
Day 1 (no drugs)		
Familiarize	Null	198
Familiarize	Null	198
Train	A	198
Train	A	198
Train	A	198
Day 2		
Refamiliarize	Null	18
Recall	A	102
Test	B	198
Test	B	198
Test	B	198
Recall	A	198

Table shows the protocol each subject was given on the two consecutive days of experimentation.

a straight line that connected its start and endpoints. The distance was computed at a time 300 ms after the beginning of the movement. For this purpose, beginning of movement was determined off-line using a velocity threshold at 15% of the peak velocity for the movement.

A theoretical model of learning has suggested that formation of an internal model in this task should have two prominent characteristics: with practice, the PDs in fielded movements should gradually decrease, and the PDs in the catch trials should gradually increase (and move in the opposite direction to the PDs in the fielded trials) (Shadmehr and Mussa-Ivaldi 1994). We therefore thought that if a single measure is to be used to quantify learning (termed a learning index, LI), it would be reasonable to use a ratio of the PDs during fielded and catch trials

$$LI = \frac{|PD_{catch}|}{|PD_{fielded}| + |PD_{catch}|} \quad (1)$$

Early in training, when we have small PDs in the catch trials and large PDs in fielded movements, the LI would be close to 0. Late in training, PDs in catch trials should be large and PDs in fielded movements should be small, so LI should be close to 1. LI was calculated on PDs averaged over 50 consecutive movements, which would include, on average, eight catch trials and 42 fielded movements. As the target sets were not divisible by 50, the last bin of the set was slightly smaller or larger than 50 targets.

Of course, it is possible, in theory, that the LI would increase because catch trial PDs became larger while fielded trial PDs remained unchanged or catch trial PDs remained unchanged while fielded trials PDs got smaller. However, an examination of our data revealed that catch trial and fielded trial PDs generally changed together.

### Statistical analysis

To compare performance across groups, we applied regression and ANOVA techniques described by Glanz and Slinker (2001). The statistical model was a linear one in which LI for a given subject from a particular group at a given sample (bin) was a sum of effects due to the categorical variable group, the discrete variable time, and the interaction of group and time. Therefore the model included parameters to explain effects of time (a "within subjects" effect, assumed to be linear), group (a "between subjects" effect), and the group by time interaction. A separate ANOVA was performed on each target set. While this prevented comparison of data across sets, it allowed us to make the approximation that time could be represented as a linear effect, significantly reducing the degrees of freedom in the analysis. Within each set, the LI behaved in a way that was compatible with an assumption of linear evolution in time. Thus we did not compare the data from different sets, and the effects of time we report here are all

the effects within a single set. The same methods were used to test for statistical differences in the analysis of the maximum velocity.

Post hoc testing was performed using the Holm test (Holm 1979). This is a reasonably conservative method for correcting *t*-test results for multiple comparisons. If the time-by-group interaction for a set was significant, we performed the post hoc test on the group data for each time step separately. Otherwise, if there was a significant effect of group, we performed the post hoc test on the group data averaged over time. If there was no significant effect of group, no post hoc analysis was performed. Effects with  $P < 0.05$  were deemed to be significant.

## RESULTS

### Subject performance during day 1

On day 1 (train day), subjects began by training in the null field. Performance of one subject in each of five groups in the null field is shown in Fig. 2, *left*. Generally, after a brief period of practice in the null field, all subjects were able to make fairly straight movements. Subjects then began training in field A. The *next two columns* of Fig. 2 show performance early and late in training. Fielded movements early in training had significant deviations from a straight line (thin red lines) while catch trials (in which the field was not applied, thick blue lines) were essentially straight. This contrasts with movements late in training where catch trials deviated from a straight line and fielded trials did not.

If subjects were learning an internal model, we expected to see the displacements in fielded movements decline while displacements in catch trials increase in the opposite direction to the field. To quantify this, we used a measure called the LI (Eq. 1). As this measure is the ratio of displacements in catch trials (i.e., aftereffects) to the sum of displacements in catch and fielded trials, we expected the index to increase from a number close to 0 toward 1. We quantified the performance of subjects in different groups in the group averages of LI (Fig. 3). We observed that performance during training on day 1 was quite similar among groups. LI started around 0.35 and doubled by the end of the third training set. Statistics of the comparisons among groups are shown in Table 3. We found no significant differences among the groups on day 1.

### Test of recall on day 2

The testing began with 18 movements in the null field. We had previously observed that subjects who trained in a force field displayed aftereffects one day after training (Shadmehr et al. 1998), indicating retention of the field learned on the previous day. Figure 4 demonstrates that all groups showed similar aftereffects. Comparing the last two plots in the figure demonstrates that the perpendicular displacements (PDs, displacements perpendicular to the direction of target) during the re-familiarization set on day 2 are consistent across groups and that these initial null PDs are in the same direction as PDs of the catch trials at the end of training on day 1. Furthermore, the PDs of these day 2 re-familiarization null movements are larger than the PDs at the end of familiarization on day 1 (as is seen by comparing them with the data in the 2nd plot of Fig. 4), suggesting that the training sets which intervened between familiarization and re-familiarization caused an increase in PD. The consistency across groups is an indication that the field

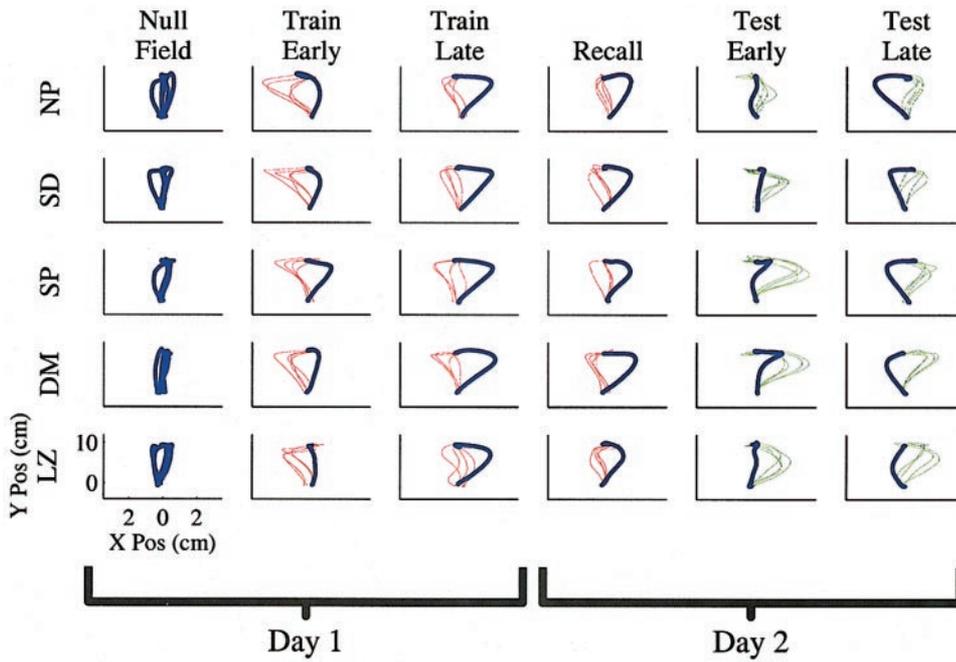


FIG. 2. Data from typical subjects. Typical movement paths during movements made by individual subjects when target was at 90°. In each row, the movements shown are movements from 1 subject. In each set 1 catch trial (thick line, blue) and 2 fielded movements before and after the catch trial are shown (thin lines, red for field A and green for field B). The no protocol (NP) and sleep-deprived (SD) subjects show normal learning in train, recall, and test sets. The scopolamine (SP), dextromethorphan (DM), and lorazepam (LZ) subjects show normal learning in train and recall but not in the test sets. For the SP and DM subjects, catch trials show normal aftereffects (curved outward) in late learning but the fielded trials are not as close to straight as the SD or NP subjects. For the LZ subject, the catch trial is much closer to straight than the fielded trials.

learned on the previous day was affecting all groups similarly. It also indicates a preserved ability in all groups to perform under the influence of treatment.

However, the aftereffects in the field on day 1 are also in the same direction as the errors made early in the null field training on day 1 (1st plot of Fig. 4). This raises the alternate hypothesis that errors on the null field testing on day 2 do not reflect aftereffects for field A. Instead they may reflect a loss of the training effect both for the null field and field A. Three con-

siderations argue against this interpretation. First, in earlier research where subjects were trained in either field A or field B, the direction of PDs during null movements 1 day later were consistent with the trained field and not with subjects' initial errors when they first performed null field movements (Shadmehr and Brashers-Krug 1997). Second, the variance during the day 2 null field movements is significantly reduced relative to the early day 1 null field movements and is similar to the variance on day 1 following training. Third, when we tested

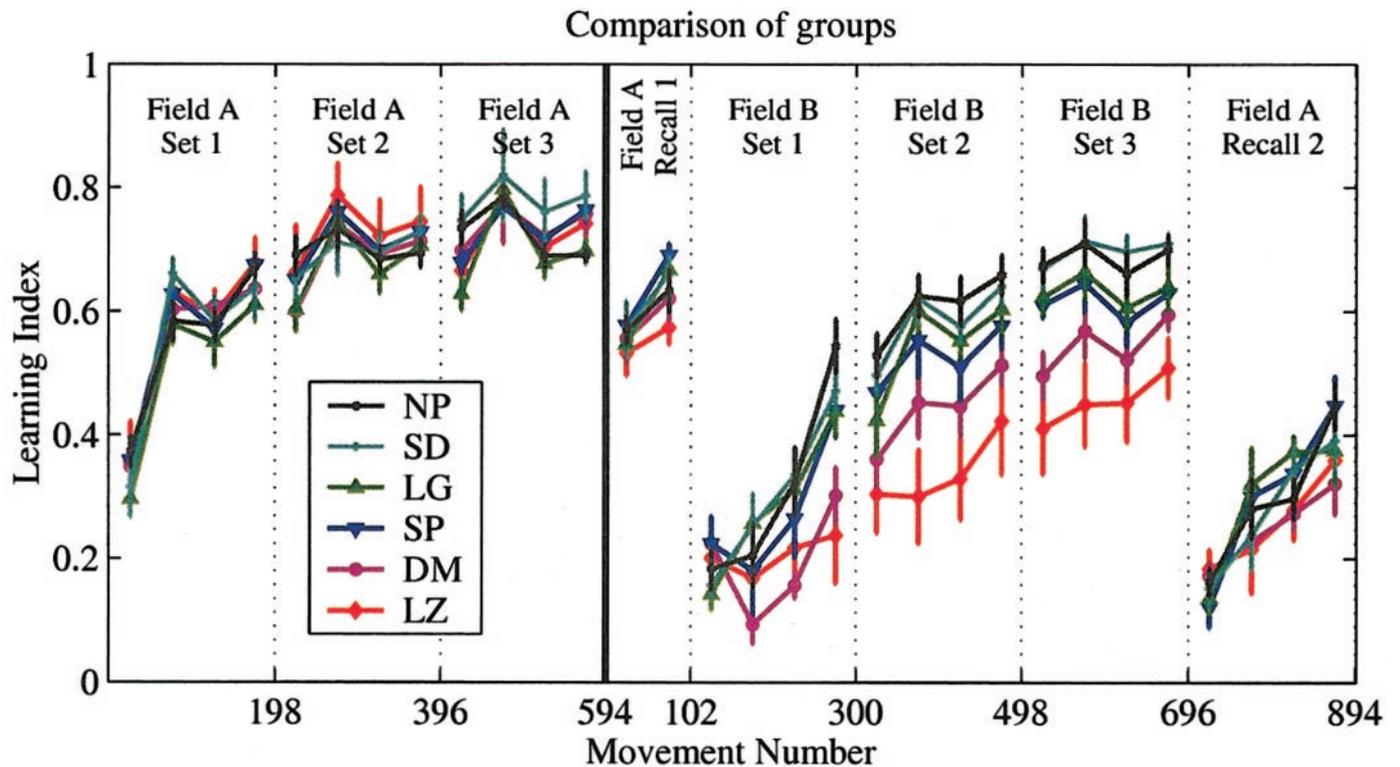


FIG. 3. Learning Index (LI) on train and test days. A comparison of the LI (*Eq. 1*) among different groups. Each point represents an average of data from 50 consecutive movements, ~8 catch trials and 42 fielded movements.

TABLE 3. Statistical results for each set

Set	Group Effect (5)	Time Effect (1)	Group $\times$ Time Interaction (5)
Train 1	0.47	180.46**	0.01
Train 2	0.31	18.91**	1.35
Train 3	0.67	1.84	1.67
Recall 1	0.84	60.60**	1.43
Recall 2	0.64	125.41**	1.26
Test 1	3.10**	100.43**	5.59**
Test 2	5.56**	74.87**	0.38
Test 3	6.51**	8.84**	2.03

This table shows the  $F$  statistics from the tests performed on the generalized model fit to the data in Fig. 2. The degrees of freedom are in parentheses. A group effect appears only in the test sets, although the existence of learning is indicated by the significance of the time effect in most sets. \*\*  $P < 0.05$ .

subjects on field A on day 2, their performance suggested retention, as quantified in the following text.

On day 2, after the brief null set, subjects were re-tested on field A for 102 movements. We observed that all subjects could make accurate movements to targets and all had aftereffects. This is shown for typical subjects in Fig. 2, and across all subjects in Fig. 3. The LI suggested better performance during recall on day 2 than during initial exposure on day 1. An ANOVA performed on LI for the first two bins of set train 1 (field A, set 1, day 1) and the two bins of set recall 1 (field A, set 1, day 2) gave a significant effect of day (train 1 vs. recall 1,  $F = 161$ ,  $P < 0.05$ ) and time (1st vs. 2nd data point in each set,  $F = 342$ ,  $P < 0.05$ ), but no significant effect of group ( $F = 0.24$ ,  $P > 0.4$ ). Therefore performance improved from day 1 to day 2 regardless of group assignment, and there was no significant difference among the groups during field A testing on day 2 (recall 1 in Fig. 3).

However, we did find that the group  $\times$  day interaction was marginally significant ( $F = 3.03$ ,  $P < 0.05$ ). Post hoc testing on the difference between days 1 and 2, compared across groups, did not reveal any group that had significantly more or less change than any other group ( $P > 0.2$  after correction for all tests). On the other hand, visual inspection of the LI data (Fig. 3) suggests that the significant group  $\times$  day interaction may be the result of reduced performance by the LZ group in the recall 1 set. It is not clear how to interpret the discrepancy between the significant group  $\times$  day interaction and the failure of the pairwise comparison of the interaction among groups to

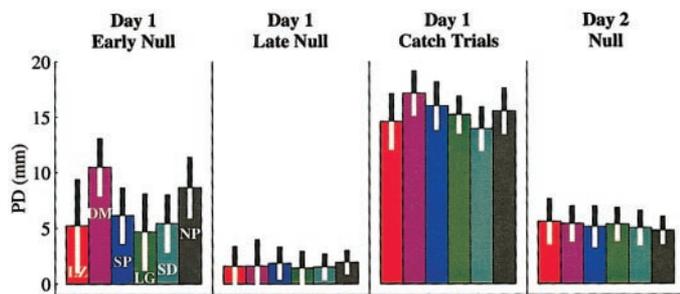


FIG. 4. Perpendicular displacements (PDs) in null movements. Comparison of means of PDs from 18 movements made during familiarization sets early and late on day 1 and at the beginning of day 2. Early in day 1, inter-subject variability is large (subject performance was not matched across groups), but the variability and the performance errors both drop with training. The 18 re-familiarization movements performed at the beginning of day 2 show a tendency to have PDs opposite to the direction of the learned field (like catch trials). This tendency is the same for subjects in all treatment groups. Lines over the histograms connect pairs of histograms that are significantly different ( $P < 0.05$ ).

achieve significance. Because our other measures of motor performance and recall (the PDs in the initial null set and the LI in the 2nd recall set at the end of the day 2 testing—see following text) suggests that performance in field A on day 2 was not different in the LZ subjects as compared with our control group, and because the significance of the group  $\times$  day interaction is relatively weak, we suggest that while LZ may have had some effect on recall, this effect was at most a subtle one.

After subjects trained in field B for  $\sim 600$  targets, they were re-tested on field A. Training in field B caused anterograde interference that inhibited the ability of subjects to perform in the original field. In all subjects, performance dropped significantly from their earlier performance in field A that day and was significantly worse than their performance during initial training on day 1. As this was the condition where the most amount of error was present in subjects' movements, it provided a strong test of the ability of subjects to recall the internal model of field A that they had learned before. We asked whether there was a difference among the groups in their rate of recovery of this internal model. We found that the group by time interaction was not significant, suggesting that all groups made this recovery at approximately the same rate.

#### Test of new learning on day 2

While recall of field A on day 2 did not introduce differences in LI among groups, differences became apparent when subjects attempted to learn a new field. We found that two groups, LZ and DM, were significantly impaired in new learning. Movements of typical subjects are shown in Fig. 2 and group LIs are compared in Fig. 3. In field B, LZ and DM subjects had generally small aftereffects, indicating an impaired ability to learn. In contrast, behavior of SD subjects was indistinguishable from that of control subjects.

The statistical analysis of the data showed that among all sets, only the sets in field B showed a significant effect of group (Table 3). In the first set of field B, there was also a significant interaction between group and time, prompting post hoc analysis on each time bin for this set. The result of the post hoc analysis is summarized in Fig. 5, and significant differ-

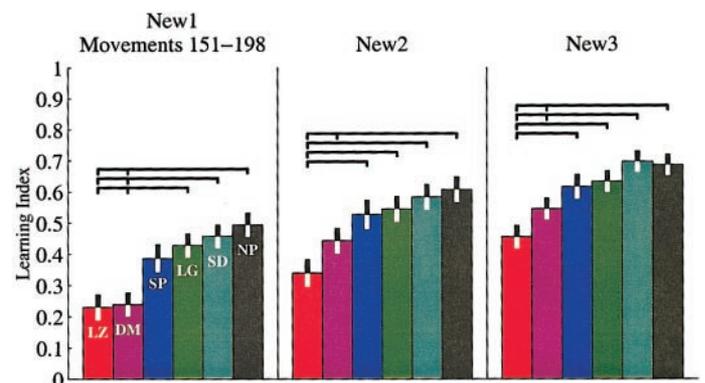


FIG. 5. Groupwise comparison of LIs. For those comparisons that produced a significant effect of group in the generalized linear model, this figure shows a comparison of the mean values for each group. Pairwise post hoc comparisons among groups that produced a significant difference are shown with connecting lines above the histograms. The connecting lines always indicate a single group with higher LI that is different from one or more groups with lower LI. Significance was determined using the Holm test for post hoc pairwise comparisons ( $P < 0.05$ ).

TABLE 4. Side effects

	SD	LG	DM	SP	LZ
Drowsiness	2.4 (0.6)	1.5 (0.4)	1.8 (0.6)	1.1 (0.6)	2.8 (0.4)
Dizziness	0.1 (0.1)	0.9 (0.5)	1.4 (0.4)	0.4 (0.3)	0.6 (0.4)
Jitters	0.5 (0.5)	0.0 (0.0)	0.9 (0.6)	0.0 (0.0)	0.0 (0.0)
Fatigue	1.9 (0.6)	0.6 (0.5)	0.8 (0.5)	1.0 (0.7)	1.6 (0.5)
Nausea	0.1 (0.1)	0.3 (0.2)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)

Self-assessed discomfort (mean  $\pm$  SE) experienced during treatment by the different experimental manipulations. Scale is from 0 (no effect) to 5 (extreme effect).

ences are apparent among the groups from the middle of set 1 through sets 2 and 3. The pattern of results for the post hoc testing varies slightly when going from sets 1 to 2 to 3; however, it seems fair to summarize the results by saying that we found that the LZ and DM groups were consistently impaired in their ability to learn field B and that LZ was more impaired than DM.

#### Preserved recall and learning in SD subjects

The SD group was included because some of the drugs administered in this study are known to cause drowsiness and other side effects. Indeed, we found that LZ subjects rated their state of drowsiness at a level comparable to the SD subjects (Table 4). Nevertheless, we found that while performance in field A was quite comparable among the SD, LZ, and NP groups on both days, learning of field B was dramatically impaired in LZ while learning in SD was indistinguishable from the NP. This result is particularly remarkable because of the evidence suggesting a role for sleep in formation of memories in certain perceptual tasks (see DISCUSSION).

#### Drug side effects

Assessment of side effects was done on day 2 when subjects were already under the effects of the different drugs and immediately before testing (Table 4). Subjects in the LZ group experienced primarily drowsiness and fatigue, whereas those in the DM group reported occasional dizziness and jitters. However, other groups that performed similarly to controls also reported similar side effects. Subjects in the LG group reported

dizziness while those in the SD group indicated drowsiness, fatigue, and jitters. There was no significant correlation between performance, as measured by LI, and side effects (Spearman's nonparametric rank order). Because drowsiness may result in slower movements, which could effect the resultant forces imposed by the field, we tested for differences in maximum velocity across groups (Fig. 6). There was no significant effect of group on movement speeds.

#### DISCUSSION

The main result of this study is that drugs blocking NMDA receptors or enhancing GABA<sub>A</sub> receptor function impaired motor learning. This effect was specific to new learning, as the drugs had no significant effect on performance of the task or on the ability to recall a previously learned internal model. The result is consistent with the known effect of the drugs on mechanisms of synaptic plasticity and the hypothesized relationship between synaptic plasticity and memory. The novelty of this work is in the extension of these concepts to the motor system in humans. Another new finding is the demonstration of a dissociation between the physiological mechanisms of acquisition and recall of a motor memory in humans.

The strongest effects on motor learning were obtained with LZ. This drug substantially reduced new learning on day 2, a result consistent with the finding that LZ has profound deleterious effects on use-dependent plasticity in the human motor system (Butefisch et al. 2000). LZ also influences cortical reorganization associated with deafferentation (Ziemann et al. 1998b) and with light deprivation (Boroojerdi et al., personal communication). All together, these effects are consistent with the known influence of GABAergic neurotransmission on cortical plasticity (Jacobs and Donoghue 1991), on synaptic plasticity in cortex (Artola and Singer 1987), and on recovery of motor function after cortical lesions like stroke (Goldstein 1993). The results reported in our study provide new evidence for the involvement of GABAergic neurotransmission on motor learning, results that could not be explained by the sedative effects of the drug because recall and motor performance were intact.

DM also resulted in significant disruption of motor learning, a result consistent with the inhibitory effects of this drug on

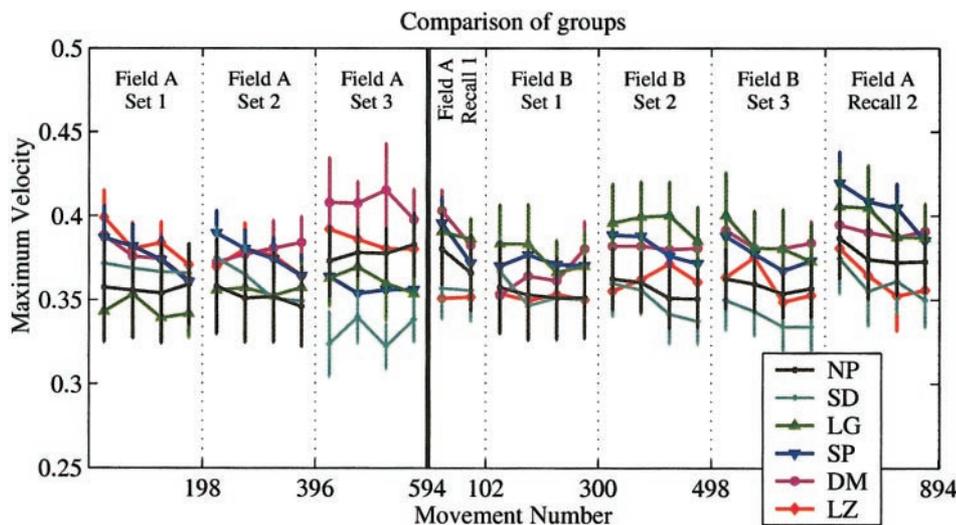


FIG. 6. Peak movement velocity on train and test days. This figure compares the peak velocity during each movement, averaged in bins of 50 consecutive movements, across groups and among training, recall, and test. The format is the same as in Fig. 3. Data from catch trials and fielded trials are combined to form the averages.

use-dependent plasticity (Butefisch et al. 2000) and motor cortex excitability (Ziemann et al. 1996). While both DM and LZ impaired the ability of subjects to acquire new internal models, neither had an effect on recall of a previously learned model. Three independent tests support this claim. First, initial null field movements on day 2 showed aftereffects that suggest recall of the field learned on day 1 (Fig. 4). Second, in a set of field A movements before testing field B, all subjects showed similar ability to perform in field A (Fig. 3). Finally, also shown in Fig. 3, despite introduction of large errors in performance of field A following testing in field B, all subjects quickly returned to the internal model for field A. This result is consistent with other studies in which these and similar drugs were shown to impair the formation of new memories but not the recall of memories that were established prior to drug administration (Bane et al. 1996; Danion 1994; Vidailhet et al. 1994).

One might expect that DM and LZ subjects would perform significantly better than controls when returning to field A after the reduced learning in field B. We found no such evidence of reduced anterograde interference. One possible explanation is that the experience of field B and the significant improvement that did take place in that field (Fig. 3) are sufficient to create anterograde interference of recall. A second possibility is to interpret this result and the somewhat reduced recall of LZ subjects in the first recall set (revealed by the significant group-by-time interaction in the ANOVA on this set) as showing consistent slight reduction in performance of LZ subjects relative to expectation. This interpretation suggests that LZ has an effect on either performance or recall in addition to the more pronounced effect on learning.

In contrast to DM and LZ, performance in the SD group was indistinguishable from controls (NP). The SD and NP subjects were consistently the two groups with the best performance levels (Fig. 5). Indeed, groups that were statistically different from NP (DM and LZ) were also statistically different from the SD group. These findings further support the contention that sedation was not a fundamental factor influencing our results.

The findings in the SD group are interesting for one additional reason. A number of studies have found a role for sleep in consolidation of certain kinds of perceptual skills (Eggermont and Smith 1995; Gais et al. 2000; Stickgold et al. 2000). In those studies, sleep, and not simply the passage of time, has been shown to be required for changes in performance between end of training and test of recall. In the force-field learning task, while we found no significant effect of sleep on performance, we had observed that simple passage of time has a significant effect on the functional properties of the internal model (Shadmehr and Brashers-Krug 1997). Although the current study was not originally designed to address the role of sleep in the consolidation of motor memories, our data do raise the hypothesis that sleep may not have a uniform, consolidating effect on all forms of memories.

The results with the other two groups—SP and LG—are less unequivocal. Learning in both groups is different from learning in the LZ group, and learning in the LG group was also different from learning in the DM group in the last quarter of the first set. However, they did not differ significantly from the controls. This is interesting because a recent study in a cognitive memory task showed SP causing a learning impairment that was similar to the one caused by LZ (Thiel et al. 2001) and

SP also depresses use-dependent plasticity (Sawaki et al. 2002).

There is now significant evidence linking forms of synaptic plasticity like long-term potentiation (LTP) and the creation of memories (for a recent review, see Martin et al. 2000). NMDA-mediated synaptic plasticity affects hippocampus-dependant explicit memory, amygdala-dependant fear conditioning, and cortically based tasks that involve habituation and adaptation. When D-2-amino-5-phosphonopentanoic acid, an NMDA blocker with action similar to DM, is administered either systemically or iontophoretically, it is effective in blocking learning, but not recall, in a variety of animal models. Similarly, GABA agonists have been shown to block LTP induction in slice preparations (Evans and Viola-McCabe 1996) and learning in animal models (Thiebot 1985). NMDA blockers and GABA agonists have also been shown to induce amnesic effects in humans, suggesting that LTP-like mechanisms may serve the same memory function in humans that they do in animals (Lister 1985; Rammsayer et al. 2000). This hypothesis finds further support in evidence that events known to induce cortical plasticity are negatively influenced by these drugs (Butefisch et al. 2000; Ziemann et al. 1998b), as is cortical excitability (Ziemann et al. 1996). While most of this research has focused on hippocampal or cortical slice, we emphasize that our results do not rule out the possibility that plasticity associated with our task takes place in the cerebellum. Similarly, it is possible that the drugs we applied influenced this cerebellar plasticity. Thus while our results support a hypothesis of shared mechanisms of plasticity in motor learning and other forms of learning, they do not permit firm localization of the site of this plasticity.

There are few studies of drug effectiveness in motor learning. In the only extended discussion of the question that we uncovered, Lister (1985), came to the conclusion that it is most likely that “benzodiazepine-induced amnesia seems to be characterized by intact procedural knowledge. . . but impaired declarative knowledge.” Thus the novelty of our results is in addressing two important issues.

In the first issue, two researchers before us addressed the question of the effects of drugs that block synaptic plasticity on psychomotor tasks in humans, although the tasks in both of these studies were quite different from ours (Ghoneim et al. 1984; Rammsayer et al. 2000). Ghoneim et al. measured repetitive tapping speed under the influence of diazepam (a GABA agonist), finding that the speed increase with practice was blocked in subjects treated with diazepam. Rammsayer et al. showed that improvement in a tracking task caused by practice is blocked by midazolam (a GABA agonist), haloperidol (a dopamine blocker), and SP. However, both of these studies suffer from a possible shortcoming that was pointed out by Lister (1985). Neither one controlled for the possibility that drug effects on psychomotor performance confound drug effects on learning. While this is a difficult confound to control, the flaw does undermine the results and was the basis of Lister’s conclusion that motor learning was being masked by direct drug effects on performance. In contrast, our study was specifically designed to control for this issue.

As for the second issue, our finding that sleep deprivation does not adversely affect recall of the task is surprising given the extensive literature showing a dependence of recall on sleep. Just as the effects of the drugs are important for showing

a link between motor learning and cognitive learning, this result is important for highlighting a difference between motor and cognitive learning. We are not aware of other reports showing that sleep is not important for the recall of skills or the consolidation of motor memories.

The task used in this study is in a class of new paradigms in motor learning where dynamics of reaching movements are altered. Recently, these paradigms have become the target of research efforts that combine theoretical, physiological, and psychophysical approaches (For reviews see: Flash and Sejnowski 2001; Sabes 2000; Wolpert and Ghahramani 2000). Because knowledge about motor learning lags far behind knowledge regarding other forms of learning, any link between a well-studied motor learning task and the mechanisms of more cognitive learning tasks could be important in advancing our knowledge and understanding of learning and memory in general.

We thank our volunteers for participating in this study.

Research at the Laboratory for Computational Motor Control is supported by grants from the National Institute of Neurological Disorders and Stroke (NS-37422) and the Office of Naval Research (N000140110534). O. Donchin was also supported by National Institute of Neurological Disorder and Stroke Fellowship NS-11163 and a distinguished postdoctoral fellowship from the Johns Hopkins Biomedical Engineering department. L. Sawaki was supported by a generous grant from the National Center for Complementary and Alternative Medicine, National Institutes of Health.

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