Brief Communication

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 1, 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 ($F_{1,237} = 21.39$, $p < 0.0001$). 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-

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Figure 1 Maximum speed at the time of falling off the rotarod is plotted against trial number for the selected training session. (A) Improvement in rotarod performance can be observed in vehicle-injected animals within and between training sessions. (B) Bold y-axes indicate sessions with CHX injection 1 h prior to training. Skill acquisition within session 1 is unaffected by CHX. Then, however, the skill cannot be retained and falls back to early session 1 levels. (C) Later application of CHX results in a delayed failure of retention. (D) CHX application to overtrained animals in their plateau phase of performance does not significantly affect performance. (Error bars represent the standard error of mean.)

mental groups showed significant effects of *session* ($F_{6,132} = 3.26$, $p = 0.005$), and *group* ($F_{3,22} = 3.82$, $p = 0.024$). 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]: $F_{1,22} = 0.03$, ns). In session 3, mean performance dropped significantly as compared with session 2 (contrast *session* [0 1 - 1 0 0 0]: $F_{1,22} = 6.25$, $p = 0.020$) and as compared with controls (contrast *group* [1 - 1 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: $F_{1,22} = 0.76$, 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 - 1]: $F_{1,22} = 0.21$, 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]: $F_{1,22} = 0.14$, ns). Worsening of performance was seen between sessions 2 and 3 (contrast *session* [0 1 - 1 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]: $F_{1,22} = 10.7$, $p = 0.003$). 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]: $F_{1,22} = 0.27$, 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 ($F_{6,18} = 0.83$, $p = 0.57$). 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-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 48h (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 and trains a specific skill and therefore can be regarded as a paradigm for learning of a complex movement: (1) the rat changes its gait pattern over subsequent training sessions; and (2) prior unspecific 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

Figure 2 Mean session performance is plotted over session number. CHX injections are indicated by arrows. (A) Early injection of CHX (*pre_1,2*, dashed line) abolishes intersession improvement between sessions 1 and 2. Subsequently, performance drops below baseline (performance comparable to early session 1; cf. Fig. 1C). After CHX is discontinued, improvement resumes. Later application of CHX (*pre_2,3*, dotted line) does not interfere with improvement between sessions 1 and 2 but causes loss of skill thereafter. (B) In contrast, CHX application during the plateau phase of performance (over-trained state) may lead to fluctuations but does not significantly affect the rotarod skill. (Error bars represent the standard error of mean.)

independent of reward, of spatial, and of instrumental/operand features. Falling off the rod may represent a weak aversive stimulus. However, the distance to fall was small (40 cm), and we avoided additional aversive stimuli such as electric shocks, which are often used during rotarod testing. Therefore, motor skill learning can be assumed as the major component responsible for improvements in rotarod performance. We argue that CHX effects on this performance reflect impairments of motor skill learning.

Intrasession improvements in performance seemed unaffected by PSI. This idea is supported by the session 1 data of *pre_1,2* animals (Fig. 1C). But, for later training sessions, intrasession improvement was abolished in *pre_1,2* and in *pre_2,3* rats. This may be explained by a combination of the following: (1) a reduction or loss of intrasession improvement as training progresses and performance becomes better (ceiling effect; Buitrago et al. 2004); (2) a cumulative effect of two doses of CHX that impair motor performance (see below); or (3) unspecific long-term effects of CHX (see below). That PSI has no effect on intrasession (short-term) learning is consistent with published data on spatial learning (Chiba et al. 2000), working memory (Cariga et al. 2002), or fear conditioning (Taniguchi et al. 2002). The short-lasting form of LTP (early LTP), in contrast to late LTP, is not sensitive to PSI (Frey et al. 1993). It is therefore assumed that short-term changes in synaptic strength are mediated by pre-existing synaptic machinery, whereas long-term modifications require structural adaptations through de novo protein synthesis.

Between-session improvement was impaired by CHX. This may be interpreted as a retention deficit. In group *pre_1,2*, the skills acquired during session 1 are incompletely recalled in session 2. The mean performance drop that occurs in session 3 does not go significantly below the initial performance in session 1 (first five trials). Therefore, the drop in mean session 3 performance could reflect a complete “forgetting” of skills acquired during session 1. In contrast, giving CHX just before session 2 does not interfere with the retention of skills acquired during session 1. Subsequently, however, performance drops. This could, in analogy to *pre_1,2* animals, be interpreted as the delayed interference with a consolidation process. It is well established that consolidation depends on protein synthesis in various types of memory paradigms (Davis and Squire 1984).

Alternatively, 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 over-trained 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. Kleim 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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REFERENCES

- Brandon, E.P., Logue, S.F., Adams, M.R., Qi, M., Sullivan, S.P., Matsumoto, A.M., Dorsa, D.M., Wehner, J.M., McKnight, G.S., and Idzerda, R.L. 1998. Defective motor behavior and neural gene expression in RII β -protein kinase A mutant mice. *J. Neurosci.* **18**: 3639–3649.
- Buitrago, M., Schulz, J., Dichgans, J., and Luft, A. 2004. Short and long-term motor skill learning in an accelerated rotarod training paradigm. *Neurobiol. Learn. Mem.* **81**: 211–216.
- Cariga, P., Catley, M., Nowicky, A.V., Savic, G., Ellaway, P.H., and Davey, N.J. 2002. Segmental recording of cortical motor evoked potentials from thoracic paravertebral myotomes in complete spinal cord injury. *Spine* **27**: 1438–1443.
- Chiba, A., Hiruma, S., Hashimoto, S., and Inase, M. 2000. Changes in spinal cord function caused by injuries at different levels of the lumbar spinal cord. *Neuroreport* **11**: 2163–2166.
- Davey, K., Epstein, C.M., George, M.S., and Bohning, D.E. 2003. Modeling the effects of electrical conductivity of the head on the induced electric field in the brain during magnetic stimulation. *Clin. Neurophysiol.* **114**: 2204–2209.
- Davis, H.P. and Squire, L.R. 1984. Protein synthesis and memory: A review. *Psychol. Bull.* **96**: 518–559.
- Frey, U., Huang, Y.Y., and Kandel, E.R. 1993. Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* **260**: 1661–1664.

- Gould, T.J., Rowe, W.B., Heman, K.L., Mesches, M.H., Young, D.A., Rose, G.M., and Bickford, P.C. 2002. Effects of hippocampal lesions on patterned motor learning in the rat. *Brain Res. Bull.* **58**: 581–586.
- Gupta, R.C. and Dettbarn, W.D. 1987. Interaction of cycloheximide and diisopropylphosphorofluoridate (DFP) during subchronic administration in rat. *Toxicol. Appl. Pharmacol.* **90**: 52–59.
- Hikosaka, O., Nakamura, K., Sakai, K., and Nakahara, H. 2002. Central mechanisms of motor skill learning. *Curr. Opin. Neurobiol.* **12**: 217–222.
- Kleim, J.A., Bruneau, R., Calder, K., Pocock, D., VandenBerg, P.M., MacDonald, E., Monfils, M.H., Sutherland, R.J., and Nader, K. 2003. Functional organization of adult motor cortex is dependent upon continued protein synthesis. *Neuron* **40**: 167–176.
- Pavlik, A. and Teisinger, J. 1980. Effect of cycloheximide administered to rats in early postnatal life: Prolonged inhibition of DNA synthesis in the developing brain. *Brain Res.* **192**: 531–541.
- Snider, B.J., Du, C., Wei, L., and Choi, D.W. 2001. Cycloheximide reduces infarct volume when administered up to 6 h after mild focal ischemia in rats. *Brain Res.* **917**: 147–157.
- Taniguchi, S., Tani, T., Ushida, T., and Yamamoto, H. 2002. Motor evoked potentials elicited from erector spinae muscles in patients with thoracic myelopathy. *Spinal Cord* **40**: 567–573.

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