Inducible, pharmacogenetic approaches to the study of learning and memory

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Here we introduce a strategy in which pharmacology is used to induce the effects of recessive mutations. For example, mice heterozygous for a null mutation of the K-*ras* gene (K-*ras*$^{+/-}$) show normal hippocampal mitogen-activated protein kinase (MAPK) activation, long-term potentiation (LTP) and contextual conditioning. However, a dose of a mitogen-activated/extracellular-signal-regulated kinase (MEK) inhibitor, ineffective in wild-type controls, blocks MAPK activation, LTP and contextual learning in K-*ras*$^{+/-}$ mutants. These indicate that K-Ras/MEK/MAPK signaling is critical in synaptic and behavioral plasticity. A subthreshold dose of NMDA receptor antagonists triggered a contextual learning deficit in mice heterozygous for a point mutation (T286A) in the αCaMKII gene, but not in K-*ras*$^{+/-}$ mutants, demonstrating the specificity of the synergistic interaction between the MEK inhibitor and the K-*ras*$^{+/-}$ mutation. This pharmacogenetic approach combines the high temporal specificity that pharmacological manipulations offer, with the molecular specificity of genetic disruptions.

It is well established that inter-individual differences in the efficacy and toxicity of many medications are related to genetic diversity. Over the past 50 years, there has been significant progress in understanding the clinical relevance of genetic determinants of drug responses[1,2]. Here we used pharmacogenetic interactions to induce the phenotypes of recessive mutations.

Gene targeting in embryonic stem cells has been used to determine the role of biochemical pathways, such as second-messenger signaling cascades, in a variety of biological processes[3,4]. For example, gene knockout studies in mice have implicated a variety of neuronal proteins including neurotransmitter receptors, protein kinases, phosphatases and transcription factors in learning and memory[5,6]. However, this methodology does not allow for temporal control of these mutations, thus limiting both the design and the interpretation of the experiments. Although inducible genetic systems[7,8] promise to circumvent some of these problems, the usefulness of these systems remains limited in contrast to the great number of conventional gene targeting mutants available. It is estimated that there are more than 4,000 targeted mutants already derived. In the heterozygote state, most of these mutations are recessive, and therefore, could be used with the pharmacogenetic approach introduced here. We used this approach to induce the effects of two recessive mutations in mice. This approach takes advantage of synergism between pharmacological and genetic manipulations. For example, the K-*ras*$^{+/-}$ mutation studied here did not affect hippocampal MAPK activation, LTP or learning. Similarly, the dose of the MAPK kinase (MEK) inhibitor that we used in the pharmacogenetic studies also did not affect these phenomena in wild-type mice. However, the combination of these genetic and pharmacological manipulations had a profound effect on hippocampal MAPK activation, LTP and learning. The studies presented here show the importance of K-Ras/MEK/MAPK signaling in synaptic and behavioral plasticity. Similarly, we also used this pharmacogenetic method to show that *N*-methyl-D-aspartate (NMDA) receptor-dependent autophosphorylation at T286 of α-calcium/calmodulin protein-dependent kinase II (αCaMKII) is required for contextual learning. These results indicate that this pharmacogenetic procedure may be widely applicable.

Results

MEK inhibition in K-ras$^{+/-}$ mice: biochemistry

The Ras/MAPK cascade is important in many biological functions[9–11]. The K-*ras* gene is essential for mouse embryogenesis, as K-Ras-deficient embryos die[12,13]. However, K-*ras* heterozygous null mutants (K-*ras*$^{+/-}$) grow and develop normally[12,13]. MEK is downstream of Ras, and activates MAPK in hippocampal neurons[9,14]. Therefore, we applied a pharmacogenetic approach that combined K-*ras*$^{+/-}$ mutation and a MEK inhibitor (SL327)[15,16], to explore the function of K-Ras/MEK/MAPK signaling in the adult brain.

Phorbol esters (for example, phorbol diacetate, or PDA) can activate the MAPK cascade in hippocampal neurons[14,17–19]. Exposure to PDA (10 μM) produced similar levels of p42 MAPK activation in the hippocampus of K-*ras*$^{+/-}$ mutants and wild-type littermates (**Fig. 1a** and **b**). However, application of the MEK inhibitor SL327 (1 μM) attenuated MAPK activation produced by PDA in the hippocampus of K-*ras*$^{+/-}$ but not in wild-type littermates ($F_{3,24} = 13.2$, $p < 0.05$; **Fig. 1a** and **b**). Neither 1 μM SL327 nor the K-*ras*$^{+/-}$ mutation alone affected MAPK phosphorylation in response to PDA. The baseline levels of phosphorylated p42 MAPK were also not altered by exposure to 1 μM SL327 in wild-type or K-*ras*$^{+/-}$ slices ($F_{3,24} = 1.6$, $p > 0.05$). Additional-

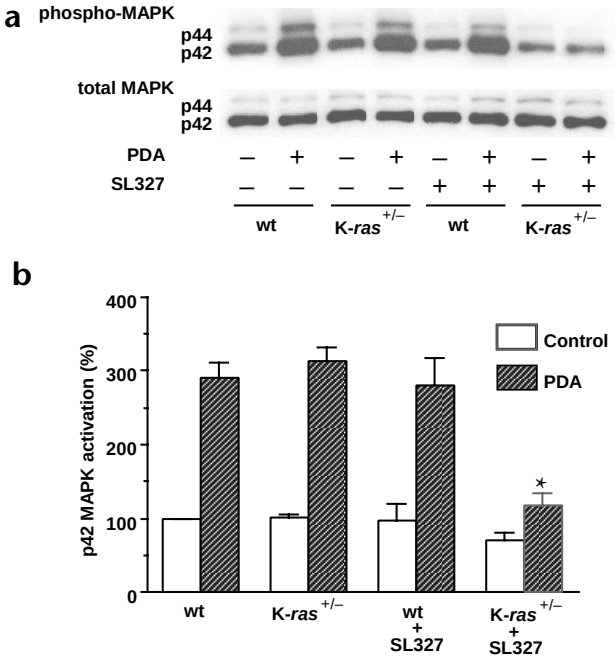

Fig. 1. A subthreshold dose of a MEK inhibitor induces less MAPK activation in K-*ras*$^{+/-}$ mutants. (**a**) Representative western blots indicating protein bands visualized with antibodies to dually phosphorylated p42/p44 MAPK and total p42/p44 MAPK. Hippocampal slices from K-*ras*$^{+/-}$ and wild-type mice were exposed to 10 μM PDA or normal ACSF (control) under the presence or absence of 1 μM SL327. The total MAPK levels indicate equal protein loading. (**b**) Average results (±s.e.m.) of phospho-p42 MAPK levels normalized with the values of the wild-type control group. The SL327-treated K-*ras*$^{+/-}$ group showed a significantly smaller increase in phospho-p42 MAPK levels induced by PDA than each the other three groups (*$p < 0.05$), whereas the other groups did not differ each other.

ly, total MAPK levels were not different among the eight groups examined (**Fig. 1a**). These data show that a subthreshold dose of the MEK inhibitor triggers a MAPK phosphorylation deficit in K-*ras*$^{+/-}$ mutants. This suggests that K-Ras signaling is important in regulating MAPK activation in the hippocampus. The synergistic interaction between the subthreshold dose of the MEK inhibitor SL327 and the K-*ras*$^{+/-}$ mutation is not unlike previously described epistatic interactions between different mutations.

MEK inhibition in K-ras$^{+/-}$ mice: LTP

MAPK signaling is important in synaptic plasticity; LTP-inducing stimuli elicit hippocampal MAPK activation, and the inhibition of MAPK activation blocks LTP induction[17,20]. Thus, we used the pharmacogenetic approach outlined above to test whether K-Ras is involved in LTP, a cellular model of learning and memory. K-*ras*$^{+/-}$ mutants exhibited normal hippocampal LTP ($F_{1,19} = 0.02$, $p > 0.05$; **Fig. 2a**), just as they showed normal MAPK activation. In wild-type slices, the MEK inhibitor SL327 prevented the induction of LTP in a concentration-dependent manner ($F_{2,28} = 9.6$, $p < 0.05$) without affecting baseline synaptic transmission at Schaffer collateral–CA1 synapses (**Fig. 2b** and **c**). Pre-tetanic application of 1 μM SL327 did not affect LTP in wild-type slices, but significantly inhibited the induction of hippocampal LTP in slices from K-*ras*$^{+/-}$ mice ($F_{3,38} = 8.5$, $p < 0.05$; **Fig. 2c** and **d**). Neither 1 μM SL327 nor K-*ras*$^{+/-}$ mutation alone affected LTP. Just as with the MAPK phosphorylation experiments, only the combination of these two treatments was effective.

Post-tetanic application of 1 μM SL327 did not affect the expression or maintenance of established LTP in K-*ras*$^{+/-}$ slices ($F_{1,12} = 0.5$, $p > 0.05$; **Fig. 2e**), indicating a temporally specific role of K-Ras/MEK/MAPK signaling in the induction but not in the expression or maintenance of LTP. These results are consistent with the observation that MAPK is activated 2 minutes after LTP induction and returns to baseline levels by 45 minutes[17,20].

MEK inhibition in K-ras$^{+/-}$ mice: behavior

Contextual fear conditioning is a robust and long-lasting form of learning, which is sensitive to hippocampal manipulations[21–24]. In this test, mice learn to associate a distinct context (conditioned stimulus; CS) with an aversive stimulus such as foot shock (unconditioned stimulus; US). When placed back in the same training context, the mice exhibit a range of conditioned fear responses, including freezing[25,26]. Contextual conditioning specifically activates p42 MAPK signaling in the hippocampus[15], and MEK inhibitors disrupt contextual condition-

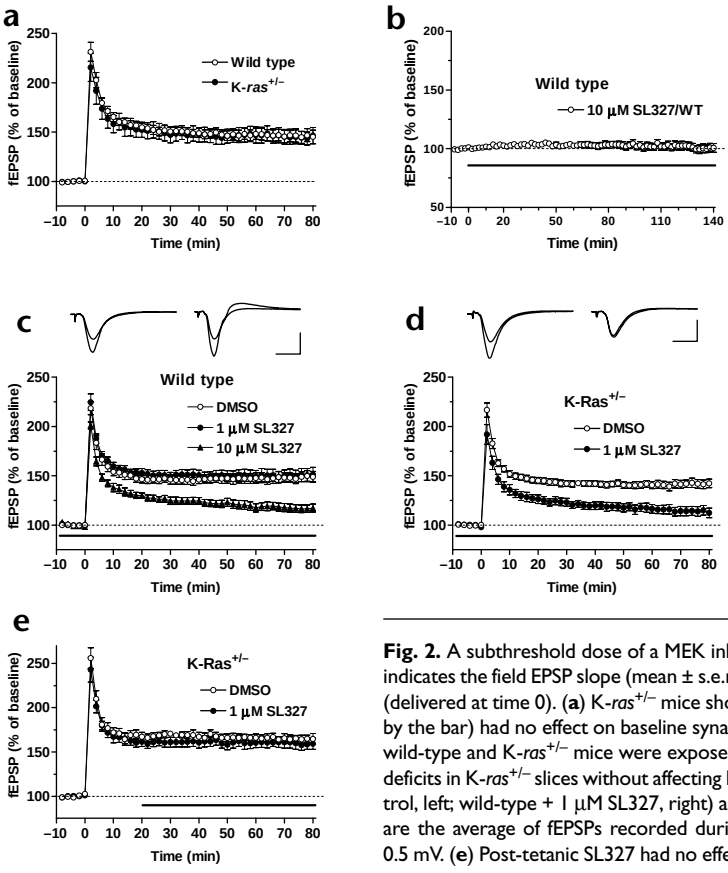

Fig. 2. A subthreshold dose of a MEK inhibitor induces an LTP impairment in K-*ras*$^{+/-}$ mutants. Each point indicates the field EPSP slope (mean ± s.e.m.) normalized to the average baseline response before the tetanus (delivered at time 0). (**a**) K-*ras*$^{+/-}$ mice showed normal hippocampal LTP. (**b**) Application of SL327 (indicated by the bar) had no effect on baseline synaptic transmission at CA1 synapses. (**c**, **d**) Hippocampal slices from wild-type and K-*ras*$^{+/-}$ mice were exposed to pre-tetanic application of SL327. SL327 (1 μM) triggered LTP deficits in K-*ras*$^{+/-}$ slices without affecting LTP induction in wild-type slices. Traces in panel (**c**) (wild-type control, left; wild-type + 1 μM SL327, right) and in panel (**d**) (K-*ras*$^{+/-}$ control, left; K-*ras*$^{+/-}$ + 1 μM SL327, right) are the average of fEPSPs recorded during baseline and 70–80 min after tetanization. Scale bars, 10 ms, 0.5 mV. (**e**) Post-tetanic SL327 had no effect on hippocampal LTP in slices from K-*ras*$^{+/-}$ mutants.

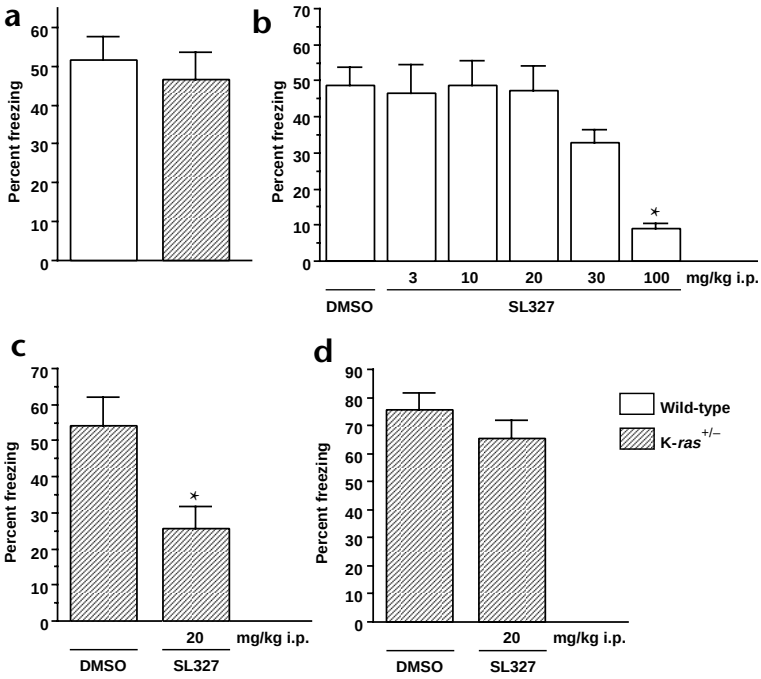

Fig. 3. A subthreshold dose of a MEK inhibitor induces a contextual conditioning impairment in K-*ras*$^{+/-}$ mutants. Each column represents the percentage of freezing (mean ± s.e.m.) tested 24 h after training. (**a**) K-*ras*$^{+/-}$ mice showed normal levels of contextual freezing. (**b**) Pre-training administration of SL327 decreased contextual freezing in wild-type mice in a dose-dependent manner. *$p < 0.05$ versus DMSO-treated group. (**c**) K-*ras*$^{+/-}$ mice were administered DMSO or 20 mg/kg of SL327 1 h before training, which had no effect on freezing in wild-type littermates. SL327 (20 mg/kg) significantly decreased contextual freezing in K-*ras*$^{+/-}$ mice (*$p < 0.05$). (**d**) SL327 (20 mg/kg) administered 2 h post-training had no effect on contextual freezing in K-*ras*$^{+/-}$ mice.

ing[15,16,27]. Therefore, we used the pharmacogenetic approach outlined above to explore the role of K-Ras/MEK/MAPK signaling in contextual learning and memory.

K-*ras*$^{+/-}$ mice tested 24 hours after training showed normal contextual conditioning ($F_{1,18} = 0.3$, $p > 0.05$; **Fig. 3a**). Pre-training administration of the MEK inhibitor SL327 impaired contextual conditioning in wild-type mice (24-h test) in a dose-dependent manner ($F_{5,63} = 4.4$, $p < 0.05$; **Fig. 3b**). Administration of 20 mg/kg of SL327 did not affect contextual conditioning in wild-type littermates, but significantly impaired it in K-*ras*$^{+/-}$ mutants ($F_{1,16} = 7.8$, $p < 0.05$; **Fig. 3c**). Thus, only the combination of the MEK inhibitor SL327 (20 mg/kg) and the K-*ras*$^{+/-}$ mutation disrupted contextual conditioning; neither manipulation alone had an effect. The unconditioned response (UR) to shock was not affected by SL327 in either K-*ras*$^{+/-}$ mutants or wild-type mice (data not shown), suggesting that this pharmacogenetic manipulation did not disrupt the mouse's ability to perceive the foot shock.

Post-training (2 h) administration of 20 mg/kg of SL327 did not affect contextual conditioning in K-*ras*$^{+/-}$ mutants ($F_{1,18} = 1.4$, $p > 0.05$; **Fig. 3d**), just as post-tetanic application of this compound failed to affect established LTP. These results indicate that K-Ras/MEK/MAPK signaling at the time of training, but not some time afterwards, is critical for formation of contextual memory.

A weak training protocol (30-s placement-to-shock) was unable to trigger a contextual conditioning deficit in K-*ras*$^{+/-}$ mutants. K-*ras*$^{+/-}$ mice exhibited contextual freezing (23.5 ± 4.4%) comparable to that of wild-type littermates (21.9 ± 4.4%; $F_{1,38} = 0.07$, $p > 0.05$), suggesting that the K-*ras*$^{+/-}$ mutation alone does not weaken contextual conditioning. Whereas contextual learning is normal in K-*ras*$^{+/-}$ mutants, more complex hippocampus-based learning such as spatial learning in water maze is affected in these mice (data not shown).

Specificity of pharmacogenetic interactions

Previous studies suggested that NMDA receptor-dependent activation of hippocampal MAPK is critical for contextual fear conditioning[15]. However, it is unknown how NMDA receptor function is coupled to MAPK activation during learning. Therefore, we next tested whether a subthreshold dose of an NMDA receptor antagonist could induce a contextual conditioning deficit in K-*ras*$^{+/-}$ mice. Pre-training administration of 10 mg/kg but not 5 mg/kg CPP disrupted contextual conditioning (24-h test) in both wild-type ($F_{2,34} = 8.4$, $p < 0.05$) and K-*ras*$^{+/-}$ mice ($F_{2,32} = 8.8$, $p < 0.05$; **Fig. 4**). These two doses of CPP affected wild-type and K-*ras*$^{+/-}$ mice in the same manner, demonstrating that decreases in NMDA receptor signaling do not affect K-*ras*$^{+/-}$ mice more severely than their wild-type littermates.

There is much evidence for a close structural and functional link between the NMDA receptor and αCaMKII[28–32]. NMDA-receptor-dependent autophosphorylation of αCaMKII at T286 is required for the activation of this kinase[32–35]. Our previous studies demonstrated that mice homozygous for a point mutation at T286 that blocks the autophosphorylation of αCaMKII (αCaMKII$^{T286A-/-}$) have normal NMDA function, but have severe impairments in hippocampal plasticity and hippocampus-dependent learning and memory[36]. Similarly, this homozygous mutation completely blocked contextual fear conditioning (data not shown). In contrast, the heterozygous αCaMKII$^{T286A+/-}$ mutants showed normal levels of contextual conditioning tested 24 h after training (**Fig. 5b** and **d**). In wild-type mice, contextual fear conditioning was disrupted by pre-training administration of NMDA receptor antagonists CPP ($F_{3,62} = 3.7$, $p < 0.05$) or MK-801 ($F_{4,48} = 3.8$, $p < 0.05$) in a dose-dependent manner (**Fig. 5a**). Administration of doses of CPP (5 mg/kg) and MK-801 (0.01 mg/kg), which were behaviorally ineffective in wild-type

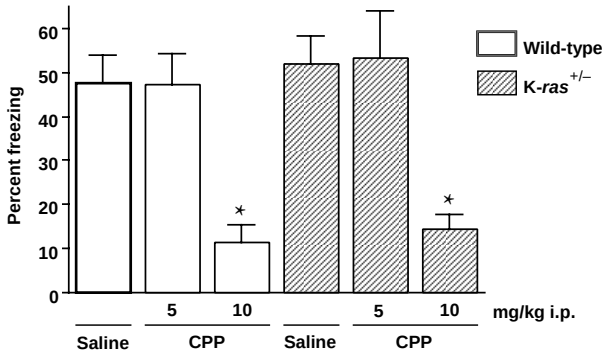

Fig. 4. A subthreshold dose of an NMDA receptor antagonist does not induce a contextual conditioning impairment in K-*ras*$^{+/-}$ mutants. Each column represents the percentage of freezing (mean ± s.e.m.) tested 24 h after training. Pre-training administration of CPP affected contextual learning in K-*ras*$^{+/-}$ mutants and wild-type littermates similarly.

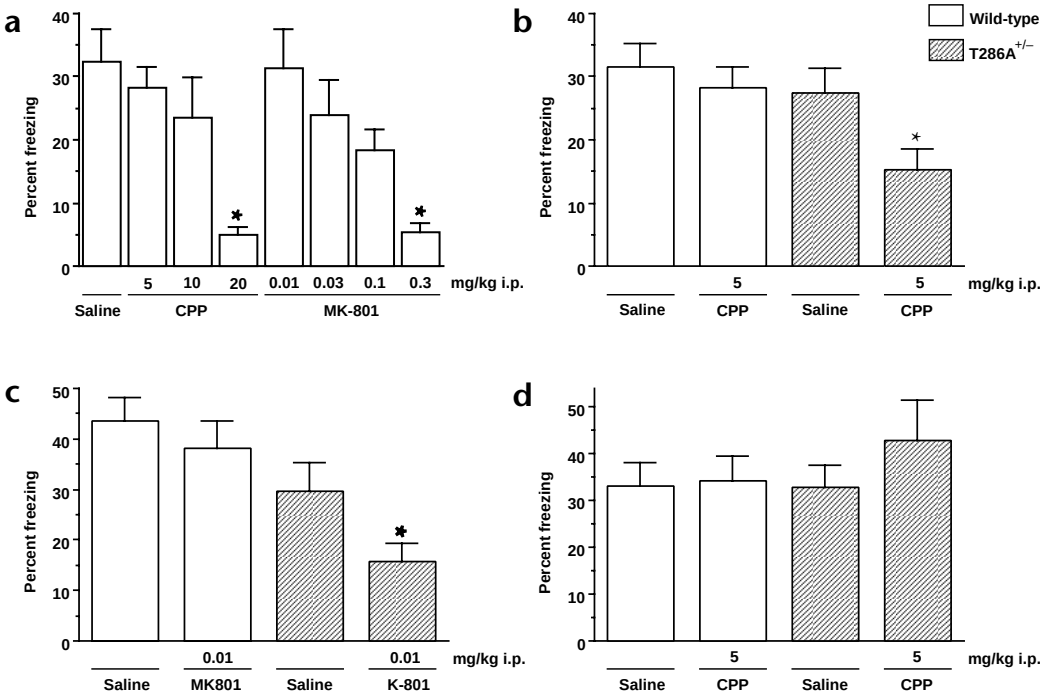

Fig. 5. Subthreshold doses of NMDA receptor antagonists induce a contextual conditioning impairment in αCaMKII^{T286A} heterozygotes, but not in K-*ras*$^{+/-}$ mutants. Each column represents the percentage of freezing (mean ± s.e.m.) tested 24 h after training. (**a**) Pre-training administration of CPP and MK-801 decreased contextual freezing in wild-type mice in a dose-dependent manner. *$p < 0.05$ versus saline-injected group. (**b**, **c**) αCaMKII$^{T286A+/-}$ mice and wild-type littermates were administered saline, 5 mg/kg CPP or 0.01 mg/kg MK-801 30 min before training. The CPP- or MK-801-injected αCaMKII$^{T286A+/-}$ group showed significantly smaller freezing responses than each the other three groups (*$p < 0.05$), whereas the other groups did not differ from each other. (**d**) CPP (5 mg/kg) administered 2 h post-training had no effect on contextual freezing in αCaMKII$^{T286A+/-}$ mice or wild-type littermates.

mice, disrupted contextual fear conditioning in αCaMKII$^{T286A+/-}$ mutants (CPP, $F_{3,115} = 3.9$, $p < 0.05$, **Fig. 5b**; MK-801, $F_{3,56} = 6.4$, $p < 0.05$, **Fig. 5c**). Therefore, 5 mg/kg of CPP triggered a contextual conditioning deficit in αCaMKII$^{T286A+/-}$ mutants, but not in K-*ras*$^{+/-}$ mice. Therefore, the effects of this dose of CPP are specific to the αCaMKII$^{T286A+/-}$ mutants, reflecting the close interaction between NMDA receptors and αCaMKII. Similarly, our data also suggest that K-Ras is critical in MEK-dependent activation of MAPK. These findings attest to the specificity of the synergistic interactions at the heart of this pharmacogenetic approach.

The conditioning deficits in αCaMKII$^{T286A+/-}$ mutants treated with either CPP or MK-801 were not due to changes in nociception, as neither of these pharmacogenetic manipulations affected unconditioned responses to foot shock (data not shown). Post-training injection of 5 mg/kg CPP also had no effect on contextual learning in αCaMKII$^{T286A+/-}$ mutants ($F_{3,36} = 0.6$, $p > 0.05$; **Fig. 5d**), indicating that contextual conditioning requires NMDA-receptor-dependent autophosphorylation of αCaMKII, specifically around the time of training.

Discussion

Here we have introduced a way to manipulate molecular function in the central nervous system. This approach takes advantage of synergistic interactions between pharmacological and genetic manipulations to alter the function of specific signaling pathways in a temporally controlled manner. For example, the K-*ras*$^{+/-}$ mutation alone did not affect hippocampal MAPK activation. However, a subthreshold dose of an inhibitor of MEK, a component of the Ras/MEK/MAPK pathway[9], triggered a complete block of hippocampal MAPK activation in the K-*ras*$^{+/-}$ mice. The same dose of this inhibitor did not affect MAPK activation in wild-type littermate controls.

MEK and MAPK have previously been shown to be involved in LTP[17,20] and learning[15,16,27,37]. Our pharmacogenetic approach indicated that K-Ras signaling is critical for the activation of the MAPK cascade during synaptic and behavioral plasticity. Although the K-*ras*$^{+/-}$ mutants showed normal hippocampal LTP and contextual conditioning, a dose of the MEK inhibitor ineffective in wild types induced both LTP and learning deficits in these mutants. Previous studies indicated that a H-*ras* mutation enhanced hippocampal NMDA currents and LTP[38]. However, it is unclear whether this enhancement in LTP is a compensatory reaction to the homozygous loss of H-*ras* during development. The advantage of our pharmacogenetic approach is that it circumvents developmental confounds. Unlike H-*ras* homozygous null mutants, the K-*ras*$^{+/-}$ mice show normal LTP; their deficits are observed only after drug treatment.

The pharmacological induction of the K-*ras*$^{+/-}$ phenotype showed temporal specificity, as pre-training but not post-training (2 h) administration of the MEK inhibitor triggered the contextual learning deficit in this mutant. Similarly, the MEK inhibitor is only effective when applied before the induction of LTP. Indeed, p42 MAPK activation is significantly increased in the hippocampus one hour after training, but returns to baseline levels within two hours of contextual conditioning[15]. Taken together, these findings indicate that K-Ras/MEK/MAPK signaling is critical in both the induction of LTP andhippocampus-dependent learning.

Although we did not find any evidence linking NMDA receptor signaling and K-Ras-dependent plasticity and learning, a pharmacogenetic approach like the one described here could be used to explore the proposed connection between NMDA signaling and different Ras proteins. For example, H-Ras is thought to regulate NMDA currents[38]. Additionally, NMDA signals may activate specific Ras proteins through Ras-GRF (a Ras guanine nucleotide releasing factor) thought to have a role in plasticity and learning[39–41]. NMDA receptor activation may also amplify certain Ras signals by inhibiting SynGAP, a synaptic Ras-GTPase activating protein[42,43]. Our present findings are not inconsistent with a possible link between NMDA signals and other Ras isoforms (such as H-Ras and N-Ras) signaling to the MAPK cascade.

Although a subthreshold dose of an NMDA receptor blocker did not trigger deficits in the K-*ras*$^{+/-}$ mutants, it induced learning and LTP deficits (data not shown) in heterozygous αCaMK-II^{T286A} mice. Mice homozygous for this point mutation show profound deficits in spatial[36] and contextual conditioning learning that could be conceivably due to developmental deficits. Our present data discount this possibility. We found that although heterozygous αCaMKII^{T286A} mice did not show a contextual deficit, a dose of NMDA receptor antagonists ineffective in wild-type mice induced a contextual conditioning deficit in these mutants. Importantly, this deficit was only induced when the drug was administered before training. Therefore, these data demonstrate that the NMDA receptor-dependent autophosphorylation of αCaMKII specifically during training is critical for learning[32]. The subsequent phosphorylation of key cellular substrates, such as glutamate receptors[44], is thought to be required for early stages of memory formation[45,46]. Because the pharmacogenetic approach introduced here uses drugs at concentrations that are ineffective in wild types, the nonspecific effects of these drugs should be reduced.

The results presented here demonstrate that pharmacological manipulations can be used to induce the phenotype of recessive mutations in mice. Although neither the αCaMKII$^{T286A+/-}$ nor K-*ras*$^{+/-}$ mice showed contextual learning deficits, partial pharmacological disruption of specific signaling components upstream or downstream from those genetically targeted molecules triggered learning deficits in the mutants. Importantly, these partial pharmacological disruptions only affected contextual conditioning in the presence of the mutations. Thus, epistatic-like interactions between pharmacological and genetic manipulations can be used to induce the effects of mutations in mice in a temporally controlled manner, and to identify novel functional relations between signaling pathways. This approach combines the high temporal specificity that pharmacological manipulations offer, with the molecular specificity of genetic disruptions. Spatial control of these effects could also be accomplished by neuroanatomically guided injections of compounds of interest. Importantly, this pharmacogenetic approach will be applicable not only to neuroscience questions, but also to other biological problems and to other genetic systems (such as *Drosophila*, *Caenorhabditis elegans* and yeast).

Methods

Mice. We used K-*ras* mutants (K-*ras*$^{+/-}$)[13] that were F1 progeny derived from a cross between mice heterozygous for a null mutation in the K-*ras* gene (129T2/SvEmsJ background) and C57Bl/6N mice. Starting with the αCaMKII$^{T286A+/-}$ chimeras (contributing to 129 background), this mutation was backcrossed into the C57Bl/6 genetic background[36]. The αCaMKII$^{T286A+/-}$ mice used in the experiments were heterozygotes derived after 8–9 backcrosses into C57Bl/6N. At 4–5 weeks postnatally, the mice were weaned and their genotypes were determined with polymerase chain reaction analysis of tail DNA samples. All experiments were done with mice 3–7 months old, and a similar number of males and females were used. The mice were housed in groups and kept on a 12 h light/dark cycle, and the experiments were always conducted during the light phase of the cycle. With the exception of testing times, the mice had *ad lib* access to food and water. All the procedures used were approved by UCLA's Animal Research Committee. Animals were maintained in accordance with the applicable portions of the Animal Welfare Act and the Department of Health and Human Services *Guide to the Care and Use of Laboratory Animals*.

Drugs. SL327 (provided by DuPont Pharmaceuticals, Wilmington, Delaware) and phorbol 12,13-diacetate (PDA; Sigma, St. Louis, Missouri) were dissolved in 100% DMSO. [±]-3-[2-Carboxypiperazin-4-yl]propanephosphonic acid (CPP; Sigma) and (+)-MK-801 hydrogen maleate (RBI, Natick, Massachusetts) were dissolved in saline or artificial cerebrospinal fluid (ACSF). All biochemical, electrophysiological and behavioral experiments were conducted with the experimenter blind to the drug treatments as well as the genotype of mice.

Electrophysiology. Transverse hippocampal slices (400 μm thick) were maintained in a submerged recording chamber perfused with ACSF equilibrated with 95% O_2 and 5% CO_2 at 30°C. The ACSF contained 120 mM NaCl, 3.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$ and 10 mM D-glucose. Extracellular field EPSPs were recorded with a metallic electrode from the stratum radiatum layer of the area CA1, and the Schaffer collaterals were stimulated with a bipolar electrode. The intensity of stimulation (100-μs duration) was adjusted to give field EPSP approximately 33% of maximum. LTP was induced by a tetanic stimulation (100 Hz, 1 s) delivered at the test intensity. After the responses were monitored at least for 20 min to ensure a stable baseline, drugs were applied to hippocampal slices. SL327 (maximal final DMSO concentration, 0.1%) was applied for 1 h before tetanization, and was maintained in the bath throughout the recording period. In some slices, SL327 was applied post-tetanically (20 min after tetanization). To determine whether the magnitude of LTP differed significantly between the groups, responses from the last 10-min block of recordings (70–80 min) were compared statistically.

Western blotting. Hippocampal slices prepared from K-*ras*$^{+/-}$ mice and wild-type littermates were transferred to the same chamber as used for electrophysiology, and then exposed to PDA (10 μM) or normal ACSF for 10 min. The slices were preincubated with SL327 for 70 min. In the last 10 min of preincubation, slices were exposed to PDA or ACSF. After pharmacological activation with PDA, the CA1 subregions of hippocampal slices were dissected out on ice and homogenized in an ice-cold lysis buffer (containing 25 mM HEPES (pH 7.5), 150 mM NaCl, 2 mM EDTA, 10% glycerol, 1% Triton X-100, 100 μg/ml phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml leupeptin, 1 mM sodium orthovanadate (Na_3VO_4), 25 mM β-Na glycerophosphate and 10 mM NaF). After insoluble material was removed by centrifugation (13,000 *g* for 5 min), protein concentration of the supernatant was determined by using a PIERCE BCA Protein Assay Kit (Pierce, Rockford, Illinois). Samples containing equivalent amount of protein (20 μg) were separated by 12% SDS-PAGE gels and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, California). Membranes were blocked in TBS with 0.1% Tween-20 and 5% dry milk overnight at 4°C. Then the blots were incubated for 2 h at room temperature with the primary antibody which selectively recognizes p42/p44 MAPK dually phosphorylated at Thr^{202} and Tyr^{204} (1:1,000; New England Biolabs, Beverly, Massachusetts). Another antibody that detects both phosphorylated and unphosphorylated forms of p42/p44 MAPK (1:1,000; New England Biolabs) was used to measure total MAPK levels. After membranes were washed, the blots were incubated with HRP-conjugated secondary antibody (1:2,000; Bio-Rad). Protein signals were visualized by enhanced chemiluminescence (ECL Western Blotting Analysis system, Amersham, Arlington Heights, Illinois). Densitometric analysis for quantification of the signals was performed using a desktop scanner and ImageQuaNT software (Molecular Dynamics, Sunnyvale, California). For each experiment, both phosphorylated and total MAPK levels were normalized to those observed in the control group of wild-type mice.

Contextual fear conditioning. The contextual conditioning experiments were performed as described previously[47]. During training, mice were placed in the conditioning chamber for 2.5 min, and then αCaMKII$^{T286A+/-}$ and K-*ras*$^{+/-}$ mice were exposed to a 0.75 and 0.50 mA foot shock for 2 s, respectively. In a separate experiment, we assessed contextual fear conditioning in mice receiving a single foot shock 30 s following placement in the conditioning chamber during training. After shock delivery, mice were left in the chamber for another 30 s, and then returned to their home cage. CPP and MK-801 were administered intraperitoneally (i.p.) in a volume of 10 ml/kg 30 min before or 2 h after training. Mice received an i.p. injection of SL327 in a volume of 2 ml/kg 1 h before or 2 h after training. The mice were tested for contextual conditioning 24 h after training. Conditioning was determined by scoring freezing behavior (absence of all but respiratory movement) with automated procedures described previously[47] when the mice were placed back into the conditioning chamber.

Data analysis. The significance of differences between the groups was determined by a one-way analysis of variance followed by *post-hoc* Newman–Keuls test when F ratios reached significance ($p < 0.05$).

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