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Dopaminergic Modulation of Auditory Cortex-Dependent Memory Consolidation through mTOR

Horst Schicknick[1], Björn H. Schott[1,2], Eike Budinger[1,2], Karl-Heinz Smalla[1], Anett Riedel[3], Constanze I. Seidenbecher[1], Henning Scheich[1], Eckart D. Gundelfinger[1] and Wolfgang Tischmeyer[1]

[1]Leibniz Institute for Neurobiology, D-39118 Magdeburg, Germany, [2]Department of Neurology II and [3]Institute of Biology, Otto von Guericke University, D-39120 Magdeburg, Germany

Previous studies in the auditory cortex of Mongolian gerbils on discrimination learning of the direction of frequency-modulated tones (FMs) revealed that long-term memory formation involves activation of the dopaminergic system, activity of the protein kinase mammalian target of rapamycin (mTOR), and protein synthesis. This led to the hypothesis that the dopaminergic system might modulate memory formation via regulation of mTOR, which is implicated in translational control. Here, we report that the D1/D5 dopamine receptor agonist SKF-38393 substantially improved gerbils' FM discrimination learning when administered systemically or locally into the auditory cortex shortly before, shortly after, or 1 day before conditioning. Although acquisition performance during initial training was normal, the discrimination of FMs was enhanced during retraining performed hours or days after agonist injection compared with vehicle-injected controls. The D1/D5 receptor antagonist SCH-23390, the mTOR inhibitor rapamycin, and the protein synthesis blocker anisomycin suppressed this effect. By immunohistochemistry, D1 dopamine receptors were identified in the gerbil auditory cortex predominantly in the infragranular layers. Together, these findings suggest that in the gerbil auditory cortex dopaminergic inputs regulate mTOR-mediated, protein synthesis-dependent mechanisms, thus controlling for hours or days the consolidation of memory required for the discrimination of complex auditory stimuli.

Keywords: discrimination learning, mammalian target of rapamycin, neuromodulation, protein synthesis, SKF-38393

Introduction

New memories consolidate over time from an initially labile state to a more permanent state (Matthies 1989; McGaugh 2000; Frankland and Bontempi 2005; Izquierdo et al. 2006; Morris 2006; Wang et al. 2006a). This consolidation process depends upon de novo protein synthesis (Davis and Squire 1984; Stork and Welzl 1999). Signaling pathways that control the translation of messenger RNAs (mRNAs) are, therefore, likely to be involved in the regulation of synaptic plasticity underlying long-term memory formation (Kelleher et al. 2004; Kindler et al. 2005; Sutton and Schuman 2006).

The protein kinase mammalian target of rapamycin (mTOR) is implicated in the regulation of synaptic plasticity and of the translation of several mRNAs (Raught et al. 2001; Kelleher et al. 2004; Jaworski and Sheng 2006). Pathways involving mTOR are important for long-term potentiation (LTP) and long-term depression (LTD) (Tang et al. 2002; Huang et al. 2005; Lenz and Avruch 2005; Banko et al. 2006; Karpova et al. 2006; Tsokas et al. 2007), 2 well-characterized cellular forms of synaptic plasticity that are thought to underlie learning and memory. Intracerebral injection of rapamycin, a specific inhibitor of mTOR, impairs memory consolidation in different learning paradigms (Tischmeyer et al. 2003; Dash et al. 2006; Parsons et al. 2006; Bekinschtein et al. 2007). In neurons, mTOR activity can be regulated by various extracellular stimuli, such as trophic factors, hormones, and neurotransmitters (Jaworski and Sheng 2006).

The neurotransmitter dopamine is an important modulator of neuronal function (Tzschentke 2001). In the hippocampus, activity of the class of D1-like dopamine receptors (i.e., D1 and D5 receptors) facilitates—probably via modulation of glutamatergic neurotransmission—the expression of LTP and LTD (Frey et al. 1991; Frey et al. 1993; Otmakhova and Lisman 1996; Matthies et al. 1997; Swanson-Park et al. 1999; Lemon and Manahan-Vaughan 2006; Granado et al. 2008). The D1-like receptor agonist SKF-38393 can induce a protein synthesis-dependent late form of hippocampal LTP (Huang and Kandel 1995; Navakkode et al. 2007; but see Mockett et al. 2004). Long-term changes in synaptic efficacy are modulated by dopamine in most target regions of dopaminergic midbrain sources, including cortical structures (Law-Tho et al. 1995; Otani et al. 2003; Huang et al. 2004; Matsuda et al. 2006). Dopamine has a prominent role in post-trial reinforcement (Jay 2003; Wise 2004; Lisman and Grace 2005; El-Ghundi et al. 2007) and has been implicated in the consolidation of memory traces associated with a variety of tasks and learning in different brain regions (Bernabeu et al. 1997; Mele et al. 2004; Dalley et al. 2005; LaLumiere et al. 2005; Wittmann et al. 2005; O'Carroll et al. 2006; Nagai et al. 2007).

The auditory cortex of mammals is believed to mediate particular aspects of auditory stimulus processing, task-specific performance, and learning (Aitkin 1990; Scheich 1991; Ehret 1997; Edeline 1999; Weinberger 2004; Ohl and Scheich 2005; Scheich et al. 2007). In adult rats, associative remodeling in the auditory cortex can be mediated by stimulation of midbrain dopaminergic neurons (Bao et al. 2001), and dopaminergic inputs to the auditory cortex are required for sound sequence discrimination learning (Kudoh and Shibuki 2006). For Mongolian gerbils, it has been shown that the auditory cortex is critical for discriminating the directions of modulation of linearly frequency-modulated tones (FMs) (Ohl et al. 1999, 2001). In this learning paradigm, long-term memory formation requires N-methyl-D-aspartate (NMDA)–type glutamate receptor activity and de novo protein synthesis in the auditory cortex during the postacquisition phase (Kraus et al. 2002; Schicknick and Tischmeyer 2006). Moreover, protein synthesis inhibitors interfere with long-term memory formation for a number of training days when applied to the gerbil auditory cortex shortly after initial conditioning (Kraus et al. 2002),

implying that a protein synthesis–dependent signal is produced which prepares relevant local nodes for subsequent memory consolidation. In a particular subpopulation of gerbils, that is, in rapid learners, additionally postacquisition mTOR activity is recruited for persistent memory formation (Tischmeyer et al. 2003). In such rapidly learning gerbils, microdialysis studies have revealed increased cortical dopamine responses during and shortly after conditioning to FMs, which may be considered as an indicator for the establishment of this complex auditory discrimination behavior (Stark and Scheich 1997; Stark et al. 2004). Based on these findings we hypothesized that in this paradigm dopaminergic activity might control memory formation via mTOR-mediated signaling.

In support of this hypothesis we report here that pharmacological activation of the dopamine system with the D1-like dopamine receptor agonist SKF-38393 shortly before, shortly after, or even 1 day before differential conditioning of gerbils to FMs facilitates memory consolidation, and that the initiation of this effect is sensitive to inhibitors of mTOR activity and of protein synthesis.

Materials and Methods

Animal experimentation was approved by the animal care committee of the Land Sachsen-Anhalt (No. 203.6.1-42502/2-600 IfN) in accordance with the regulations of the German Federal Law on the Care and Use of Laboratory Animals and with National Institutes of Health guidelines.

Animals

Male 3-month-old Mongolian gerbils (*Meriones unguiculatus*) were used. The animals were housed in groups of 5 and given free access to standard laboratory chow and tap water on a 12-h light/dark cycle (light on at 6:00 A.M.).

Pharmacological Agents

The cytoplasmic protein synthesis inhibitor anisomycin, the 5-HT2 receptor agonist 1-(3-chlorophenyl)piperazine (m-CPP), the D1/D5 receptor antagonist SCH-23390, and the D1/D5 receptor agonist SKF-38393 were obtained from Sigma-Aldrich (Taufkirchen, Germany). The mTOR inhibitor rapamycin was purchased from Calbiochem–Novabiochem (La Jolla, CA). For intraperitoneal (ip) injections, drugs were dissolved in 0.9% saline; the doses used, that is, 0.1 mg/kg SCH-23390 and 5 mg/kg SKF-38393, were deduced from studies on mechanisms of emotional learning in rats (Greba and Kokkinidis 2000; O'Tuathaigh and Moran 2002). For intracortical injections, m-CPP (1 μg/μL = 3.7 mM), SCH-23390 (0.3 μg/μL = 0.9 mM, 1.2 μg/μL = 3.7 mM, 2.4 μg/μL = 7.4 mM), and SKF-38393 (0.06 μg/μL = 0.2 mM) were dissolved in 0.9% saline. The applied dose of m-CPP was within the range shown to exert, after intrastriatal infusion, similar effects as intrastriatal SKF-38393 on oral activity responses in rats (Plech et al. 1995); the doses of SCH-23390 and SKF-38393 were deduced from studies on mechanisms of working memory and attentional performance in the rat prefrontal cortex (Seamans et al. 1998; Granon et al. 2000). Anisomycin (20 μg/μL = 75 mM) and rapamycin (0.055 ng/μL = 60 nM) were dissolved as described previously (Kraus et al. 2002; Tischmeyer et al. 2003) and diluted with 0.9% saline. All solutions used for intracortical injections were adjusted to pH ≈ 7.

Surgical Procedures and Intracortical Injections

Surgery and intracortical injections were performed bilaterally as described in detail elsewhere (Budinger et al. 2000; Kraus et al. 2002). In brief, on the day before intracortical injections, gerbils were deeply anesthetized (4 mg ketamine and 3 mg xylacine per 100 g body weight, ip), the cranial skin was disinfected and incised, and 3 holes of about 1 mm in diameter were drilled per hemisphere into the skull at locations covering the primary, anterior, and posterior fields of the auditory cortex. After surgery, gerbils were allowed to recover for 1 day before injections were performed. At the indicated times in relation to the behavioral experiments, that is, 1 day prior to or shortly after conditioning to FMs, 1-μL portions of drug solution or vehicle (0.9% saline) were applied per target region under light halothane anesthesia (1.5–2% halothane in air for respiration) over a period of 4 min. The procedure of positioning the injection tracks has previously been validated (Kraus et al. 2002).

Behavioral Experiments

Gerbils were trained once per day in a 2-way shuttle box to discriminate the directions of modulation of linearly FMs as described earlier (Kraus et al. 2002). Briefly, before each training session, gerbils were allowed to habituate for 3 min to the training chamber without acoustical stimulation and foot-shock. During the sessions, the animals were trained to discriminate between sequences (250-ms tone, 250-ms pause) of an ascending (1–2 kHz, CS+) and of a descending FM (2–1 kHz, CS–). A training session always started with CS+ presentation in trial 1 and CS– presentation in trial 2 and consisted of 60 trials, that is, 30 presentations of each CS+ and CS– in a pseudorandomized order. The mean intertrial interval was 15 s. To avoid mild foot-shock, gerbils had to cross the hurdle within 6 s of CS+ presentation and to suppress this response within 6 s of CS– presentation. Hurdle crossings within 6 s upon the onset of CS+ and CS– were regarded as correct conditioned responses (CR+) and false alarms (CR–), respectively. For each experiment, the numbers of CR+ and CR– monitored per training session are documented in the Supplementary Material. To quantify the discrimination performance, the discrimination rate *D*, that is, the difference between the numbers of CR+ and CR– expressed as percentage of the maximum number of CR+, was calculated for each animal and session. Where indicated, a training session was subdivided into 5 blocks of consecutive trials, and *D* was calculated per trial block; each trial block consisted of 12 trials, that is, 6 presentations of each CS+ and CS–. To assess drug effects on arousal and activity, the numbers of hurdle crossings during the habituation period preceding each training session as well as the intertrial activity, that is, the numbers of hurdle crossings occurring between the trials of each training session, were monitored. To assess drug effects on sensory systems and motor coordination, the avoidance latencies, that is, the times required to change the compartment during CR+, and the escape latencies, that is, the times required to change the compartment after the onset of foot-shock, were recorded within the training sessions. For each experiment, these data are documented in the Supplementary Material.

Immunohistochemistry

Gerbils were deeply anesthetized (5 mg ketamine and 3 mg xylacine per 100 g body weight, ip) and perfused transcardially with 50 mL of phosphate-buffered saline (PBS, pH 7.4) followed by 200 mL of 4% paraformaldehyde in PBS. The brains were removed, postfixed overnight in the same fixative at 4 °C, and cryoprotected in PBS containing 30% sucrose at 4 °C for 48 h. Fifty-micrometer-thick horizontal or frontal sections were cut on a freezing microtome (Leica CM 3050 S, Germany) and collected in 0.1 M PBS. After preincubation at room temperature in 1% $NaBH_4$ in PBS for 20 min, in 1% H_2O_2 in methanol/PBS for 20 min, and in RotiImmunoBlock (Roth, Germany, 1:10 in aqua dest.) for 30 min, sections were incubated with rabbit polyclonal antibody raised against amino acids 338–446 (Santa Cruz Biotechnology, diluted 1:200) of the human D1 dopamine receptor in RotiImmunoBlock (1:10 in 0.01% Triton) for 48 h. After 3 washes of 5 min in PBS, slices were incubated for 2 h with biotinylated anti-rabbit secondary antibody (Sigma-Aldrich, diluted 1:200) and visualized using the avidin–biotin–peroxidase method (ABC-kit, Vector Laboratories) with diaminobenzidine as chromogen. Appropriate controls without primary antibody were performed (Supplementary Fig. S1). The sections were mounted and coverslipped with Entellan (Merck, Germany) and examined using the light microscope Axioscope 2 (Zeiss, Germany). Regions of interest were digitally photographed (Leica DCS 500). Photographs were arranged for illustrations using the Adobe Photoshop software.

Statistical Analysis

All behavioral data are presented as group means ± standard error of the mean (SEM). For statistical evaluation, a repeated-measures analysis of variance (ANOVA) was performed. Fisher's protected least significant difference test or Dunnett's test for multiple comparisons to a control were used for post hoc comparisons, where appropriate. Student's 2-tailed *t*-test for paired comparisons was used to compare the numbers of CR+ and CR–. *P* values of <0.05 were considered as statistically significant.

Results

Effects of Presession Application of Dopamine Agonists and Antagonists

Experiment 1 was designed as a pilot study with only 4 gerbils per group for an initial assessment of the role of dopamine in FM discrimination learning and performance. To this end, presession intraperitoneal injections of the D1-like dopamine receptor agonist SKF-38393 and, later in the well-trained animals, of the D1-like dopamine receptor antagonist SCH-23390 were performed. Gerbils were randomly assigned to group A or B and trained on the FM discrimination task once per day for a total of 18 sessions with training-free intervals of 2 days after sessions 5, 10, and 15. The 2 groups were pharmacologically treated and behaviorally tested following the scheme of Figure 1*A*. The mean discrimination rates *D* calculated per group and training session are shown in Figure 1*B*.

To examine effects of D1-like receptor activation during acquisition, vehicle (group A) or SKF-38393 (group B) was infused 30 min prior to session 1. ANOVA comparison of *D* values over sessions 1–5 across treatment groups showed no significant group effect ($F_{1,6}$ = 1.51, P = 0.265), but a significant session effect ($F_{4,24}$ = 3.10, P = 0.034), and a significant group × session interaction ($F_{4,24}$ = 2.98, P = 0.039). Further examinations revealed that group B performed the discrimination significantly better than group A in session 5—here, the group means of *D* differed by a factor of approximately 3.

To assess whether SKF-38393 affects learning and performance in repeatedly pretrained gerbils, the vehicle-treated group A received the D1-like agonist 30 min prior to session 6, whereas group B received vehicle. Later in the experiment, that is, 30 min prior to session 11, both groups received SKF-38393. ANOVA comparing *D* over sessions 5–10 and 10–15 across groups revealed significant group effects (sessions 5–10: $F_{1,6}$ = 14,83, P = 0.008; sessions 10–15: $F_{1,6}$ = 16.06, P = 0.007), indicating the persistence of the group difference that had developed over sessions 1–5. However, no significant session effects and group × session interactions were observed.

In contrast to group B, group A did not show a significant increase in *D* even after 15 training sessions. This implies that these gerbils might have failed in discrimination learning. However, paired comparison of the numbers of CR+ and CR– within each group revealed a significant FM discrimination in session 2 (Supplementary Table S1). Moreover, later in the experiment, each group showed a stable FM discrimination on consecutive training sessions. This was the case for group A after the tenth session and for group B already after the third one. Thus, both groups were able to learn the discrimination task and to form a stable memory of it. These abilities, however, appeared to be substantially facilitated in group B animals, which received SKF-38393 prior to the first conditioning.

The final part of Experiment 1 addressed the question whether D1-like receptor activity in well-trained gerbils is required to perform the established FM discrimination. To this end the gerbils of group B received the dopamine antagonist SCH-23390 30 min prior to the beginning of session 17 (Fig. 1). ANOVA comparing *D* over sessions 16–18 revealed a significant session effect ($F_{2,6}$ = 10.02, P = 0.012). Post hoc comparisons revealed that in session 17, but not in session 18, *D* was significantly lower than in session 16.

To summarize this pilot study, when SKF-38393 was systemically applied 30 min prior to the initial session of FM discrimination training, gerbils progressively improved their discrimination performance over that of vehicle-treated controls on subsequent training days. SKF-38393 was ineffective when applied to repeatedly trained gerbils, which required dopamine activity merely for sensory processing and/or behavioral expression of the established discrimination.

A

Group	I.p. injection 30 min prior to the beginning of			
	session 1	session 6	session 11	session 17
A (n = 4)	Veh ○	SKF ●	SKF ●	
B (n = 4)	SKF ■	Veh □	SKF ■	SCH ⊠

B

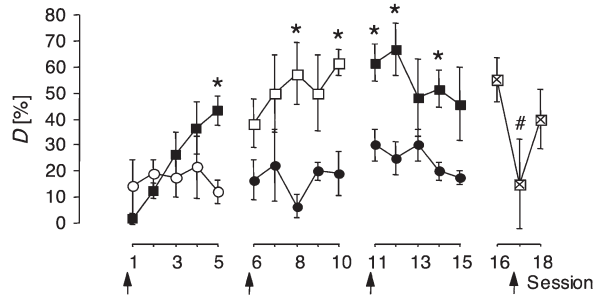

Figure 1. Preconditioning ip injection of the D1/D5 agonist SKF-38393 into naïve gerbils improves learning, and presession ip injection of the D1/D5 antagonist SCH-23390 into well-trained gerbils impairs performance of the FM discrimination. Data were collected in Experiment 1. (*A*) Design of pharmacological treatment. Gerbils were randomly assigned to 1 of 2 groups, A and B, and trained on the FM discrimination task once per day for a total of 18 sessions. Training-free intervals of 2 days were interspersed after sessions 5, 10, and 15. Injections of vehicle (Veh), SKF-38393 (SKF; 5 mg/kg), or SCH-23390 (SCH; 0.1 mg/kg) were performed 30 min prior to the indicated training sessions. (*B*) Discrimination rates *D* per training session. Arrows indicate the approximate injection times. All data points represent group means ± SEM; (*) significantly different from the value of group A; ($^{\#}$) significantly different from the value in session 16.

Postconditioning Effect of SKF-38393 and its Antagonism by the mTOR Inhibitor Rapamycin

In Experiment 2, local infusions into the auditory cortex were utilized to test whether SKF-38393 might also affect retention and/or retrieval of the FM discrimination if applied after the first training session. In another group of the same experiment we tested how the mTOR inhibitor rapamycin might influence this effect. Gerbils were trained on the FM discrimination task every 24 h for 3 days. Vehicle, or SKF-38393, or a combination of SKF-38393 and rapamycin were applied to the auditory cortex twice, that is, immediately after and 2 h after the completion of the first training session. This injection protocol was analogous to the pharmacological application regime in our previous studies (Kraus et al. 2002; Tischmeyer et al. 2003; Schicknick and Tischmeyer 2006). There, we have demonstrated amnesic effects on FM conditioned memory retention of global protein synthesis inhibitors, of the mTOR inhibitor

rapamycin, and of the NMDA-type glutamate receptor antagonist D-AP5 when applied to the gerbil auditory cortex.

The mean discrimination rates *D* calculated per training session are shown in Figure 2. ANOVA comparing *D* over sessions 1–3 across treatment groups revealed significant effects of group ($F_{2,30}$ = 5.37, P = 0.010) and session ($F_{2,60}$ = 24.06, P < 0.001), and no significant group × session interaction ($F_{4,60}$ = 1.72, P = 0.157). Post hoc comparison revealed that the effect of group indicates a significant increment in the discrimination rates of SKF-38393–treated gerbils over those of vehicle-treated controls. Such an increment was not evident when rapamycin was coinjected with SKF-38393. Comparison of individual data points revealed significant group differences in session 2, in which the mean discrimination rate of SKF-38393–treated gerbils reached approximately twice the value of controls, but not in session 1. In session 3, the difference between groups was no longer statistically significant, implying that locally applied SKF-38393 might improve the efficiency rather than the overall capacity of FM discrimination learning.

Thus, gerbils locally infused with SKF-38393 into the auditory cortex shortly after differential conditioning to FMs showed profoundly higher discrimination rates than vehicle-treated controls in session 2, that is, 1 day after injections. This effect was antagonized by coinfusion of the mTOR inhibitor rapamycin that, when applied alone into the gerbil auditory cortex, does not impair FM discrimination learning and performance monitored 24 h later (Tischmeyer et al. 2003). Therefore, unspecific side effects or a sustained presence of the dopamine agonist are unlikely to explain its behavioral effect in the FM discrimination paradigm. Together, the findings of Experiment 2 suggest that post-training SKF-38393 infusions activate mTOR-mediated processes that support FM discrimination learning. This might be accomplished, for example, by enhancing the consolidation of memory acquired shortly before injections and/or by facilitating learning during subsequent training (see below).

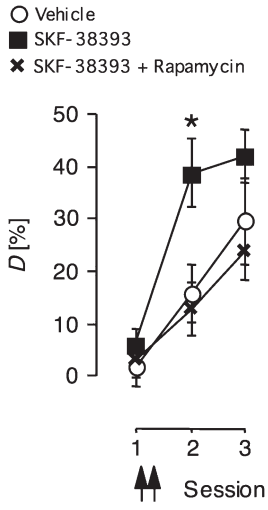

Figure 2. Postconditioning local injections of SKF-38393 into the gerbil auditory cortex induce a rapamycin-sensitive improvement in the FM discrimination monitored during retraining 24 h later. Data were collected in Experiment 2. Gerbils were trained on the FM discrimination task every 24 h for 3 days. Vehicle (n = 8), or 0.2 mM SKF-38393 (n = 16), or a combination of 0.2 mM SKF-38393 and 60 nM rapamycin (n = 9) was applied twice, that is, immediately and 2 h after completion of the first training session. Discrimination rates *D* per training session are shown. Arrows indicate the approximate injection times. All data points represent group means ± SEM; (*) significantly different from the value of vehicle-treated controls.

Effect of SKF-38393 Applied 1 Day Before Conditioning

In Experiment 3 we examined whether SKF-38393 may influence FM discrimination learning on subsequent days when applied to naïve gerbils 1 day before conditioning. Gerbils were trained on the FM discrimination task every 24 h for 3 days. The mean discrimination rates *D* calculated per session are shown in Figure 3 for gerbils locally infused into the auditory cortex with SKF-38393 or vehicle twice, that is, 24 and 22 h before the beginning of the first training session. ANOVA comparing *D* over sessions 1–3 across treatment groups showed no significant group effect ($F_{1,15}$ = 0.64, P = 0.437), a significant session effect ($F_{2,30}$ = 22.03, P < 0.001), and a significant group × session interaction ($F_{2,30}$ = 5.10, P = 0.012). Within-group analyses of *D* revealed significant session effects in both the vehicle-treated control group ($F_{2,16}$ = 10.59, P = 0.002) and the group of SKF-38393–treated gerbils ($F_{2,14}$ = 16.58, P < 0.001), indicating that, over sessions 1–3, both groups improved their performance. To further analyze the significant interaction, an ANOVA was performed to compare *D* over sessions 1–2 and over sessions 2–3 separately. ANOVA over sessions 1–2 across treatment groups revealed no significant group effect ($F_{1,15}$ = 2.37, P = 0.144), a significant session effect ($F_{1,15}$ = 31.20, P < 0.001), and a significant group × session interaction ($F_{1,15}$ = 11.43, P = 0.004). Within-group analyses of *D* over sessions 1–2 revealed a significant session effect in SKF-38393–treated gerbils ($F_{1,7}$ = 37.99, P < 0.001), but not in vehicle-treated controls ($F_{1,8}$ = 2.58, P = 0.147). ANOVA over sessions 2–3 across treatment groups revealed no significant effects of group ($F_{1,15}$ = 1.79, P = 0.201) or session ($F_{1,15}$ = 2.62, P = 0.126), but again a significant group × session interaction was observed ($F_{1,15}$ = 6.36, P = 0.024). In this case, within-group ANOVA of *D* revealed a significant session effect in vehicle-treated controls ($F_{1,8}$ = 12.16, P = 0.009) but not in SKF-38393–treated gerbils ($F_{1,7}$ = 0.30, P = 0.602). Thus, the discrimination rates of

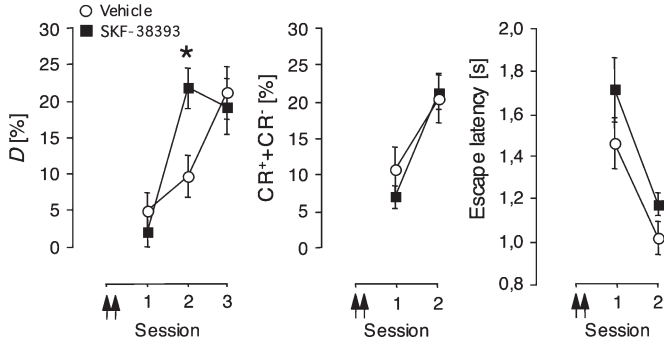

Figure 3. Local injections of SKF-38393 into the gerbil auditory cortex 1 day before conditioning improve the FM discrimination reaction monitored during retraining 2 days after injections. Data were collected in Experiment 3. Gerbils were trained on the FM discrimination task every 24 h for 3 days. Vehicle (n = 9) or 0.2 mM SKF-38393 (n = 8) was applied twice, that is, 24 and 22 h prior to the beginning of the first training session. *Left:* Discrimination rates *D* per training session. *Middle:* Numbers of hurdle crossings in response to FMs, that is, the sums of correct conditioned responses and false alarms, expressed as percent of total trial number. *Right:* Escape latencies, that is, the times required to change the compartment after the onset of foot-shock. Arrows indicate the approximate injection times. All data points represent group means ± SEM; (*) significantly different from the value of vehicle-treated controls.

vehicle-treated controls increased significantly between sessions 2 and 3 but not between sessions 1 and 2. In contrast, the discrimination rates of SKF-38393–treated gerbils increased already between sessions 1 and 2 and remained stable in session 3.

Comparisons of individual data points revealed that pharmacological treatment 1 day before conditioning had no significant impact on acquisition performance in session 1. In session 2, however, the discrimination rates of SKF-38393–treated gerbils significantly exceeded those of vehicle-treated controls. This is due to both increases in the numbers of CR+ and decreases in the numbers of CR– (Supplementary Table S3). Consequently, as shown in Figure 3, similar increasing total numbers of hurdle crossings in response to tones, that is, the sum of CR+ and CR–, were observed in both groups over sessions 1 and 2 (ANOVA, group effect: $F_{1,15} = 0.22$, $P = 0.643$; session effect: $F_{1,15} = 21.84$, $P < 0.001$; group × session: $F_{1,15} = 0.78$, $P = 0.392$). Both treatment groups also showed similar decreasing escape latencies, that is, the times required to cross the hurdle after the onset of foot-shock, over sessions 1 and 2 (ANOVA, group effect: $F_{1,15} = 3.38$, $P = 0.086$; session effect: $F_{1,15} = 23.98$, $P < 0.001$; group × session: $F_{1,15} = 0.22$, $P = 0.645$). Thus, pharmacological treatment did not affect learning and performance of the hurdle reaction in response to the tones per se and to the foot-shocks.

In summary, this experiment showed that SKF-38393 infused into the auditory cortex 1 day prior to initial conditioning to FMs did not affect acquisition performance monitored 24 h later but caused a discrimination increment detectable during retraining 48 h after injections.

Rapamycin and Global Protein Synthesis Blockade Antagonize the Effect of SKF-38393 Applied 1 Day before Conditioning

In Experiment 4, rapamycin and the global protein synthesis inhibitor anisomycin were utilized to assess the role of mTOR activity and protein synthesis for the SKF-38393–induced FM discrimination increment. The long-lasting persistence of the SKF-38393–induced effect shown in Experiment 3 provided the opportunity to analyze its induction separately from its action on postacquisition memory processing. Therefore, injections were applied to the auditory cortex 24 and 22 h before the beginning of the first training session as in Experiment 3; where indicated (Experiments 4B and 4D), additional injections were applied immediately and 2 h after completion of the first training session. Gerbils were trained on the FM discrimination task every 24 h for 2 days. The mean discrimination rates D calculated per training session and treatment group are shown in Figure 4.

In Experiment 4A, gerbils received SKF-38393 either alone or in combination with rapamycin 1 day prior to, and no further injections after the first training session. ANOVA comparing D over sessions across treatment groups revealed no significant group effect ($F_{1,20} = 2.47$, $P = 0.132$), a significant session effect ($F_{1,20} = 23.01$, $P < 0.001$), and a significant group × session interaction ($F_{1,20} = 5.75$, $P = 0.026$). Further evaluation revealed no significant group difference in session 1. In session 2, however, gerbils infused with the mixture of SKF-38393 and rapamycin showed significantly lower discrimination rates than gerbils infused with SKF-38393 alone (Fig. 4*A*).

In Experiment 4B, SKF-38393 was applied 1 day prior to, and either vehicle or rapamycin shortly after the initial training. An

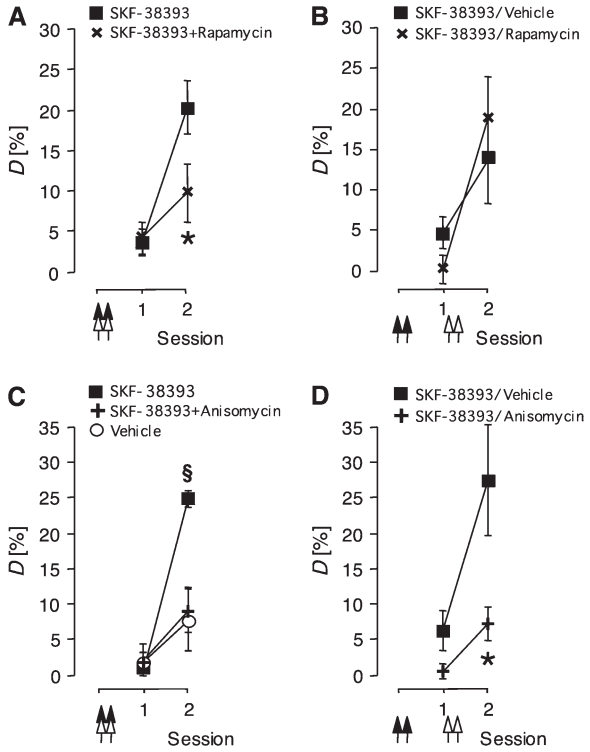

Figure 4. The SKF-38393–induced increment in FM discrimination learning requires mTOR activity and protein synthesis. Data were collected in Experiment 4. Gerbils were trained on the FM discrimination task every 24 h for 2 days. Injections were locally applied to the auditory cortex. (*A*) Experiment 4A: 0.2 mM SKF-38393 was applied either alone ($n = 10$) or in combination with 60 nM rapamycin ($n = 12$) twice, 24 and 22 h prior to initial training. (*B*) Experiment 4B: 0.2 mM SKF-38393 was applied twice, that is, 24 and 22 h prior to initial training; vehicle ($n = 7$) or 60 nM rapamycin ($n = 14$) was applied twice, that is, immediately and 2 h after completion of initial training. (*C*) Experiment 4C: Vehicle ($n = 5$), or 0.2 mM SKF-38393 ($n = 3$), or 0.2 mM SKF-38393 in combination with 75 mM anisomycin ($n = 8$) was applied twice, 24 and 22 h prior to initial training. (*D*) Experiment 4D: 0.2 mM SKF-38393 was applied twice, that is, 24 and 22 h prior to initial training; vehicle ($n = 8$) or 75 mM anisomycin ($n = 9$) was applied twice, that is, immediately and 2 h after completion of initial training. Discrimination rates D per training session are shown. Filled arrows indicate the approximate injection times for SKF-38393, open arrows indicate the approximate injection times for vehicle or inhibitors. All data points represent group means ± SEM; (*) significantly different from the value in SKF-38393-treated gerbils; (§) significantly different from the value in vehicle-treated gerbils.

ANOVA comparing D over sessions across treatment groups revealed no significant group effect ($F_{1,19} < 0.01$, $P = 0.960$), a significant session effect ($F_{1,19} = 11.47$, $P = 0.003$), and no significant group × session interaction ($F_{1,19} = 1.36$, $P = 0.257$). Thus, gerbils that received SKF-38393 one day prior to and rapamycin shortly after initial training were similarly effective in learning the discrimination as gerbils that received SKF-38393 followed by vehicle (Fig. 4*B*).

In Experiment 4C, gerbils received either vehicle, or SKF-38393, or SKF-38393 in combination with anisomycin 1 day prior to, and no further injections after the first training session. ANOVA comparing D over sessions across treatment groups revealed no significant group effect ($F_{2,13} = 2.08$, $P = 0.164$), a significant session effect ($F_{1,13} = 31.95$, $P < 0.001$), and a significant group × session interaction ($F_{2,13} = 5.78$, $P = 0.016$). Further examination revealed no significant group differences in session 1. In session 2, the discrimination rates monitored in SKF-38393–treated gerbils significantly exceeded those of vehicle-treated controls. This effect was not evident in

gerbils that received the combination of SKF-38393 and anisomycin (Fig. 4*C*).

In Experiment 4D, gerbils received SKF-38393 one day prior to, and either vehicle or anisomycin shortly after initial training. ANOVA comparing *D* over sessions across treatment groups revealed significant effects of group ($F_{1,15}$ = 7.45, P = 0.016) and session ($F_{1,15}$ = 18.27, P < 0.001), and a significant group × session interaction ($F_{1,15}$ = 4.99, P = 0.041). Comparison of individual data points revealed no significant group difference in *D* in session 1. In session 2, however, gerbils infused with SKF-38393 followed by anisomycin showed significantly lower discrimination rates than gerbils infused with SKF-38393 followed by vehicle (Fig. 4*D*).

To summarize this set of experiments, when coinjected with SKF-38393 into the gerbil auditory cortex 1 day prior to the initial conditioning to FMs, rapamycin and anisomycin prevented the FM discrimination increment, which was normally found after infusion of the dopamine agonist, with similar efficiencies. This suggests that the induction of the long-lasting effect of SKF-38393 on memory formation requires mTOR-dependent translational changes in the cortex. When applied shortly after conditioning, that is, 1 day after SKF-38393 injections, the general protein synthesis blocker anisomycin (not rapamycin, which interferes only with the mTOR-controlled subset of translation) abolished this increment. This suggests that—when SKF-38393 has already induced mTOR-dependent translational changes in the cortex before the learning event—additional mTOR-dependent, that is, rapamycin-sensitive, translation is not required during postacquisition memory formation, whereas other anisomycin-sensitive protein synthesis still is necessary.

The D1-Like Antagonist SCH-23390 Counteracts SKF-38393 Applied 1 Day before Conditioning

In Experiment 5, gerbils were trained on the FM discrimination task every 24 h for 3 days. In Experiments 5A–C, naïve gerbils received infusions of 0.2 mM SKF-38393 in combination with increasing doses of the D1-like dopamine receptor antagonist SCH-23390 into the auditory cortex twice, that is, 24 and 22 h prior to initial training. When compared with vehicle-treated controls, gerbils infused with a mixture of SKF-38393 and either 0.9 mM (Table 1, Experiment 5A) or 3.7 mM SCH-23390 (Table 1, Experiment 5B) showed significant increments in *D* during session 2, performed 2 days after injections. These increments resembled that monitored in Experiment 3, when SKF-38393 was applied alone (compare Fig. 3). In contrast, coinjection of 7.4 mM SCH-23390 with SKF-38393 antagonized the discrimination increment (Table 1, Experiment 5C). Injection of 7.4 mM SCH-23390 alone into the auditory cortex 24 and 22 h before initial training did not significantly affect FM discrimination learning and performance (Table 1, Experiment 5D). Finally, injection of the 5-HT2 receptor agonist m-CPP into the auditory cortex of naïve gerbils 24 and 22 h before the initial FM discrimination training did not improve FM discrimination learning and performance when compared with vehicle-treated controls (Table 1, Experiment 5E).

Together, this set of experiments showed that, when coinfused with SKF-38393 into the auditory cortex 1 day

Table 1

Discrimination rates *D* recorded during 3 sessions of FM discrimination training of gerbils infused with vehicle or the indicated drugs or drug combinations into the auditory cortex twice, 24 and 22 h prior to the beginning of session 1

Experiment	Treatment group	*n*	Session	*D* (%)	ANOVA#		
					Main effect of treatment	Main effect of session	Treatment × session
5A	0.2 mM SKF	5	1	3.33 ± 2.36	$F_{1,9}$ = 6.71	$F_{2,18}$ = 5.86	$F_{2,18}$ = 2.52
	+ 0.9 mM SCH		2	20.00 ± 6.75*	P = 0.029	P = 0.011	P = 0.109
			3	18.00 ± 6.63			n.s.
	Vehicle	6	1	1.11 ± 1.86			
			2	1.67 ± 2.40			
			3	11.11 ± 1.65			
5B	0.2 mM SKF	3	1	−1.11 ± 2.94	$F_{1,4}$ = 0.06	$F_{2,8}$ = 17.11	$F_{2,8}$ = 5.50
	+ 3.7 mM SCH		2	25.56 ± 2.22*	P = 0.820	P = 0.002	P = 0.032
			3	17.78 ± 5.56	n.s.		
	Vehicle	3	1	2.22 ± 2.22			
			2	11.11 ± 4.01			
			3	31.11 ± 5.88			
5C	0.2 mM SKF	6	1	2.78 ± 2.34	$F_{1,10}$ = 0.02	$F_{2,20}$ = 8.11	$F_{2,20}$ = 0.31
	+ 7.4 mM SCH		2	11.11 ± 7.54	P = 0.878	P = 0.003	P = 0.736
			3	31.11 ± 11.50	n.s.		n.s.
	Vehicle	6	1	4.44 ± 5.42			
			2	12.78 ± 4.08			
			3	24.44 ± 7.63			
5D	7.4 mM SCH	3	1	6.67 ± 3.33	$F_{1,4}$ = 0.15	$F_{2,8}$ = 4.65	$F_{2,8}$ = 0.84
			2	7.78 ± 2.94	P = 0.718	P = 0.045	P = 0.464
			3	14.44 ± 2.94	n.s.		n.s.
	Vehicle	3	1	3.33 ± 1.92			
			2	8.89 ± 4.84			
			3	23.33 ± 11.55			
5E	3.7 mM m-CPP	3	1	2.22 ± 4.01	$F_{1,4}$ = 0.31	$F_{2,8}$ = 4.95	$F_{2,8}$ < 0.01
			2	12.22 ± 5.56	P = 0.606	P = 0.040	P = 0.992
			3	18.89 ± 9.69	n.s.		n.s.
	Vehicle	3	1	0.00 ± 3.85			
			2	10.00 ± 3.33			
			3	15.56 ± 2.22			

Note: SCH, SCH-23390; SKF, SKF-38393. (#) Values relating to ANOVA comparing *D* over sessions across treatment groups for either experiment; n.s., not significant. (*) Significantly different from the corresponding value of vehicle-treated controls.

before initial training, the dopamine antagonist SCH-23390 dose-dependently antagonized the FM discrimination increment. SCH-23390 did not affect normal FM discrimination learning and performance when applied alone 1 day before conditioning. Infusion of the 5-HT2 receptor agonist m-CPP into the gerbil auditory cortex 1 day prior to initial training was also ineffective.

Effect of SKF-38393 on Intersession Performance Changes

To assess whether SKF-38393 infused into the auditory cortex 1 day prior to initial conditioning to FMs affects retention and retrieval of memory acquired already during session 1 or only additional learning during session 2, data collected in Experiments 3–5 were pooled and subdivided into 5 blocks of 12 trials per training session, referred to as trial blocks 1–5. Included in the statistical analysis were data derived from gerbils that received either vehicle or SKF-38393–only injections 1 day before, and either vehicle or no injections shortly after initial training. Data were assigned to 1 of 2 groups according to the preconditioning treatments. Figure 5 shows the mean discrimination rates *D* calculated per group and trial block. An ANOVA comparing *D* over sessions and trial blocks across treatment groups revealed significant effects of group ($F_{1,69}$ = 13.60, P < 0.001) and session ($F_{1,69}$ = 78.69, P < 0.001), a significant group × session interaction ($F_{1,69}$ = 19.97, P < 0.001), and, importantly, a significant group × session × trial block interaction ($F_{4,276}$ = 3.20, P = 0.013).

The significant interactions imply that the behavioral effect of pharmacological treatment is confined to particular trial blocks of a particular training session. Indeed, comparisons of *D* of individual trial blocks across treatment groups revealed no significant group differences during the course of session 1. In trial blocks 1 and 2 of session 2, however, SKF-38393–treated gerbils performed the discrimination task significantly better than controls. When compared with the level reached in trial block 5 of session 1, SKF-38393–treated gerbils, but not controls, showed a significant increment in *D* already in trial block 1 of session 2.

Comparison of the numbers of CR+ and CR– demonstrated that both groups showed a significant FM discrimination in session 1 (vehicle: CR+ = 2.29 ± 0.46, CR– = 1.42 ± 0.29, t_{34} = 2.35, P < 0.025; SKF-38393: CR+ = 2.08 ± 0.33, CR– = 0.97 ± 0.18, t_{35} = 3.83, P < 0.001) as well as in session 2 (vehicle: CR+ = 5.46 ± 0.61, CR– = 2.89 ± 0.37, t_{34} = 6.06, P < 0.001; SKF-38393: CR+ = 8.81 ± 0.79, CR– = 2.44 ± 0.31, t_{35} = 9.17, P < 0.001). However, during the first 2 trials of session 2, SKF-38393–treated gerbils performed significantly more correct conditioned responses than false alarms (CR+ = 0.61 ± 0.08, CR– = 0.33 ± 0.08; t_{35} = 2.94, P < 0.01), whereas vehicle-treated controls did not (CR+ = 0.54 ± 0.08, CR– = 0.37 ± 0.08, t_{34} = 1.64, P > 0.1).

Together, these findings indicate that a significant increment of FM discrimination was evident in SKF-38393–treated gerbils already at the beginning of session 2.

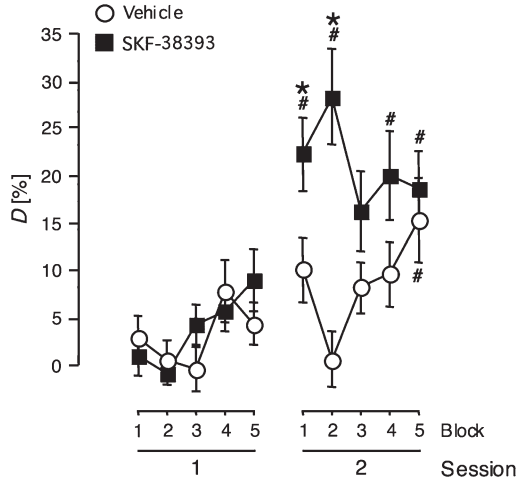

Figure 5. Local injections of SKF-38393 into the gerbil auditory cortex 1 day before differential conditioning to FMs contribute to between-session information processing. Data collected in Experiments 3–5 were pooled for groups that received local injections of either vehicle (n = 35) or SKF-38393 (n = 36) one day before, and no injections or vehicle injections shortly after initial training. Each training session was subdivided into 5 blocks of 12 trials. Discrimination rates *D* per trial block are shown. All data points represent group means ± SEM; (*) significantly different from the value of vehicle-treated controls; ($^{\#}$) significantly different from the corresponding value in trial block 5 of session 1.

Distribution of D1 Dopamine Receptors in the Gerbil Auditory Cortex

Our data suggest that D1-like dopamine receptors mediate the action of SKF-38393 on FM discrimination learning. To confirm that corresponding receptors are present in the gerbil auditory cortex, the distribution of D1 dopamine receptors in the gerbil brain was investigated using immunohistochemistry.

At the subcortical level, D1 receptor immunoreactivity was recognized in various structures that were previously shown in other species to contain or bind dopamine (e.g., Paxinos 2004), such as in the substantia nigra (SN, Fig. 6*A*), ventral tegmental area, striatum (caudate putamen, CPu, Fig. 6*D*; nucleus accumbens), and pallidum (globus pallidus, ventral pallidum). In most cases D1 receptor immunoreactivity appeared as stained punctae that were densely distributed within the labeled structures.

At the cortical level, D1 receptor immunoreactivity was detected in all layers, appearing mainly as labeled punctae but also as labeled somata (Fig. 6*A–C*). In auditory and other sensory cortices, strongest labeling was observed in the infragranular layer V (Fig. 6*A,B*). Here, the perikarya of many pyramidal cells, mainly of layer V large pyramidal cells, were labeled with punctae (Fig. 6*C*). In addition, we also observed D1-immunoreactive Golgi-like labeled multiform and horizontal cells in layer VI (Fig. 6*E*).

Discussion

The major findings of this study are that the D1-like dopamine receptor agonist SKF-38393 significantly and strongly improved the performance of gerbils in the FM discrimination task when applied either systemically or directly into the auditory cortex shortly before, shortly after, or 1 day before conditioning, but not when applied to repeatedly trained gerbils. Although acquisition performance during the initial training session was normal, the accuracy of discriminating the FMs was enhanced during retraining performed hours or days after agonist injection in comparison to control-injected gerbils. The dopamine antagonist SCH-23390, the mTOR inhibitor rapamycin, and the global protein synthesis blocker anisomycin suppressed the induction of this effect.

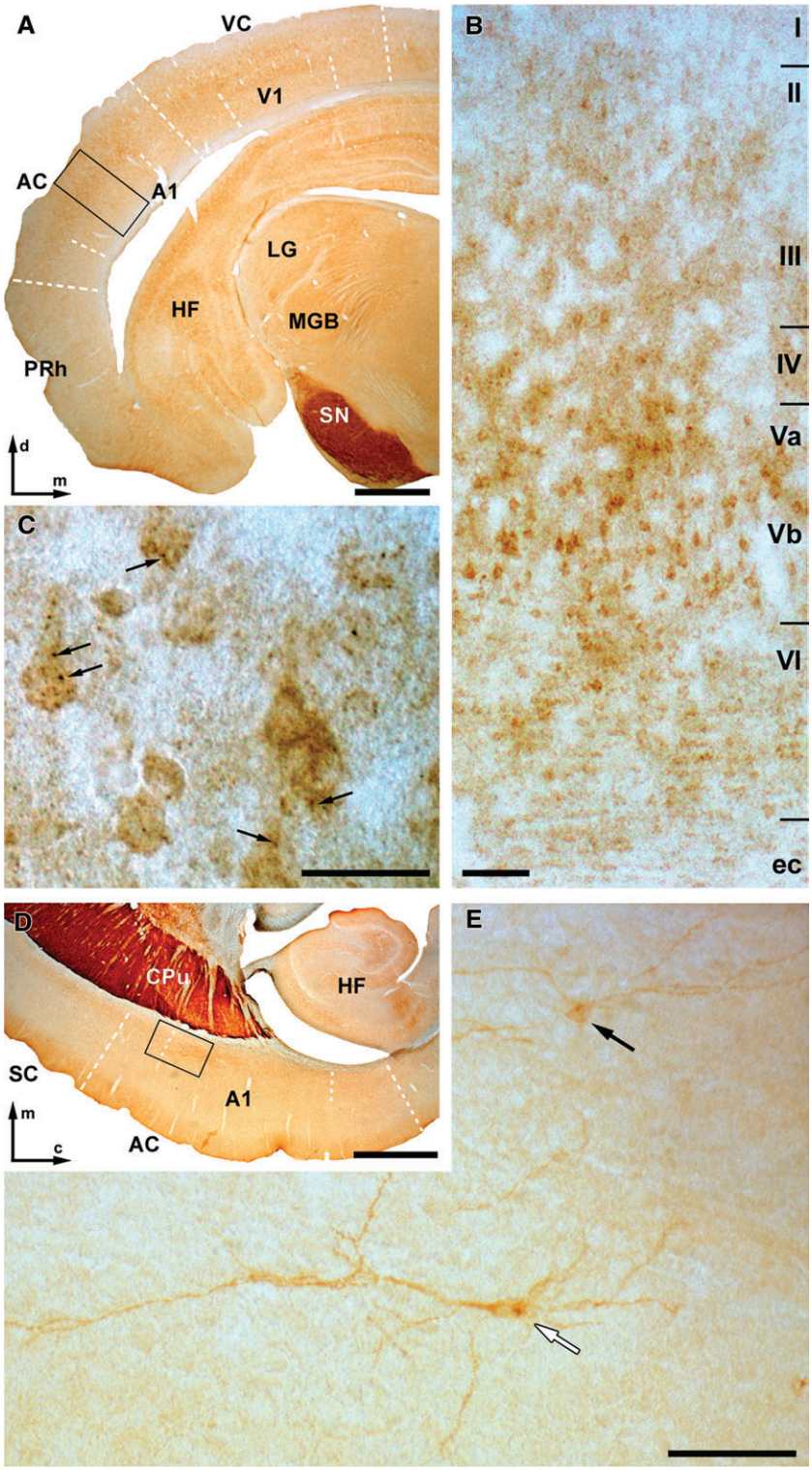

Figure 6. Photomicrographs demonstrating the distribution of D1 dopamine receptor immunoreactivity in the gerbil brain. (*A*) Low-power view of a frontal section showing the D1 receptor staining patterns in several cortical areas, including the auditory (AC), visual (VC), and perirhinal cortex (PRh), in the hippocampal formation (HF), in the thalamus (LG, lateral geniculate body; MGB, medial geniculate body), and in the SN. (*B*) Higher magnification of the area boxed in (*A*), showing the laminar staining pattern in the primary auditory cortex (field A1). Note the labeled somata of pyramidal neurons in infragranular layers V and VI. (*C*) High-power view demonstrating D1 receptor–positive large pyramidal cells of layer V. The staining often appears as little punctae at the perikarya of these cells (arrows). (*D*) Low-power view of a horizontal section showing the D1 receptor staining patterns in several cortical areas, including the auditory (AC) and somatosensory cortex (SC), in the hippocampal formation (HF), and in the CPu complex. (*E*) High-power view of the area boxed in (*D*), showing 2 labeled nonpyramidal cells in layer VI of the AC that have the appearance of a multiform (white arrow) and a horizontal cell (black arrow). Orientation in (*A*) applies to (*A*–*C*), orientation in (*D*) applies to (*D*, *E*). Scale bars: 1 mm (*A*, *D*), 100 μm (*B*), and 50 μm (*C*, *E*). Other abbreviations: c, caudal; d, dorsal; ec, external capsule; m, medial; V1, primary visual cortex.

Changes in an animal's behavior measured in relearning sessions some time after initial learning may reflect effects on many processes, including performance as well as acquisition, consolidation, retention, and retrieval of memory (Abel and Lattal 2001; Cahill et al. 2001; Wang et al. 2006b). To control for potentially confounding effects of SKF-38393 on mechanisms that may interact with the learning performance, general parameters, such as exploratory activities, intertrial crossings, avoidance latencies, and escape latencies, were monitored during the behavioral experiments. In the present study, neither of these parameters was significantly affected in SKF-38393–treated gerbils when compared with vehicle-treated controls (Fig. 3 and Supplementary Material), suggesting that SKF-38393 did not cause alterations in the states of arousal and activity or in the sensitivities of sensory and motor systems.

Effects of preconditioning systemic injection of naïve gerbils with SKF-38393 and effects of presession systemic injection of well-trained gerbils with SCH-23390 point to a functional importance of D1-like dopamine receptor activity for both learning and performance of the FM discrimination (Fig. 1). However, presession systemic injection of already repeatedly trained gerbils with SKF-38393 was ineffective, irrespective of the actual discriminative performance level of the animals. Therefore, the facilitating action of the dopamine agonist cannot be explained by a general effect on the abilities to acquire, retain, and retrieve the information and on mechanisms required for behavioral expression of the discrimination. Rather, these findings imply that the enhancing action of SKF-38393 is confined to neural processes that result from some initial experience with the learning situation. This is in accordance with microdialysis studies which suggest that strong dopaminergic signaling in the gerbil auditory cortex is involved at a specific stage of auditory learning in a shuttle box, namely, when after the first tone-shock associations successful avoidances systematically increase (Stark and Scheich 1997).

Postconditioning local infusions of SKF-38393 into the gerbil auditory cortex revealed that drug-dependent improvements of acquisition of the FM discrimination reaction during the first training session are not required for the facilitating effect of the dopamine agonist on discriminative performance in the second session (Fig. 2). Gerbils trained for the first time 1 day after local injections of SKF-38393 performed normally during session 1 but showed a discrimination increment in session 2, 2 days after injections, when compared with session 1 and with control-injected gerbils (Fig. 3). Analysis of blocks of trials indicated that this increment was already evident at the beginning of session 2 (Fig. 5). Therefore, effects of SKF-38393 on processes that support the acquisition of the discrimination and the formation of a short-term memory within session 1 as well as within session 2 are unlikely to explain the discrimination increment. Instead, SKF-38393 applied to the gerbil auditory cortex shortly after—or even 1 day before—the first session of differential conditioning to FMs may act on the consolidation of newly acquired memory during the postacquisition intersession interval. In session 3, control-injected gerbils reached performance levels comparable to those of SKF-38393–treated gerbils (Figs 2 and 3), suggesting that locally applied SKF-38393 might improve the efficiency rather than the overall capacity of FM discrimination learning. Together, these findings may be explained by postulating that, in the FM discrimination paradigm, the D1-like dopamine receptor agonist SKF-38393 applied to the gerbil auditory cortex induces a signaling trace that can persist for hours or even days and accelerates consolidation-relevant processes, that is, processes required for a progressive stabilization of newly acquired memory and/or its subsequent retrieval.

When injected in combination with SKF-38393 into the auditory cortex of naïve gerbils a day before differential conditioning to FMs, the mTOR inhibitor rapamycin or the global protein synthesis inhibitor anisomycin prevented the facilitating effect on FM discrimination memory that was monitored when SKF-38393 was applied alone (Fig. 4*A*,*C*). Administration of rapamycin or anisomycin alone to the auditory cortex of naïve gerbils does not impair FM discrimination learning on subsequent days (Kraus et al. 2002; Tischmeyer et al. 2003). Therefore, the above findings suggest that D1-like dopamine receptor activation induces mTOR-mediated and protein synthesis-dependent signaling traces which are relevant for this memory consolidation. Intracellular pathways that link cortical D1-like dopamine receptors with mTOR are currently not known. Recent evidence that the extracellular signal-regulated kinase (ERK) might play a role in the synaptically induced activation of mTOR (Kelleher et al. 2004; Jaworski and Sheng 2006) may be of particular interest, because this kinase, which has been implicated in LTP and learning, can be regulated by dopaminergic and glutamatergic signals (Brami-Cherrier et al. 2002; Papadeas et al. 2004; Lenz and Avruch 2005; Banko et al. 2006; Nagai et al. 2007). In the rat auditory cortex, dopamine is involved in the modulation of glutamate release (Atzori et al. 2005), and in the gerbil, NMDA-type glutamate receptor activity is required for auditory cortex-dependent memory (Schicknick and Tischmeyer 2006). Therefore, regulation of ERK/mTOR signaling by interactions of the dopaminergic and glutamatergic systems at pre- and/or post-synaptic sites might play a role in long-term FM discrimination memory in the gerbil auditory cortex.

The putative mTOR-mediated, protein synthesis-dependent trace induced when SKF-38393 was locally applied a day before conditioning did not supersede the need for other anisomycin-sensitive protein synthesis in the gerbil auditory cortex during postacquisition memory consolidation in the FM discrimination paradigm (Fig. 4*D*). However, the SKF-38393–induced discrimination increment was sensitive to the mTOR inhibitor rapamycin given after the first session only when the dopamine agonist was also applied shortly after the first session (Fig. 2) but not when applied 1 day before the first session (Fig. 4*B*). This suggests that mTOR activity controls the generation of the SKF-38393–induced signal but not its action on memory consolidation. Among the cellular functions of mTOR is the translational control of distinct classes of mRNAs, including transcripts that encode constituents and regulators of the translational machinery (Kelleher et al. 2004; Wullschleger et al. 2006). Therefore, activation of D1-like dopamine receptors in the gerbil auditory cortex might induce an increase in the translational capacity of relevant local nodes (e.g., neurons and/or neuronal compartments), which, in turn, might enhance the availability of plasticity-related proteins during current and future processes of long-term memory consolidation. This could be accomplished by a widespread synthesis of plasticity-related proteins, which can be used at synapses that have been "tagged" by appropriate stimulation (Frey and Morris 1997; Barco et al. 2002; Sajikumar and Frey 2004; Govindarajan et al. 2006). Alternatively, the distribution of translational capacity could enable a potentially large

number of synapses to synthesize plasticity-related proteins rapidly, locally, and on demand (Krichevsky and Kosik 2001; Blitzer et al. 2005). In hippocampal neurons, for example, an interaction of ERK and mTOR in increasing the translational capacity has recently been demonstrated after LTP-inducing stimulation and activation of neuromodulatory transmitter receptors (Gelinas et al. 2007; Tsokas et al. 2007), and a dopaminergic stimulation of rapamycin-sensitive local protein synthesis has been implicated in the activation of previously silent synapses (Smith et al. 2005). However, differential pathways may control the translational machinery in hippocampal and cortical neurons (Sutton and Schuman 2006), and the molecular mechanisms of FM discrimination memory and protein synthesis in the auditory cortex remain to be determined.

The total numbers of hurdle crossings performed in response to FM tones were very similar in SKF-38393–treated gerbils and controls (Fig. 3). This suggests that the differences in *D* neither reflect the probability of response initiation nor the learning of the hurdle crossings in response to the tones per se. Similarly, learning and performance of the hurdle crossings in order to escape from the foot-shock—as measured by the escape latencies—were not affected. Therefore, the initial tone-shock associations that enable the animals to decrease the response latency to the shock were not affected. These findings suggest that, in this paradigm, SKF-38393 locally applied to the auditory cortex preferentially improves learning of aspects of the conditioned stimuli that are related to the differences in the directions of the frequency modulations, that is, aspects required for discriminating the conditioned stimuli and/or for associating them with their meanings. In gerbils, processing and/or storage of information about those aspects of FMs were previously shown to critically depend on the auditory cortex, whereas learning the discrimination of pure tone pitches in a shuttle box and, consequently, learning the hurdle crossing in response to tones and to foot-shocks, remained unaffected after ablation of the auditory cortex (Ohl et al. 1999).

Both SKF-38393 and SCH-23390 bind with low affinity to 5-HT2 subtypes of serotonin receptors (Briggs et al. 1991; Woodward et al. 1992), and some of the oral motor effects of intrastriatal SKF-38393 in rats are mediated by 5-HT2 receptors (Plech et al. 1995). Therefore, it is not excluded that serotonin receptors mediate the effect of SKF-38393 on FM discrimination learning. However, the D1-like dopamine receptor antagonist SCH-23390 dose-dependently blocked the actions of SKF-38393, whereas the 5-HT2 receptor agonist m-CPP did not show a similar effect (Table 1), suggesting that the memory-enhancing properties of SKF-38393 are indeed mediated by cortical D1-like dopamine receptors. The effect of other dopamine agonists, including D1-like agonists supposed to discriminate between receptors coupled to distinct intracellular signaling pathways, will be subject of future studies.

The cerebral cortex is innervated by dopaminergic terminals that emanate from cell groups in the ventral tegmental area of the midbrain (Tzschentke 2001; Gu 2002). In monkeys and ferrets, sparsely innervated cortical regions, such as the primary auditory cortex, have the highest density of tyrosine hydroxylase-labeled fibers in infragranular layers V and VI (Lewis et al. 1987; Harper and Wallace 1995). Accordingly, in the rat cerebral cortex, layers V and VI contain more D1-like dopamine receptors than the superficial layers (Tzschentke 2001; Gu 2002). Projections of cortical layers V and VI are regarded as modulators of thalamic relay activity, which may underlie attentional regulation for the selection of relevant sensory signals and suppression of distractors (Guillery and Sherman 2002; Cudeiro and Sillito 2006; Zikopoulos and Barbas 2006). In the present study, D1 receptor immunoreactivity was mainly localized at the perikarya of layer V pyramidal cells and in nonpyramidal neurons of layer VI in the gerbil auditory cortex. Considering the supposed corticofugal functions of the infragranular projection pathways, the D1-containing neurons might play a key role in the modulation of FM discrimination behavior.

To summarize, based on our previous studies on the FM discrimination paradigm we have hypothesized that increased cortical dopamine responses and mTOR-dependent translation during and shortly after conditioning might facilitate memory formation in rapidly learning gerbils. In support of this hypothesis, the present findings indicate that pharmacological activation of cortical D1-like dopamine receptors shortly after conditioning of gerbils to FMs causes mTOR-mediated accelerations of memory consolidation. This suggests that—when applied to a group of gerbils with heterogeneous learning abilities—the dopamine agonist facilitates memory formation in individuals that were otherwise learning rather slowly. This, in turn, could be accomplished by mTOR-mediated, translation-dependent changes in the cortex that resemble some of the changes supposed to occur in rapid learners due to increased cortical dopamine responses during learning. Moreover, the efficacy of the dopamine agonist even when applied well in advance of discrimination learning indicates that the relevance of the translation-dependent change for memory consolidation lasts for hours or days. Therefore, increased cortical dopamine responses, previously shown to occur in rapidly learning gerbils during and shortly after conditioning to FMs, might induce mTOR-dependent translational changes that prepare relevant local nodes for the actual demands of memory consolidation and, potentially, also for demands in the near future.

In conclusion, the present results suggest that D1-like dopaminergic neurotransmission in the gerbil auditory cortex is involved in the control of mTOR-mediated, protein synthesis-dependent mechanisms which support processes of memory consolidation in the FM discrimination paradigm for hours or even days. Thus, one of the functions of cortical dopamine activity appears to be paving the way for the formation of long-term memory required for the discrimination of complex sensory stimuli.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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Notes

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Address correspondence to Wolfgang Tischmeyer, Leibniz Institute for Neurobiology, Brenneckestr. 6, D-39118 Magdeburg, Germany. Email: tischmeyer@ifn-magdeburg.de.

References

Abel T, Lattal KM. 2001. Molecular mechanisms of memory acquisition, consolidation and retrieval. Curr Opin Neurobiol. 11: 180–187.

Aitkin L. 1990. The auditory cortex. London: Chapman and Hall.

Atzori M, Kanold PO, Pineda JC, Flores-Hernandez J, Paz RD. 2005. Dopamine prevents muscarinic-induced decrease of glutamate release in the auditory cortex. Neuroscience. 134:1153–1165.

Banko JL, Hou L, Poulin F, Sonenberg N, Klann E. 2006. Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. J Neurosci. 26:2167–2173.

Bao S, Chan VT, Merzenich MM. 2001. Cortical remodelling induced by activity of ventral tegmental dopamine neurons. Nature. 412: 79–83.

Barco A, Alarcon JM, Kandel ER. 2002. Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. Cell. 108:689–703.

Bekinschtein P, Katche C, Slipczuk LN, Igaz LM, Cammarota M, Izquierdo I, Medina JH. 2007. mTOR signaling in the hippocampus is necessary for memory formation. Neurobiol Learn Mem. 87:303–307.

Bernabeu R, Bevilaqua L, Ardenghi P, Bromberg E, Schmitz P, Bianchin M, Izquierdo I, Medina JH. 1997. Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. Proc Natl Acad Sci USA. 94:7041–7046.

Blitzer RD, Iyengar R, Landau EM. 2005. Postsynaptic signaling networks: cellular cogwheels underlying long-term plasticity. Biol Psychiatry. 57:113–119.

Brami-Cherrier K, Valjent E, Garcia M, Pages C, Hipskind RA, Caboche J. 2002. Dopamine induces a PI3-kinase-independent activation of Akt in striatal neurons: a new route to cAMP response element-binding protein phosphorylation. J Neurosci. 22:8911–8921.

Briggs CA, Pollock NJ, Frail DE, Paxson CL, Rakowski RF, Kang CH, Kebabian JW. 1991. Activation of the 5-HT1C receptor expressed in Xenopus oocytes by the benzazepines SCH 23390 and SKF 38393. Br J Pharmacol. 104:1038–1044.

Budinger E, Heil P, Scheich H. 2000. Functional organization of auditory cortex in the Mongolian gerbil (Meriones unguiculatus). III. Anatomical subdivisions and corticocortical connections. Eur J Neurosci. 12:2425–2451.

Cahill L, McGaugh JL, Weinberger NM. 2001. The neurobiology of learning and memory: some reminders to remember. Trends Neurosci. 24:578–581.

Cudeiro J, Sillito AM. 2006. Looking back: corticothalamic feedback and early visual processing. Trends Neurosci. 29:298–306.

Dalley JW, Laane K, Theobald DE, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW. 2005. Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. Proc Natl Acad Sci USA. 102:6189–6194.

Dash PK, Orsi SA, Moore AN. 2006. Spatial memory formation and memory-enhancing effect of glucose involves activation of the tuberous sclerosis complex—mammalian target of rapamycin pathway. J Neurosci. 26:8048–8056.

Davis HP, Squire LR. 1984. Protein synthesis and memory: a review. Psychol Bull. 96:518–559.

Edeline JM. 1999. Learning-induced physiological plasticity in the thalamo-cortical sensory systems: a critical evaluation of receptive field plasticity, map changes and their potential mechanisms. Prog Neurobiol. 57:165–224.

Ehret G. 1997. The auditory cortex. J Comp Physiol. 181:547–557.

El-Ghundi M, O'Dowd BF, George SR. 2007. Insights into the role of dopamine receptor systems in learning and memory. Rev Neurosci. 18:37–66.

Frankland PW, Bontempi B. 2005. The organization of recent and remote memories. Nat Rev Neurosci. 6:119–130.

Frey U, Huang YY, Kandel ER. 1993. Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science. 260:1661–1664.

Frey U, Matthies H, Reymann KG, Matthies H. 1991. The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region in vitro. Neurosci Lett. 129:111–114.

Frey U, Morris RG. 1997. Synaptic tagging and long-term potentiation. Nature. 385:533–536.

Gelinas JN, Banko JL, Hou L, Sonenberg N, Weeber EJ, Klann E, Nguyen PV. 2007. ERK and mTOR signaling couple beta-adrenergic receptors to translation initiation machinery to gate induction of protein synthesis-dependent long-term potentiation. J Biol Chem. 282:27527–27535.

Govindarajan A, Kelleher RJ, Tonegawa S. 2006. A clustered plasticity model of long-term memory engrams. Nat Rev Neurosci. 7:575–583.

Granado N, Ortiz O, Suarez LM, Martin ED, Cena V, Solis JM, Moratalla R. 2008. D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-induced arc and zif268 expression in the hippocampus. Cereb Cortex. 18:1–12.

Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ, Robbins TW. 2000. Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. J Neurosci. 20:1208–1215.

Greba Q, Kokkinidis L. 2000. Peripheral and intraamygdalar administration of the dopamine D1 receptor antagonist SCH 23390 blocks fear-potentiated startle but not shock reactivity or the shock sensitization of acoustic startle. Behav Neurosci. 114:262–272.

Gu Q. 2002. Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. Neuroscience. 111:815–835.

Guillery RW, Sherman SM. 2002. Thalamic relay functions and their role in corticocortical communication: generalizations from the visual system. Neuron. 33:163–175.

Harper MS, Wallace MN. 1995. Changes in density of brainstem afferents in ferret primary auditory cortex (AI) during postnatal development. J Anat. 186(Pt 2):373–382.

Huang F, Chotiner JK, Steward O. 2005. The mRNA for elongation factor 1alpha is localized in dendrites and translated in response to treatments that induce long-term depression. J Neurosci. 25:7199–7209.

Huang YY, Kandel ER. 1995. D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. Proc Natl Acad Sci USA. 92:2446–2450.

Huang YY, Simpson E, Kellendonk C, Kandel ER. 2004. Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. Proc Natl Acad Sci USA. 101:3236–3241.

Izquierdo I, Bevilaqua LR, Rossato JI, Bonini JS, Medina JH, Cammarota M. 2006. Different molecular cascades in different sites of the brain control memory consolidation. Trends Neurosci. 29:496–505.

Jaworski J, Sheng M. 2006. The growing role of mTOR in neuronal development and plasticity. Mol Neurobiol. 34:205–219.

Jay TM. 2003. Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. Prog Neurobiol. 69:375–390.

Karpova A, Mikhaylova M, Thomas U, Knopfel T, Behnisch T. 2006. Involvement of protein synthesis and degradation in long-term potentiation of Schaffer collateral CA1 synapses. J Neurosci. 26:4949–4955.

Kelleher RJ, 3rd, Govindarajan A, Tonegawa S. 2004. Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron. 44:59–73.

Kindler S, Wang H, Richter D, Tiedge H. 2005. RNA transport and local control of translation. Annu Rev Cell Dev Biol. 21:223–245.

Kraus M, Schicknick H, Wetzel W, Ohl F, Staak S, Tischmeyer W. 2002. Memory consolidation for the discrimination of frequency-modulated tones in Mongolian gerbils is sensitive to protein-synthesis inhibitors applied to the auditory cortex. Learn Mem. 9: 293–303.

Krichevsky AM, Kosik KS. 2001. Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. Neuron. 32:683–696.

Kudoh M, Shibuki K. 2006. Sound sequence discrimination learning motivated by reward requires dopaminergic D2 receptor activation in the rat auditory cortex. Learn Mem. 13:690–698.
LaLumiere RT, Nawar EM, McGaugh JL. 2005. Modulation of memory consolidation by the basolateral amygdala or nucleus accumbens shell requires concurrent dopamine receptor activation in both brain regions. Learn Mem. 12:296–301.
Law-Tho D, Desce JM, Crepel F. 1995. Dopamine favours the emergence of long-term depression versus long-term potentiation in slices of rat prefrontal cortex. Neurosci Lett. 188:125–128.
Lemon N, Manahan-Vaughan D. 2006. Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. J Neurosci. 26:7723–7729.
Lenz G, Avruch J. 2005. Glutamatergic regulation of the p70S6 kinase in primary mouse neurons. J Biol Chem. 280:38121–38124.
Lewis DA, Campbell MJ, Foote SL, Goldstein M, Morrison JH. 1987. The distribution of tyrosine hydroxylase-immunoreactive fibers in primate neocortex is widespread but regionally specific. J Neurosci. 7:279–290.
Lisman JE, Grace AA. 2005. The hippocampal-VTA loop: controlling the entry of information into long-term memory. Neuron. 46:703–713.
Matsuda Y, Marzo A, Otani S. 2006. The presence of background dopamine signal converts long-term synaptic depression to potentiation in rat prefrontal cortex. J Neurosci. 26:4803–4810.
Matthies H. 1989. In search of cellular mechanisms of memory. Prog Neurobiol. 32:277–349.
Matthies H, Becker A, Schroeder H, Kraus J, Hollt V, Krug M. 1997. Dopamine D1-deficient mutant mice do not express the late phase of hippocampal long-term potentiation. Neuroreport. 8: 3533–3535.
McGaugh JL. 2000. Memory—a century of consolidation. Science. 287:248–251.
Mele A, Avena M, Roullet P, De Leonibus E, Mandillo S, Sargolini F, Coccurello R, Oliverio A. 2004. Nucleus accumbens dopamine receptors in the consolidation of spatial memory. Behav Pharmacol. 15:423–431.
Mockett BG, Brooks WM, Tate WP, Abraham WC. 2004. Dopamine D1/D5 receptor activation fails to initiate an activity-independent late-phase LTP in rat hippocampus. Brain Res. 1021:92–100.
Morris RG. 2006. Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. Eur J Neurosci. 23:2829–2846.
Nagai T, Takuma K, Kamei H, Ito Y, Nakamichi N, Ibi D, Nakanishi Y, Murai M, Mizoguchi H, Nabeshima T, et al. 2007. Dopamine D1 receptors regulate protein synthesis-dependent long-term recognition memory via extracellular signal-regulated kinase 1/2 in the prefrontal cortex. Learn Mem. 14:117–125.
Navakkode S, Sajikumar S, Frey JU. 2007. Synergistic requirements for the induction of dopaminergic D1/D5-receptor-mediated LTP in hippocampal slices of rat CA1 in vitro. Neuropharmacology. 52:1547–1554.
O'Carroll CM, Martin SJ, Sandin J, Frenguelli B, Morris RG. 2006. Dopaminergic modulation of the persistence of one-trial hippocampus-dependent memory. Learn Mem. 13:760–769.
O'Tuathaigh CM, Moran PM. 2002. Evidence for dopamine D(1) receptor involvement in the stimulus selection task: overshadowing in the rat. Psychopharmacology (Berl). 162:225–231.
Ohl FW, Scheich H. 2005. Learning-induced plasticity in animal and human auditory cortex. Curr Opin Neurobiol. 15:470–477.
Ohl FW, Scheich H, Freeman WJ. 2001. Change in pattern of ongoing cortical activity with auditory category learning. Nature. 412:733–736.
Ohl FW, Wetzel W, Wagner T, Rech A, Scheich H. 1999. Bilateral ablation of auditory cortex in Mongolian gerbil affects discrimination of frequency modulated tones but not of pure tones. Learn Mem. 6:347–362.
Otani S, Daniel H, Roisin MP, Crepel F. 2003. Dopaminergic modulation of long-term synaptic plasticity in rat prefrontal neurons. Cereb Cortex. 13:1251–1256.
Otmakhova NA, Lisman JE. 1996. D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. J Neurosci. 16:7478–7486.
Papadeas ST, Blake BL, Knapp DJ, Breese GR. 2004. Sustained extracellular signal-regulated kinase 1/2 phosphorylation in neonate 6-hydroxydopamine-lesioned rats after repeated D1-dopamine receptor agonist administration: implications for NMDA receptor involvement. J Neurosci. 24:5863–5876.
Parsons RG, Gafford GM, Helmstetter FJ. 2006. Translational control via the mammalian target of rapamycin pathway is critical for the formation and stability of long-term fear memory in amygdala neurons. J Neurosci. 26:12977–12983.
Paxinos G. 2004. The rat nervous system. San Diego: Elsevier Academic Press.
Plech A, Brus R, Kalbfleisch JH, Kostrzewa RM. 1995. Enhanced oral activity responses to intrastriatal SKF 38393 and m-CPP are attenuated by intrastriatal mianserin in neonatal 6-OHDA-lesioned rats. Psychopharmacology (Berl). 119:466–473.
Raught B, Gingras AC, Sonenberg N. 2001. The target of rapamycin (TOR) proteins. Proc Natl Acad Sci USA. 98:7037–7044.
Sajikumar S, Frey JU. 2004. Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. Neurobiol Learn Mem. 82:12–25.
Scheich H. 1991. Auditory cortex: comparative aspects of maps and plasticity. Curr Opin Neurobiol. 1:236–247.
Scheich H, Brechmann A, Brosch M, Budinger E, Ohl FW. 2007. The cognitive auditory cortex: task-specificity of stimulus representations. Hear Res. 229:213–224.
Schicknick H, Tischmeyer W. 2006. Consolidation of auditory cortex-dependent memory requires N-methyl-D-aspartate receptor activation. Neuropharmacology. 50:671–676.
Seamans JK, Floresco SB, Phillips AG. 1998. D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. J Neurosci. 18:1613–1621.
Smith WB, Starck SR, Roberts RW, Schuman EM. 2005. Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. Neuron. 45:765–779.
Stark H, Rothe T, Wagner T, Scheich H. 2004. Learning a new behavioral strategy in the shuttle-box increases prefrontal dopamine. Neuroscience. 126:21–29.
Stark H, Scheich H. 1997. Dopaminergic and serotonergic neurotransmission systems are differentially involved in auditory cortex learning: a long-term microdialysis study of metabolites. J Neurochem. 68:691–697.
Stork O, Welzl H. 1999. Memory formation and the regulation of gene expression. Cell Mol Life Sci. 55:575–592.
Sutton MA, Schuman EM. 2006. Dendritic protein synthesis, synaptic plasticity, and memory. Cell. 127:49–58.
Swanson-Park JL, Coussens CM, Mason-Parker SE, Raymond CR, Hargreaves EL, Dragunow M, Cohen AS, Abraham WC. 1999. A double dissociation within the hippocampus of dopamine D1/D5 receptor and beta-adrenergic receptor contributions to the persistence of long-term potentiation. Neuroscience. 92: 485–497.
Tang SJ, Reis G, Kang H, Gingras AC, Sonenberg N, Schuman EM. 2002. A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. Proc Natl Acad Sci USA. 99:467–472.
Tischmeyer W, Schicknick H, Kraus M, Seidenbecher CI, Staak S, Scheich H, Gundelfinger ED. 2003. Rapamycin-sensitive signalling in long-term consolidation of auditory cortex-dependent memory. Eur J Neurosci. 18:942–950.
Tsokas P, Ma T, Iyengar R, Landau EM, Blitzer RD. 2007. Mitogen-activated protein kinase upregulates the dendritic translation machinery in long-term potentiation by controlling the mammalian target of rapamycin pathway. J Neurosci. 27:5885–5894.
Tzschentke TM. 2001. Pharmacology and behavioral pharmacology of the mesocortical dopamine system. Prog Neurobiol. 63:241–320.
Wang H, Hu Y, Tsien JZ. 2006a. Molecular and systems mechanisms of memory consolidation and storage. Prog Neurobiol. 79:123–135.

Wang H, Wang X, Wetzel W, Scheich H. 2006b. Rapid-rate transcranial magnetic stimulation of animal auditory cortex impairs short-term but not long-term memory formation. Eur J Neurosci. 23:2176–2184.
Weinberger NM. 2004. Specific long-term memory traces in primary auditory cortex. Nat Rev Neurosci. 5:279–290.
Wise RA. 2004. Dopamine, learning and motivation. Nat Rev Neurosci. 5:483–494.
Wittmann BC, Schott BH, Guderian S, Frey JU, Heinze HJ, Duzel E. 2005. Reward-related FMRI activation of dopaminergic midbrain is associated with enhanced hippocampus-dependent long-term memory formation. Neuron. 45:459–467.
Woodward RM, Panicker MM, Miledi R. 1992. Actions of dopamine and dopaminergic drugs on cloned serotonin receptors expressed in Xenopus oocytes. Proc Natl Acad Sci USA. 89:4708–4712.
Wullschleger S, Loewith R, Hall MN. 2006. TOR signaling in growth and metabolism. Cell. 124:471–484.
Zikopoulos B, Barbas H. 2006. Prefrontal projections to the thalamic reticular nucleus form a unique circuit for attentional mechanisms. J Neurosci. 26:7348–7361.