Research

Chronically increased $G_s\alpha$ signaling disrupts associative and spatial learning

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The cAMP/PKA pathway plays a critical role in learning and memory systems in animals ranging from mice to *Drosophila* to *Aplysia*. Studies of olfactory learning in *Drosophila* suggest that altered expression of either positive or negative regulators of the cAMP/PKA signaling pathway beyond a certain optimum range may be deleterious. Here we provide genetic evidence of the behavioral and physiological effects of increased signaling through the cAMP/PKA pathway in mice. We have generated transgenic mice in which the expression of a constitutively active form of $G_s\alpha$ ($G_s\alpha^*$ Q227L), the G protein that stimulates adenylyl cyclase activity, is driven in neurons within the forebrain by the promoter from the CaMKII α gene. Despite significantly increased adenylyl cyclase activity, $G_s\alpha^*$ transgenic mice exhibit PKA-dependent decreases in levels of cAMP due to a compensatory up-regulation in phosphodiesterase activity. Interestingly, $G_s\alpha^*$ transgenic mice also exhibit enhanced basal synaptic transmission. Consistent with a role for the cAMP/PKA pathway in learning and memory, $G_s\alpha^*$ transgenic mice show impairments in spatial learning in the Morris water maze and in contextual and cued fear conditioning tasks. The learning deficits observed in these transgenic mice suggest that associative and spatial learning requires regulated $G_s\alpha$ protein signaling, much as does olfactory learning in *Drosophila*.

Genetic, pharmacological, and electrophysiological studies in a variety of organisms have underscored the importance of the cAMP/PKA pathway in synaptic plasticity, learning, and memory (Barco et al. 2002; Bourtchouladze 2002; Lonze and Ginty 2002; Tully et al. 2003). Experiments in *Aplysia* and *Drosophila* provided the initial evidence that G-protein-coupled cAMP signaling pathways play an important role in simple forms of learning and memory. At the sensorimotor synapse in *Aplysia*, serotonin stimulates a G-protein-coupled receptor that activates the cAMP/PKA signaling pathway. PKA then acts through the transcription factor cAMP response element binding protein (CREB) to mediate long-lasting changes in the synaptic strength in response to the facilitatory neurotransmitter serotonin (Abel and Kandel 1998). To determine the role of cAMP signaling pathways in synaptic plasticity, learning, and memory in mammals, we have turned to the study of genetically modified mice. The analysis of these mice allows one to test whether a particular gene product is important for behavior and synaptic function and provides a useful bridge between molecules and behavior. Within the hippocampus, the cAMP/PKA signaling pathway plays a crucial role in synaptic plasticity at several synapses (Huang et al. 1996). In the Schaffer collateral pathway, the late phase of long-term potentiation (L-LTP) differs from shorter lasting forms of LTP in requiring PKA activity, protein synthesis, and transcription. Previously, we and others found alterations in synaptic plasticity and memory storage in mice in which the function of CREB has been impaired (Bourtchouladze et al. 1994; Kogan et al. 1997;

Gass et al. 1998; Kogan et al. 2000; Graves et al. 2002; Pittenger et al. 2002; Frankland et al. 2004; Brightwell et al. 2005). Similarly, transgenic mice expressing an inhibitory form of the regulatory subunit of PKA have reduced hippocampal PKA activity and exhibit selective impairments in hippocampus-dependent long-term memory and L-LTP (Abel et al. 1997). Mice lacking both of the calcium-stimulated adenylyl cyclases type 1 and 8 also show deficits in L-LTP and in long-term memory for contextual fear conditioning, a hippocampus-dependent task (Wong et al. 1999).

Understanding the role of the cAMP/PKA pathway in hippocampal function may be especially important for elucidating the physiological and molecular mechanisms of age-related memory loss. Drugs that enhance this pathway by activating D1/D5 receptors or by inhibiting a cAMP phosphodiesterase (PDE) attenuate the L-LTP and memory deficits that accompany aging in rodents (Bach et al. 1999). Pharmacological experiments in mice have explored the effects of amplifying the cAMP pathway using the type IV-specific PDE inhibitor rolipram (Barad et al. 1998; Bourtchouladze et al. 2003; Tully et al. 2003; Alarcon et al. 2004; Gong et al. 2004). Treatment with levels of rolipram that amplified signaling through the cAMP/PKA pathway without altering basal levels of cAMP resulted in persistent LTP in area CA1 with stimuli that normally produce only transient potentiation. Behaviorally, rolipram treatment increased long-term but not short-term memory for contextual fear conditioning and object recognition, tasks sensitive to lesions of the hippocampus (Barad et al. 1998; Bourtchouladze et al. 2003).

Forward and reverse genetic approaches in *Drosophila* have been particularly powerful ways to investigate the role of the cAMP/PKA pathway in learning. Olfactory learning in *Drosophila* requires regulated signaling through the cAMP/PKA pathway because mutations in either adenylyl cyclase (*rutabaga*) or cAMP

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PDE (*dunce*) result in learning deficits (Dubnau and Tully 1998). Thus, decreases or increases in the basal levels of cAMP in *Drosophila* result in learning impairments in olfactory conditioning. Furthermore, the transgenic overexpression of a constitutively active form of $G_s\alpha$, the G protein that stimulates adenylyl cyclase, selectively in the mushroom body of *Drosophila* disrupts associative learning (Connolly et al. 1996). Taken together, results from studies of olfactory learning in *Drosophila* suggest that beyond a certain optimum range, altered expression of either positive or negative regulators of the cAMP signaling pathway may be deleterious.

To test whether this phenomenon applies to mice, we genetically altered the cAMP/PKA signaling pathway by generating transgenic mice that express a constitutively active form of $G_s\alpha$ ($G_s\alpha^*$ Q227L) in neurons within the forebrain. Previously, we demonstrated that $G_s\alpha^*$ mice exhibit significant increases in adenylyl cyclase activity in cortex, hippocampus, and striatum (Wand et al. 2001; Kelly et al. 2006). As predicted from this increase in cyclase activity, $G_s\alpha^*$ transgenic mice exhibit significantly increased cAMP levels in striatum; however in cortex and hippocampus, $G_s\alpha^*$ mice show significantly reduced cAMP levels due to a PKA-dependent compensatory up-regulation in total cAMP PDE activity (Kelly et al. 2006). Here we show that $G_s\alpha^*$ transgenic mice exhibit learning deficits in the spatial version of the water maze as well as impairments in cued and contextual fear conditioning. In contrast, we found significantly enhanced basal synaptic transmission in hippocampal area CA1 slices from transgenic animals. The $G_s\alpha^*$ transgenic mice provide evidence that both associative and spatial learning in mice require regulated $G_s\alpha$ signaling, much as does olfactory learning in *Drosophila*. These mice provide a model system in which to explore the behavioral and physiological effects of persistently enhanced $G_s\alpha$ and adenylyl cyclase signaling.

Results

$G_s\alpha^*$ transgenic mice

To explore the behavioral effects of chronically stimulating the cAMP/PKA system, we have generated transgenic mice that express a constitutively active form of $G_s\alpha$ ($G_s\alpha$ Q227L; $G_s\alpha^*$) (Landis et al. 1989). Expression of this mutant cDNA was driven by an 8.5-kb portion of the CaMKII α promoter, containing upstream control regions and the transcriptional initiation site. The CaMKII α promoter drives expression postnatally in the hippocampus, neocortex, amygdala, and striatum (Mayford et al. 1996). The $G_s\alpha^*$ cDNA (Landis et al. 1989) was flanked by a SV40 polyadenylation signal at the 3' end and by a 5' untranslated leader containing a heterologous intron, elements which have been shown to enhance expression of transgenes (Choi et al. 1991). This hybrid $G_s\alpha^*$ construct was then placed under the control of the CaMKII α promoter (Fig. 1), and transgenic mice were generated by pronuclear injection into fertilized eggs obtained from a cross of C57BL/6/J and CBA/J mice. Six independent founder animals were obtained, three of which bred successfully and transmitted the transgene. These lines, designated $G_s\alpha^*$ -1, $G_s\alpha^*$ -2, and $G_s\alpha^*$ -3 carried 10 copies, two copies, and one copy of the transgene, respectively, as determined by Southern blot analysis (data not shown). Northern blot analysis of forebrain RNA, using a probe specific for the 3' end of the transgene, revealed that a transcript of ~2.4 kb is expressed at highest levels in the $G_s\alpha^*$ -1 transgenic line (data not shown). Based on this high level of transgene expression, our studies have focused on the detailed analysis of this line; however, many of the same trends were observed in the $G_s\alpha^*$ -2 line (data not shown). Comparison of Nissl-stained as well as hematoxylin and eosin-stained sagittal sections of brains from transgenic and wild-type mice revealed

Figure 1. Regional distribution of $G_s\alpha^*$ transgene expression. Sagittal sections from the brains of $G_s\alpha^*$ -1 transgenic mice were hybridized with a probe specific for the transgene. Expression of the transgene is seen throughout the hippocampus and cortex, olfactory bulb, amygdala, and striatum. No expression is seen in wild-type control animals (data not shown). The $G_s\alpha^*$ -1 transgene contains an 8.5-kb CaMKII α promoter, a hybrid intron in the 5' untranslated leader (filled boxes), the coding region of $G_s\alpha^*$, and a polyadenylation signal from SV40.

no gross anatomical abnormalities within the hippocampus or other regions of adult transgenic animals (data not shown).

As previously described (Wand et al. 2001; Gould et al. 2004; Kelly et al. 2006), expression of the $G_s\alpha^*$ transgene is limited to the hippocampus, neocortex, olfactory bulb, striatum, and amygdala (Fig. 1). This mutant form of $G_s\alpha$ has reduced GTPase activity, enhancing its ability to activate adenylyl cyclase (Landis et al. 1989). Interestingly, despite increased adenylyl cyclase activity, $G_s\alpha^*$ transgenic mice show a PKA-dependent decrease in levels of cAMP in hippocampus and cortex (Kelly et al. 2006).

General behavioral observations and neurological tests

To evaluate gross neurological function, we tested $G_s\alpha^*$ -1 transgenic mice and wild-type mice ($n = 7$ in each group) in a subset of tests described by Irwin (1968) and Crawley and Paylor (1997). We observed that $G_s\alpha^*$ -1 transgenic mice have normal postural, righting, eye blink, ear twitch, and whisker orienting reflexes, as well as a normal visual response to light; they did not exhibit abnormal spontaneous behaviors such as running, excessive grooming, freezing, or altered body posture; they did not exhibit obvious ataxia when tested in the hind paw footprint test; and they responded similarly to wild-type mice in the visual cliff test and in response to a novel object. However, $G_s\alpha^*$ -1 transgenic mice did take longer than did wild-type mice to adapt to a handling procedure and appear more nervous and "jumpy" when handled. Overall, no gross neurological impairments are present in the $G_s\alpha^*$ -1 transgenic mice, and thus, we carried out further behavioral testing to examine learning and memory in these mutant mice.

$G_s\alpha^*$ -1 transgenics exhibit deficits in spatial learning

Because $G_s\alpha^*$ -1 transgenic mice exhibited biochemical changes within the hippocampus, we began to explore potential behavioral effects of this transgene on spatial learning. We trained mice on the hidden platform version of the Morris water maze task, a hippocampus-dependent task that depends on the ability of the animal to learn and remember the relationships between multiple distal cues and the platform (Morris et al. 1982). Figure 2 shows that the overall performance of $G_s\alpha^*$ -1 transgenics during

Figure 2. G_{α}^{*-1} transgenic mice exhibit learning deficits in the hidden platform version of the Morris water maze. (A) The average distance traveled to reach the platform is plotted versus trial day. ANOVA with repeated measures showed that the overall performance of the G_{α}^{*-1} mice was significantly worse than that of controls ($F_{(1,12)} = 5.58$; $P = 0.03$; $n = 7$ in each group). (B) The average time (latency) to reach the platform is plotted versus trial day. The overall performance of the G_{α}^{*-1} mice was not significantly different from controls ($F_{(1,12)} = 4.62$; $P = 0.05$). However, there was a significant difference in the time to reach the platform on days 9 and 10 ($P < 0.01$ and $P < 0.005$, respectively). (C) No significant differences were observed in swim speed between the genotypes ($F_{(1,12)} = 0.42$; $P = 0.52$). (D) The percentage of time spent in each quadrant for wild-type and transgenic mice during the probe trial given 1 h after the end of training on day 10 is shown. Wild-type mice searched more selectively than did transgenic mice for the absent platform and spent significantly more time in the training quadrant (TQ; $42.7 \pm 0.8\%$ vs. $27.8 \pm 3.5\%$ for wild-type and transgenic mice, respectively, $P < 0.05$) than in the adjacent quadrant to the right (AR), the adjacent quadrant to the left (AL), or in the quadrant opposite (OQ) the training quadrant. (E) The number of times the platform position (or a similar position in each of the other quadrants) was crossed during the probe trial is shown for wild-type and transgenic mice. Wild-type mice searched more selectively than did transgenic mice for the absent platform and crossed the location of the platform significantly more times in the TQ (4.3 ± 0.9 crosses vs. 1.4 ± 0.5 crosses for wild-type and transgenic mice, respectively; $P < 0.05$) than a similar position in the AR, the AL, or the OQ training quadrants.

training (four trials per day with a 60-sec intertrial interval for 10 d) was significantly worse than that of controls ($P < 0.05$; $n = 7$ in each group). To more directly assess memory for the spatial task, we tested the mice in a probe trial, during which the platform was removed from the pool and the mice were allowed to search for 60 sec. The time spent in each quadrant measures the spatial bias of an animal's search pattern and is thought to reflect ex-

plicit aspects of long-term spatial memory (Schenk and Morris 1985). The mutants spent significantly less time than did wild-type controls in the target quadrant ($42.3\% \pm 5.8\%$ and $27.8\% \pm 3.5\%$ for controls and transgenics, respectively, $P < 0.05$) (Fig. 2) and crossed the exact site of the platform during training fewer times than did wild-type controls (4.3 ± 0.9 vs. 1.4 ± 0.5 crosses for wild-types and transgenics, respectively, $P < 0.02$). No differences were observed in swim speed between the genotypes ($P > 0.05$).

To examine the possibility that this deficit may be due to poor vision, motor coordination, motivation, or procedural learning, we tested naive mice on a visual discrimination task in which mice learned to discriminate between two visible cues: one of which was attached to a real platform; the other, to a false platform. The position of these cues varied from trial to trial, but the same cue was associated with the platform in each trial. The mice were trained in this task with two trials per day for 7 d with a 60-sec intertrial interval. G_{α}^{*-1} mice were not different from control mice in latencies to find the platform in the visual discrimination task ($P > 0.05$; $n = 7$ and $n = 6$, respectively) (Fig. 3).

G_{α}^{*-1} transgenic mice exhibit deficits in fear conditioning

Because the Morris water maze task requires repeated training over several days, this task does not provide the temporal resolution necessary to distinguish between different phases of memory storage. We therefore turned to fear conditioning, a task in which robust learning can be triggered by a single trial, to determine whether the G_{α}^{*-1} transgenics exhibit short-term or long-term memory deficits. In fear conditioning, animals learn to fear a new environment or an emotionally neutral conditioned stimulus (CS), such as a tone, because of its temporal association with an aversive unconditioned stimulus (US), usually footshock. When exposed to the same context or the same CS, conditioned animals show freezing behavior. In addition to allowing good temporal resolution, these two forms of fear conditioning are thought to recruit distinct neuroanatomical substrates: Contextual conditioning is sensitive to lesions of

both the hippocampus and the amygdala, whereas cued conditioning is disrupted by lesions of the amygdala (for review, see Holland and Bouton 1999).

Mice were trained with a single exposure to a novel environment and a discrete CS (tone) paired with a US as described previously (Bourtchouladze et al. 1994). To assess memory for contextual conditioning, we tested different groups of mice in

Figure 3. G_{α^*1} transgenic mice exhibit normal performance in the visual discrimination task in the water maze. Time to reach the platform (latency) is plotted versus trial day. Overall performance of the G_{α^*1} transgenic mice ($n = 7$) was not significantly different ($F_{(1,11)} = 0.008$, $P = 0.98$) from that of wild-type mice ($n = 6$).

the same context 30 min or 24 h after training with a 0.7-mA footshock. Immediately after training, G_{α^*1} transgenic mice and wild-type mice exhibited similar levels of freezing ($25.1 \pm 3.1\%$ and $24.1 \pm 3.3\%$ for control and G_{α^*1} transgenic mice, respectively; $P = 0.82$) (Fig. 4). However, G_{α^*1} transgenic mice showed significantly reduced levels of context-evoked freezing relative to wild-type control mice 30 min after training ($30.1 \pm 3.3\%$ and $9.9 \pm 1.5\%$ for control and G_{α^*1} transgenic mice, respectively; $P < 0.001$; $n = 11$ in each group) and 24 h after training ($39.0 \pm 4.8\%$ and $20.1 \pm 7.4\%$ for control and G_{α^*1} transgenic mice, $n = 18$ and $n = 11$, respectively; $P = 0.03$).

To assess memory for cued conditioning, we tested the mice by presenting the cue (tone) in a novel context at 30 min or at 24 h after training with a 0.7-mA or 1.5-mA footshock. G_{α^*1} transgenic mice showed significantly reduced levels of cue-evoked freezing relative to controls 30 min after training with a 0.7-mA footshock ($34.7 \pm 4.3\%$ and $20.1 \pm 3.0\%$ for control and G_{α^*1} transgenic mice, respectively; $n = 8$ in each group; $P < 0.01$), but only marginally reduced freezing at 24 h ($43.7 \pm 3.8\%$ and $31.3 \pm 7.7\%$ for control and G_{α^*1} transgenic mice, $n = 18$ and $n = 12$, respectively; $P = 0.12$). In contrast, when trained with a higher intensity footshock (1.5 mA), G_{α^*1} transgenic mice showed drastically reduced cue-evoked freezing relative to control mice at 24 h ($70.9 \pm 4.3\%$ and $24.2 \pm 4.2\%$ for control and G_{α^*1} transgenic mice, $n = 13$ and $n = 11$, respectively; $P < 0.0001$). To determine if G_{α^*} expression affects shock sensitivity, we measured the minimal amount of current required to elicit three stereotypical behaviors: flinching/running, jumping, and vocalizing. We found that each of these behaviors was elicited with similar current intensities in G_{α^*1} transgenic and control mice ($P > 0.05$) (data not shown). Taken together, our results suggest that enhanced G_{α} signaling results in memory impairments in contextual and cued fear conditioning without altering perception of shock or initial performance.

Basal synaptic transmission is enhanced in G_{α^*1} transgenic mice

As an initial step in the electrophysiological analysis of G_{α^*1} transgenic mice, we examined basal synaptic function at the Schaffer collateral-CA1 synapses in hippocampal area CA1. Slices obtained from wild-type and mutant animals showed a marked difference in their input-output coupling during extracellular stimulation of Schaffer collaterals (Fig. 5A). Across the input/output curve, G_{α^*1} transgenic mice exhibit enhanced basal synaptic transmission relative to wild-type mice ($P < 0.001$; Two-way ANOVA with repeated measures). We next examined paired-

pulse facilitation (PPF), a form of short-term plasticity in which the size of the postsynaptic response to the second of two closely spaced stimuli is increased, probably as a result of increased transmitter release evoked by additional calcium remaining from the first stimulus. PPF was not altered in the transgenic mice relative to wild-type controls ($P > 0.05$) (Fig. 5B).

LTP induced by one train of stimulation is normal in G_{α^*1} transgenic mice, but LTP induced by four trains of stimulation is not

We next examined a relatively short-term form of LTP (early or E-LTP) evoked by applying one 50-Hz train of stimulation to the Schaffer collateral pathway. With a single train, there were no significant differences between the mean extracellular field EPSP (fEPSP) slopes measured from wild-type and transgenic mice immediately or 30 min after tetanus ($P > 0.05$) (Fig. 6A). Thus, LTP

Figure 4. G_{α^*1} transgenic mice exhibit deficits in contextual (A) and cued (B) fear conditioning. (A) G_{α^*1} transgenic mice show deficits in short-term as well as long-term memory for contextual conditioning following one CS/US pairing. Mice were tested for contextual conditioning 30 min after training with a 0.7-mA footshock. Freezing responses of the G_{α^*1} mice were significantly less than those of wild-type mice ($P < 0.001$; $n = 11$ in each group). Mice were tested for contextual conditioning 24 h after training. The freezing responses of the G_{α^*1} mice were again significantly less from those of wild-type mice ($n = 18$ and $n = 11$ for wild-type and transgenic mice, respectively; $P = 0.03$). No significant difference between mutants and controls in freezing responses was observed immediately after the US ($n = 55$ and $n = 42$ for wild-type and transgenic mice, respectively, pooled training data from all 0.7 mA groups; $P = 0.82$). (B) G_{α^*1} transgenic mice exhibit deficits in cued fear conditioning. Mice were tested for cued conditioning 30 min after training with a 0.7-mA footshock. The G_{α^*1} transgenic mice showed significantly less freezing responses than did wild-type mice ($n = 8$ in each group; $P < 0.01$). When tested 24 h after training with a 0.7-mA footshock, there was not a significant difference between the freezing responses of the G_{α^*1} transgenic mice compared with wild-type mice ($n = 18$ and $n = 12$ for wild-type and transgenic mice, respectively; $P = 0.12$); however, after training with a 1.5-mA footshock, G_{α^*1} transgenic mice showed drastically reduced freezing to the cue relative to control mice ($n = 13$ and $n = 11$ for wild-type and transgenic mice, respectively; $P < 0.0001$).

Figure 5. G_sα*-1 transgenic mice exhibit enhanced basal synaptic transmission in hippocampal area CA1. (A) Basal synaptic transmission. Plot of fEPSP slope (mV/msec) at various stimulation intensities for hippocampal slices from wild-type and transgenic mice shows a marked increase in basal synaptic transmission in area CA1 in the G_sα*-1 transgenic mice ($P < 0.001$; two-way ANOVA with repeated measures; wild-type mice, $n = 45$ slices, 22 mice; G_sα*-1 transgenic mice, $n = 48$ slices, 26 mice). Post hoc analysis demonstrated significant differences between genotypes at stimulus voltages >15 V (Student-Newman-Keuls test, $P < 0.05$). (B) Paired-pulse facilitation. Percentage of facilitation, calculated from the ratio of the second fEPSP slope to the first fEPSP slope, is shown at interpulse intervals ranging from 25–200 msec (wild-type mice, $n = 18$ slices, 7 mice; G_sα*-1 transgenic mice, $n = 14$ slices, 6 mice). No significant differences in paired-pulse facilitation were observed ($P > 0.05$).

induced by one train of high-frequency stimulation was not affected by overexpression of a constitutively active form of G_sα. In contrast, a long-lasting form of LTP (late or L-LTP) evoked by four 50-Hz trains spaced by 5-min intervals showed a small, but significant elevation over time in slices from mutant mice ($P < 0.001$; two-way ANOVA with repeated measures) (Fig. 6B).

Discussion

We have examined the behavioral and electrophysiological effects of expressing a constitutively active form of G_sα, the G protein that stimulates adenylyl cyclase activity, in forebrain neurons of mice. Consistent with the previously observed decreases in levels of cAMP in hippocampus and cortex (Kelly et al. 2006), G_sα*-1 transgenic mice exhibit deficits in spatial learning as well as contextual and cued fear conditioning. These findings are consistent with previous studies showing that genetic and pharmacological blockade of the cAMP/PKA signaling pathway impairs learning and memory in mice (Bourtchouladze et al. 1994, 1998; Abel et al. 1997; Wong et al. 1999; Kida et al. 2002; Pittenger et al. 2002; Josselyn and Nguyen 2005). In contrast, electrophysiological studies demonstrated that these mutants showed enhanced basal synaptic transmission, and modest enhancements in L-LTP at the Schaffer collateral-CA1 pathway within the hippocampus. These results suggest that regulated and optimal signaling through G_sα-coupled pathways is required for proper neuronal function in the mammalian brain.

Behaviorally, G_sα*-1 transgenic mice exhibit impairments in spatial learning and memory relative to wild-type littermates. G_sα*-1 transgenic mice demonstrated normal performance relative to wild-types on the visual platform water maze (Fig. 2), suggesting that motor function, motivation, and procedural learning remain intact in these transgenic mice. Further support for the observation that a spatial learning deficit is caused by the constitutive activation of G_sα comes from the observations that G_sα*-1 transgenic mice exhibited deficits in the spatial version of the Barnes circular platform maze ($n = 6$ in each group, $P < 0.05$) (data not shown). Finally, mice from another transgenic line, G_sα*-2, also exhibited deficits in the hidden platform version of the water maze (R. Bourtchouladze and T. Abel, unpubl.). Taken together, the present results strongly suggest that constitutive activation of G_sα leads to spatial learning and memory deficits.

In addition to a spatial learning and memory deficit, our fear conditioning experiments suggest that chronically enhancing G_sα activity results in impairments in fear-based associative memory. G_sα*-1 transgenic mice exhibited lower levels of freezing to the conditioning context 30 min and 24 h following contextual fear conditioning (Fig. 4A). Relative to wild-type mice, G_sα*-1 transgenic mice also exhibited lower levels of cued freezing 30 min and 24 h following conditioning (Fig. 4B). Given that G_sα*-1 transgenic mice demonstrated an intact ability to freeze, as indicated by normal performance immediately following the shock, their lower levels of freezing during retrieval likely reflect impairments in short-term and long-term associative memory.

The biochemical and learning deficits noted in our G_sα*-1

Figure 6. Properties of hippocampal LTP in G_sα*-1 transgenic mice. Mean fEPSP slopes are plotted as a percentage of pretetanus baseline values. (A) G_sα*-1 transgenic mice exhibit normal E-LTP in response to one 50-Hz, 0.5-sec train in hippocampal area CA1. Synaptic facilitation induced by a single train of stimulation (50 Hz, 0.5 sec) was similar in slices from wild-type mice ($n = 12$ slices, 7 mice) and in slices from G_sα*-1 transgenic mice ($n = 11$ slices, 7 mice; $P > 0.05$). (B) Slices from G_sα*-1 transgenic mice showed a slight enhancement over time in L-LTP produced by a protocol of four 50-Hz, 0.5-sec trains separated by 5-min intervals ($P < 0.0001$; Two-way ANOVA with repeated measures, at 15-min intervals; wild-type mice, $n = 15$ slices, 11 mice; G_sα*-1 transgenic mice, $n = 16$ slices, 13 mice).

transgenic mice are consistent with previous studies showing that regulated signaling through the cAMP/PKA pathway is critical for learning and memory formation. Pharmacological experiments have shown that injections of PDE 4 inhibitors, which increase cAMP signaling, facilitated the establishment of long-term memory in rodents (Barad et al. 1998; Bourtchouladze et al. 2003; Tully et al. 2003; Gong et al. 2004). Conversely, studies of transgenic mice expressing an inhibitory form of the regulatory subunit of PKA revealed that the down-regulation of the cAMP/PKA pathway impaired spatial and long-term contextual memory in mice (Abel et al. 1997; Bourtchouladze et al. 1998). Currently, it is difficult to speculate if the observed short-term memory deficit for contextual and cued fear memory in G_{α}^* -1 transgenic mice is a direct consequence of decreased cAMP levels. In our previous studies of transgenic mice expressing an inhibitory form of the regulatory subunit of PKA, we only observed long-term (but not short-term) deficit for hippocampus-dependent contextual, but not amygdala-dependent cued memory. Importantly, in agreement with our findings, mice lacking both of the calcium-stimulated adenylyl cyclases type 1 and 8 also showed deficits in short-term memory (5 and 30 min after training) for passive avoidance task, a fear-based task that requires function of intact hippocampus and amygdala (Wong et al. 1999). Studies of *rutabaga* and *dunce* mutant flies have shown that a loss of adenylyl cyclase or PDE, respectively, both result in impairments in associative learning and short-term memory (Dubnau and Tully 1998). In addition, in experiments using a constitutively active form of G_{α} similar to that used in these studies, Connolly et al. (1996) found that constitutive activation of adenylyl cyclase specifically in the mushroom body resulted in impairments in associative olfactory learning. These effects of G_{α} activation on learning in mice and flies may result from alterations in cAMP/PKA signaling, such as those noted above, or the direct action of G_{α} on ion channels or other molecular targets (Wolfgang et al. 1996; Lader et al. 1998). Whatever the exact molecular mechanism through which G_{α}^* alters neuronal function, our results support the hypothesis that regulated signaling through the cAMP/PKA pathway is necessary for normal learning and memory.

In contrast to our behavioral results, our electrophysiological studies in hippocampal area CA1 suggest that the constitutive activation of G_{α} signaling enhanced synaptic transmission. In the experiments described here, we observed an increase in basal synaptic transmission, normal short-term synaptic plasticity (E-LTP), and a small but significant increase in L-LTP over time. These findings appear somewhat inconsistent with the fact that cAMP levels are significantly lower in G_{α}^* -1 transgenic mice in the hippocampus and cortex (Kelly et al. 2006). It should be noted, however, that our mutant mice shows a similar constellation of phenotypes (increased adenylyl cyclase activity and memory deficits) with mutant mice lacking the $G_{\alpha}1$ gene (Pineda et al. 2004). The difference between the behavioral phenotype (learning and memory impairments) and the electrophysiological phenotype (enhanced basal transmission and LTP) in our transgenic mice suggests the possibility that G_{α}^* may be acting through additional targets other than adenylyl cyclase, such as L-type calcium channels (Lader et al. 1998). Indeed, although transgenic mice overexpressing adenylyl cyclase type I (ACI) also exhibit enhancement in LTP, they do not exhibit alterations in basal synaptic transmission (Wang et al. 2004). Furthermore, mice overexpressing ACI do not exhibit impairments in spatial learning or in contextual or cued fear conditioning (Wang et al. 2004). It should be noted, however, that interpretation of our LTP data is complicated because of the large differences in basal synaptic transmission observed in the G_{α}^* -1 transgenic mice relative to wild-type mice.

Disruptions in G_{α} and cAMP signaling have also been associated with cognitive impairments in humans. A human genetic disorder associated with mild mental retardation, Fuller Albright's syndrome or Albright hereditary osteodystrophy, is caused by loss-of-function mutations in the gene encoding G_{α} (Farfel and Friedman 1986; Ringel et al. 1996). Our analysis of G_{α}^* -1 transgenic mice suggests that overactivation of G_{α} can also ultimately lead to reduced cAMP levels and learning impairments in mice. It will be interesting to determine whether human patients with McCune-Albright syndrome, who carry somatic activating mutations in G_{α} (Shenker et al. 1993; Ringel et al. 1996), exhibit similar biochemical alterations and learning deficits.

Materials and Methods

G_{α}^* transgenic mice

G_{α}^* -1 transgenic mice are maintained in the hemizygous state on a C57BL/6J background. Mice examined in this study were generated from G_{α}^* -1 transgenic mice that had been backcrossed seven to nine times onto a C57BL/6J background. For genotyping, tail DNA was prepared and analyzed by Southern blotting using a transgene-specific probe as described (Abel et al. 1997). Equal numbers of male and female mice between the ages of 2 and 6 mo were used for all behavioral experiments in this paper.

Morris water maze experiments

After 1 w of handling, mice were trained in a water maze as described previously (Bourtchouladze et al. 1994; Abel et al. 1997). Briefly, on the first day of training, the mice were placed on the platform for 30 sec, and then they were allowed one 30-sec practice swim and one platform climb. They were allowed to rest for another 30 sec on the platform, and then training was initiated. During hidden platform training, the platform was not marked by any cue and remained in the same location throughout training, 0.7 cm below the surface of the water. Each swimming trial started with the mice facing the wall of the pool at a random position and ended when they climbed the platform. After reaching the platform, the mice were allowed to remain for 30 sec. If mice did not find the platform within 60 sec, they were picked up by the experimenter and placed on the platform. The mice were trained with four trials per day with a 60-sec intertrial interval. In the probe test, we removed the platform and measured the time that the mice spent in the quadrant in which the platform was located during training. All water maze trials were analyzed using a video tracking system (VP 200, HVS Image). In the visual discrimination test, two cues were placed in the water maze: One was a small plastic cylinder painted with horizontal black and white stripes; the other, a cone painted with a vertical black and white stripes. One cue was attached to a platform that was hidden 0.7 cm below the surface of the water. Another cue was attached to a false platform that was hidden 20 cm below the surface of the water. The mice were trained with two trials per day for 7 d with a 60-sec intertrial interval.

Fear conditioning experiments

Fear conditioning experiments were carried out as described (Abel et al. 1997; Bourtchouladze et al. 1998). On the training day, the mouse was placed in the conditioning chamber (Med Associates) for 2 min before the onset of the tone (CS), which lasted for 30 sec at 2800 Hz and 85 db. The last 2 sec of the CS were paired with the US, 0.7 or 1.5 mA (where indicated) of continuous footshock. After an additional 30 sec in the chamber, the mouse was returned to a home cage. Conditioning was assessed by scoring freezing behavior, which is defined as complete lack of movement, except for respiration, in intervals of 5 sec. Thirty min or 24 h later, contextual fear conditioning was measured by placing subjects in the training chamber for 5 min, and their freezing behavior was scored. The chamber was cleaned

with 70% ethanol before each training trial and prior to each contextual test. For testing cued fear conditioning, the chamber was altered by covering the rod floor with a gray plastic panel and dividing the chamber diagonally in half with another gray plastic panel. The chamber was washed with a dilute solution of Lemon Joy before each cue trial. During the cue test, mice were scored for 5 min. For the first 2 min the CS was not presented (pre-CS test). The CS was presented continuously during the last 3 min (CS test). To avoid potential effects of repeated testing, separate groups of mice were tested 30 min and 24 h after training, and separate groups of mice were tested in contextual and cued fear conditioning. Animals were tested in a balanced fashion with wild-type and transgenic mice and each time point included in each experiment.

Electrophysiological methods

Transverse hippocampal slices (400 μm thick) from 8- to 12-wk-old male mice were prepared using conventional procedures and were maintained in an interface chamber at 28°C. Slices were perfused at a flow rate of 1 mL/min with carboxygenated artificial cerebral spinal fluid (Patterson et al. 1996). For all experiments, slices were allowed to recover for 90 min before recordings were initiated. A bipolar nickel-chromium stimulation electrode was positioned in the stratum radiatum layer of area CA1, and fEPSPs were recorded with a glass microelectrode situated in the stratum radiatum. The stimulation intensity (0.05-msec pulse width) was adjusted to give fEPSP slopes approximately one third of maximum evoked slopes, and baseline responses were elicited once per minute at this intensity. PPF of field EPSPs was tested in wild-type and mutant slices at this intensity using interpulse intervals of 25–200 msec. As a measure of basal synaptic function, the field EPSP slope was measured from sweeps obtained at different stimulus strengths. LTP was induced by applying one 0.5-sec train (50 Hz, test strength) to the stratum radiatum. Slices were tetanized using one of two protocols: one 0.5-sec train at 50 Hz was used to induce E-LTP, and four 0.5-sec trains at 50 Hz, delivered 5 min apart, were used to induce L-LTP.

Data analysis

Animals were tested in a balanced fashion with wild-type and mutant mice included in each experiment. All behavioral experiments included males and females. No effects of sex were detected, so data from male and female mice were combined. Data were analyzed by ANOVA (SYSTAT, version 7.0.1). All values reported are means ± SEM.

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