Protein Synthesis Inhibition Blocks Consolidation of an Acrobatic Motor Skill

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To investigate whether motor skill learning depends on de novo protein synthesis, adult rats were trained in an acrobatic locomotor task (accelerating rotarod) for 7 d. Animals were systemically injected with cycloheximide (CHX, 0.5 mg/kg, i.p.) 1 h before sessions 1 and 2 or sessions 2 and 3. Control rats received vehicle injections before sessions 1, 2, and 3. Although CHX did not affect improvement of performance within session I, between-session improvement was impaired. In overtrained animals, comparable injections of CHX had no effect on rotarod performance. These findings suggest that consolidation of motor skills requires protein synthesis.

Motor skill learning is an essential aspect of development, adult life, and recovery after brain lesion. It differs from hippocampus-dependent learning by the neuronal circuitry likely to be involved. Its circuits include sensorimotor cortex, cerebellum, and basal ganglia (Hikosaka et al. 2002). The hippocampus does not seem to contribute (Gould et al. 2002; Davey et al. 2003).

For hippocampus-dependent learning, the role of protein synthesis has long been established. It was found that mainly retention of learned content, not acquisition, depends on de novo synthesized proteins (Davis and Squire 1984). Depending on the paradigm, induction of protein synthesis inhibition (PSI) even after training produces amnesia, although this amnesia is not as profound as if PSI is initiated before training. Hence, establishing persistent memory traces seems to require de novo synthesized proteins. These proteins may be used to remodel the synaptic and/or dendritic structure.

Whether protein synthesis is required for motor skill learning has not been demonstrated. Here, in a reward-independent acrobatic locomotor paradigm, we show that mainly consolidation, less likely acquisition of a complex motor skill, is disrupted by PSI.

Thirty adult male Long-Evans rats (300–400 g body weight, raised at Charles River, Sulzfeld, Germany) were randomly assigned to three experimental groups (n = 10 each). All animals were trained for 7 consecutive days. An additional group of eight rats was trained for 15 d (pre_11,12). One training session was performed per day. The investigator performing the tests (M.M.B.) was blinded to groups. Rats were housed in groups of four, kept in a 12:12-h day/night cycle with ad libitum access to food and water. Body weight was recorded daily. All procedures were approved by the Animal Care and Use Committee of the University of Tübingen.

The control group was intraperitoneally injected with vehicle (normal saline, 0.5 mL, i.p.) 1 h before sessions 1, 2, and 3. Group pre_1,2 received 0.5 mg/kg of the protein synthesis inhibitor cycloheximide (CHX, Sigma-Aldrich; diluted in 0.5 mL of normal saline) 1 h before sessions 1 and 2. Group pre_2,3 received CHX 1 h before sessions 2 and 3. To test for potentially deleterious effects of CHX on motor performance, rats in group pre_11,12 received two doses of CHX 1 h prior to training on days 11 and 12. At that time, their rotarod performance had plateaued (overtrained state).

A rotarod with a 7-cm diameter rod and acceleration capability was used following previously published protocols (Buitrago et al. 2004). Briefly, rats were allowed to accommodate to the rod for 5 min before daily training. For each session, the animals were subjected to 20 rotarod trials. Constant acceleration of 1 cm/sec² was used until the rat fell from the rod and activated a light sensor that stopped rotation. The maximum tangential velocity mastered by the animal was recorded. The time interval between trials was 30 sec. Training sessions were performed during the night phase of the daily cycle, when animals were active.

Data analysis was performed using Statistica (Version 6, StatSoft Inc.). A repeated measured ANOVA model was used with “group” (four levels: control, pre_1,2, pre_2,3, pre_11,12) as between-subject factor, and “session” (seven levels) and “trial” (20 levels) as within-subject factors. The maximum rotarod “speed” per trial was entered as the dependent variable. Appropriate contrasts were formed to test for differences between prespecified groups or sessions (specified as trial [1 ... 20], session [1 ... 7], group [control pre_1,2 pre_2,3 pre_11,12]). Additionally, post hoc Tukey’s HSD tests were computed to compare groups. The slope of improvement within the 20 trials of a session was assessed using a linear model.

In vehicle-injected animals, improvements in rotarod performance were observed within (intrasession; Fig. 1A) and between training sessions (intersession; Fig. 2A). In a linear model including only data for session 1 of control animals, intrasession improvement was significant (F1,237 = 21.39, p < 0.0001). The highest between-session improvement occurred between sessions 1 and 2 (Fig. 2A). Plateau performance was reached by day 2.

The overall repeated measures model including all experi-
mental groups showed significant effects of session ($F_{3,32} = 3.26$, $p = 0.005$), and group ($F_{1,32} = 3.82$, $p = 0.024$). Interactions were insignificant. Post hoc Tukey’s HSD tests revealed a significant difference between control and pre_1,2 animals ($p = 0.041$). Univariate tests for session showed significant effects of group in session 2 ($F_{3,32} = 5.82$, $p = 0.003$), session 3 ($F_{3,32} = 12.0$, $p < 0.0001$), session 4 ($F_{3,32} = 6.40$, $p = 0.002$), session 5 ($F_{3,32} = 3.70$, $p = 0.022$), and session 6 ($F_{3,32} = 2.27$, $p = 0.013$).

Pre_1,2 animals demonstrated normal intrasession improvement during session 1 (Fig. 1B; linear model: $F_{1,138} = 5.02$, $p = 0.027$), but no intersession improvement between sessions 1 and 2 (contrast session $[1 - 1 0 0 0 0 0]$; $F_{1,23} = 0.03$, ns). In session 3, mean performance dropped significantly as compared with session 2 (contrast session $[0 1 - 1 0 0 0 0]$; $F_{1,22} = 6.25$, $p = 0.020$) and as compared with controls (contrast group $[1 - 1 0 0 0]$; $F_{1,22} = 15.7$, $p = 0.001$; Fig. 2). Analysis of intrasession data revealed that performance in session 3 was reduced to the levels of early session 1 (contrast comparing the initial five trials of sessions 1 vs. 3; $F_{1,23} = 0.76$, ns). After discontinuation of CHX, skill improvement was observed (repeated measures ANOVA including sessions 3 to 7 in group pre_1,2; effect of session: $F_{4,16} = 3.46$, $p = 0.032$). In session 7, the mean session performance of pre_1,2 rats had reached the level of session 1 (contrast session $[1 0 0 0 0 0 - 1]$; $F_{1,23} = 0.21$, ns).

Group pre_2,3 showed similar skill acquisition as compared with control during and between sessions 1 and 2 (Figs. 1C and 2A; contrast group $[1 0 - 1 0]$; session $[1 1 0 0 0 0 0]$; $F_{1,32} = 0.14$, ns). Worsening of performance was seen between sessions 2 and 3 (contrast session $[0 1 - 1 0 0 0 0]$; $F_{1,22} = 5.35$, $p = 0.03$) as well as sessions 3 and 4 (contrast session $[0 1 - 1 0 0 0 0]$; $F_{1,22} = 10.7$, $p = 0.003$). Rats somewhat improved thereafter (Fig. 2A), but only a trend was seen in the repeated measures ANOVA including sessions 4–7 in pre_2,3 animals ($F_{3,33} = 2.67$, $p = 0.064$). The overall learning curve (sessions 1–7) of pre_2,3 animals was not statistically different from controls (contrast group $[1 0 - 1 0]$; $F_{1,32} = 0.27$, ns).

In overtrained animals (pre_11,12), rotarod performance increased slightly after the first CHX injection, then dropped, and reverted to plateau levels thereafter (Fig. 2B). These fluctuations were not significant, as was the effect of session in a repeated-measures ANOVA that included data of sessions 9–15 ($F_{1,18} = 0.83$, $p = 0.57$). Plateau performance in pre_11,12 animals (sessions 9 and 10) was significantly higher than session 1 performance in controls ($t(15) = 2.6$, $p = 0.018$).

These data demonstrate that protein synthesis inhibition (PSI) impairs improvement of skill over 24 h of rest between sessions, but neither within- nor between-session improvement nor motor performance is affected. After PSI is relieved, skill learning is reinitiated.

Cycloheximide (CHX)—an inhibitor of peptidyl transferase in eukaryotes—inhibits protein synthesis in brain and other organs after systemic application. A single dose of 0.5 to 0.6 mg/kg, i.p., reportedly reduces brain protein synthesis by ∼75% within 20 min of administration (Pavlik and Teisinger 1980; Snider et al. 2001). Although most capacity for synthesis is restored by 12 h, complete normalization is reached by 48 h (Pavlik and Teisinger 1980). CHX in small doses of <0.6 mg/kg is nontoxic (Gupta and Dettbarn 1987; Snider et al. 2001). Our application scheme was designed to obtain a high level of PSI at and during the training periods.

The rotarod is commonly used to test motor function. Animals improve during repetitive testing (Brandon et al. 1998). We added high constant acceleration (1 cm/sec$^2$) to increase task complexity. We previously reported that the rotarod requires specific locomotor training (running inside a wheel) does not interfere with rotarod improvement, demonstrating that the rotarod requires specific movement strategies beyond running fast (Buitrago et al. 2004). A feature of the rotarod task is that it is largely...
Learning & Memory 381

Protein Synthesis and Motor Skill Learning

Alternately, drops in performance could reflect CHX-induced impairment of motor function. In doses higher than the one used here, CHX produces locomotor deficits (Davis and Squire 1984). We found no drop in performance in animals injected with CHX in an overtrained state (i.e., plateau phase of performance) after one dose of CHX; after a second dose, a nonsignificant drop is seen (Fig. 2B). Hence, performance deficits may be induced by an additive effect of two CHX doses. After this drop in mean performance (session 3 in pre_1,2; session 4 in pre_2,3), animals in the CHX groups improve but, over the 7-d study period, do not reach the performance of controls. Potential unspecific long-term effects of CHX may account for this finding. Klein et al. (2003) showed in a recent study that PSI in motor cortex leads to a breakdown of limb representation geometry over a prolonged period that outlasted the phase of relevant PSI.

However, despite these limitations, the conclusions of the study, that intersession motor learning but not performance depends on de novo protein synthesis, are supported by the data of sessions 1 and 2 as well as sessions 11 and 12 in overtrained animals. Further studies are needed to identify the brain areas, in which protein synthesis occurs, and to characterize the proteins necessary for motor skill learning.

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Luft et al.

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